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Comparative study on Cytotoxicity of Delfin Insecticide using Two Vital Protozoan Ciliates *Paramecium caudatum* and *Oxytricha fallax*

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Abstract : The freshwater ciliate protozoan *Paramecium caudatum and Oxytricha fallax* were used to evaluate potential cytotoxic effects of Delfin Insecticide and a comparison was made. LC_{50} value by mortality curve for three hours acute toxicity test of *Paramecium caudatum* and *Oxytricha fallax* was found to be 250.17±15.33ppm and 413.363±72.91ppm, respectively. *Paramecium caudatum* was found to be sensitive than *Oxytricha fallax* to Delfin. The shape, size and length of test organisms were reduced prominently. Similarly, changes in contractile vacuole and phagocytosis were also observed. Genotoxicity of pesticide was clearly evident from the alterations in nuclear morphology such as fragmentation, vacuolization, elongation, rod shaped deformity and complete diffusion of nucleus on exposure to Delfin in a concentration dependent manner. The results call attention to the possible role of ciliates in genotoxic studies and to their capacity as suitable toxicological tools.

Key words : *Paramecium caudatum*, *Oxytricha fallax*, Delfin, Feulgen Fast Green Technique, Low cost microbiotest, Toxicity evaluation.

Introduction

It is generally agreed that in the field of environmental biomonitoring, toxicological bioassays can provide useful information for identifying those situations requiring a close investigation at an early stage. From this point of view, it is of increasing interest to identify a panel of organisms displaying direct and sensitive responses to environmental changes. Assays with protists are regarded as valuable bioassays to be exploited in standardized laboratory procedures for evaluating the toxicity of chemical compounds or polluted waters (Daniz *et al.*, 2006; Fenech and Crott, 2002; Geetha and Fulekar, 2008; Wild, 2006). Due to their nature as a eukaryotic organism, protists exhibit a relatively simple organization and a high degree of specialization. Protists respond directly to environmental stimuli behaving like animals as selection units towards environmental challenges. On the other hand, protists are more sensitive to environmental modifications than the cells of higher organisms that have differentiated, organized complex structures and organs. Due to their small size, protists generally multiply through short cell cycles, thus making it possible to study the effects of pollutants on a large and genetically homogeneous cell population over a short time period as well as on subsequent cell generations (Fawole *et al.,* 2008; Masood Hussain *et al.*, 2008; Nageswar and Masood Hussain, 2007). In this context, the present study is an attempt to evaluate the sensitivity of *Paramecium caudatum* and *Oxytricha fallax* to Delfin and to obtain a predictive tool for hazard and risk assessment in the water quality criteria.

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Materials and Methods

Protozoan ciliates *Paramecium caudatum* and *Oxytricha fallax* were selected as test species for the present study. They were collected and isolated from fresh water pond within the vicinity of Osmania University, Hyderabad, India. The organisms were cultured in sterilized hay infusion medium in the laboratory at room temperature (Shiny *et al.*, 2005). Stock solution and experimental concentrations of Delfin were prepared as recommended by APHA (1995). Stock solution of 1000ppm of Delfin was prepared using double distilled water. Commercial grade of Delfin Insecticide used in this study was manufactured by CERTIS LLC, Columbia, USA and imported and packed by Margo biocontroles (P) Ltd, Bangalore, India. After preliminary range findings, the appropriate stock solutions and the test concentrations were selected, prepared afresh and used for further studies. Acute toxicity tests were conducted for 3 hours duration as suggested by Arshaduddin *et al.* (1998). In acute experiments 0.5 ml of pesticide solution was added to 4.5 ml of culture medium to achieve desired concentration of pesticide. 50 organisms were introduced in each cavity block. Triplicates were maintained for all test concentrations. Each cavity block, after adding pesticide was placed under binocular microscope and counting was done at 10 min interval during first 1 h and thereafter 20 min interval for the next 2 hours. LC_{50} value was calculated against the mortality curve for 3 hours. Controls devoid of pesticide, with same number of organisms were run simultaneously.

Cytochemical studies were conducted to demonstrate the nuclear morphology of *Paramecium caudatum* and *Oxytricha fallax* on exposure to sub-lethal concentrations (200, 20 and 2ppm) of Delfin for 1 hour. Nuclear staining was done after fixation of cells using Carnoy's fixative (Ethyl alcohol, Acetic acid and Methyl alcohol in the ratio of 3:1:1 respectively) by Feulgen fast green technique and it was the most suitable technique for the demonstration of nuclear apparatus. A total of 200 cells each both in control and treated were examined in one set and it was repeated 5 times. The cells were hydrolyzed first briefly in 1 N HCl maintained at room temperature and then at 60°C for exactly 8 minutes in an incubator. Hydrolysis followed by transferring the slides to Schiff's reagent and incubated for 1 h. Then the cells were immersed in three changes of sulphurous acid salt solution for 5 to 6 min, again rinsed in distilled water, dehydrated in graded alcohols, cleared in xylene and mounted in DPX.

