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Short Communication

Ecophysiological and cytopathological impact of