Results

The macronuclear staining technique has been performed on *Paramecium caudatum* and *Oxytricha fallax* in order to explore the pesticidal influence on genetic material. Various types of abnormalities in the nuclear shape, number, and size have been found. Fragmentation, rod shape, elongation, karyolysis and vacuolization were observed.

Table 1 : Delfin-induced macronuclear aberrations in *Paramecium caudatum* for one hour exposure

Con/ppm	Total abnormalities	Unevenly divided nucleus	Fragmentation of nucleus	Rod shaped nucleus	Vacuolated nucleus	Other deformities
200	72.2±2.82	19±1.22	16±0.70	13.3±1.52	15.3±2.08	9.3±2.51
20	56.1±1.85	16±1.58	12.2±1.92	11.3±0.57	12.0±1.0	5.3±1.52
2	33.0±2.16	8.2±0.83	6.2±0.83	8.0±1.01	7.33±1.52	4.3±2.51

Values are significant at P<0.05, (n= 5)

Table 2 : Delfin induced macronuclear aberrations in *Oxytricha fallax* for one hour exposure.

Con/ppm	Total abnormalities	Unevenly divided nucleus	Fragmentation of nucleus	Rod shaped nucleus	Diffusion of nucleus	Other deformities
200	60.5±1.71	14.3±1.52	16.0±1.0	9.3±0.94	11.6±1.52	10.3±2.51
20	43.2±1.81	10.2±1.83	12.3±0.57	5.6±1.52	8.3±1.52	7.0±1.0
2	26.3±0.94	6.3±2.51	7.3±1.52	3.2±1.83	5.3±0.57	5.3±1.52

Values are significant at P<0.05, (n= 5)

Such abnormalities are related to cell division failures, cell death processes, and to genotoxicity and / or mutagenicity (Tatiana da Silva and Carmen, 2006). Feulgen fast green stained preparations were used to detect the nuclear aberrations. The occurrence of macronuclear aberrations were dose dependent. The obtained data illustrates the presence of large number of fragmented and unevenly divided nuclear forms on exposure to Delfin. The highest total abnormalities (72.2±2.82) were recorded when *Paramecium caudatum* exposed against 200ppm of Delfin for one hour. In concentrations of 200, 20and 2ppm the percent abnormal forms recorded were 72.2±2.82, 56.1±1.85 and 33.0±2.16, respectively (Table-1). Whereas in the second set of experiment *Oxytricha fallax* showed 60.5±1.71, 43.2±1.81 and 26.3±0.94 abnormalities to 200, 20 and 2ppm of Delfin respectively (Table- 2).

750ppm was immediate toxic dose to *Paramecium caudatum* and cell death was exhibited with complete stopping of ciliary movements and lack of any contractile vacuole and food vacuole activity. At higher concentration cases of abnormal individuals with a disturbance in the mechanism of locomotion were observed. Shortening of longitudinal axis, speedy egesting of food vacuoles and enlargement of contractile vacuole were also observed. The calculated LC_{50} value of Delfin to *Paramecium caudatum* for 3 hours was 250.17±15.33ppm (Fig-1). *Oxytricha* exposed to 200ppm concentration showed vacuolization and

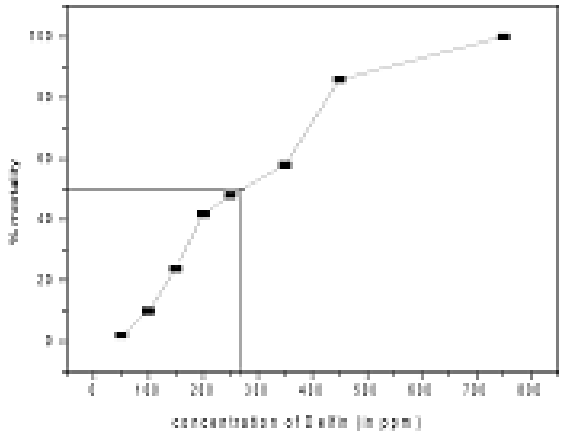

Fig. 1 : Calibration of curve showing lethal concentration and LC_{50} values of delfin to *Paramecium caudatum.*

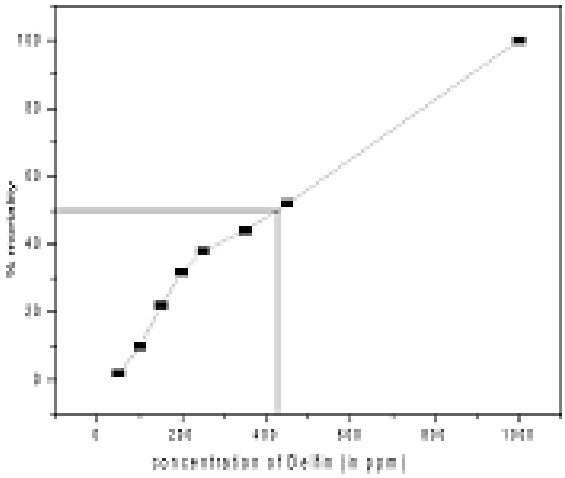

Fig. 2 : Calibration of curve showing lethal concentration and LC_{50} values of delfin to *Oxytricha fallax*.

morphological deformities such as swollen body shape and shortening of longitudinal axis. Calculated LC_{50} value of Delfin to *Oxytricha fallax* was 413.363±72.91ppm (Fig-2).

Discussion

The cytotoxicity of several pesticides was evaluated in different experimental model organisms but due to ciliate sensitivity to environmental alterations they have been proposed as biological indicators of water pollution. A detailed study was made on the physiological and toxic effect of some detergents on *Paramecium caudatum* (Dryl and Mehr, 1976). Besides the use of other protozoa as test organisms, namely *Colpidium campylum, Spirostomum ambiguum and Spirostomum teres; Paramecium caudatum and Oxytricha fallax* are some of the most commonly used ciliated protozoa for laboratory research. In these ciliates various endpoints can be used to evaluate the cytotoxic effects of xenobiotics (Sauvant *et al*., 2000). Growth rate and morphological changes have been used for some decades. Other parameters such as cell motility, swimming patterns and cytoskeleton analysis, can be assessed and have been proposed to determine the physiological and energetic state of ciliates when in contact with pollutants (Darcy *et al*., 2002; Dias and Nelson, 2003).

The monocrotophos, an organo-phosphorous pesticide, has significantly enhanced the velocities of exposed paramecia in a concentration dependent manner. At lower concentrations paramecia did not exhibit any blebbing but showed an initial enhancement in velocity. About 20% of the exposed paramecia at 45 mgl^{-1} developed minor blebbing, with reduced velocity. However, more than 60% paramecia exposed to 60 mgl^{-1} showed initial increase in their velocities but declined within few minutes due to blebbing (Venkateswara Rao *et al.*, 2007). Length of paramecia was reduced prominently by a concentration of monocrotophos above 1ppm at exposure periods of 48 to 120 hours but the degree of decrements in breadth and length of cytopharynx was dependent upon the test concentration of monocrotophos. The sub-lethal concentrations at a very low level (1, 10, 50 and 100ppm) are toxic to *Paramecium caudatum* with reference to decline in population (Ujwala *et al.*, 2007). Certain

morphological alterations and physiological responses in paramecia were observed on exposure to a biological insecticide Delfin at lower concentrations (Nageswara and Masood Hussain, 2008).

Many nuclear abnormalities such as vacuole nuclei, nuclear fragmentation, nuclear retraction, karyolysis and vacuolated cytoplasm reported in the erythrocytes of *Nile tilapia* exposed to waters affected by refinery effluent (Tatiana da Silva and Carmen, 2006). The amplified DNA is localized selectively to specific sites at the periphery of the nucleus and eliminated *via* nuclear budding to form micronuclei. The formation of these abnormalities would represent a way to eliminate any amplified genetic material from the cell nucleus (Shimizu *et al.*, 2000). The active metabolites of cypermethrin may have contributed to the formation of DNA monoadducts and DNA interstrand cross links in the primary mouse hepatocytes (Yong *et al*., 2006). If the DNA adducts are not repaired or are mistakenly repaired before DNA replication, they may lead to gene mutations and initiate Carcinogenesis (Gupta and Spencer-Beach, 1996). DNA interstrand crosslinks stimulate cells to proliferate out of control leading to tumor (Sawyer and Brown, 2000). The results obtained by analyzing nucleus of *Paramecium caudatum* and *Oxytricha fallax*, it is clear that the Delfin induces the abnormalities in nuclear shape which seems to indicate its cytogenetic effect. The macronucleus of ciliates is center of the entire series of metabolic activities and in its absence the animal soon dies.

In conclusion, our results indicate that: (1) *Paramecium caudatum* and *Oxytricha fallax*, ciliates in general can be exploited as toxicological tools for the screening of pesticides; (2) *Paramecium caudatum* was found to be more sensitive than *Oxytricha fallax* to Delfin; (3) the use of nuclear staining technique could be extended to biomonitoring programs investigating the genotoxic effects of pesticide contaminants present in fresh water environments.

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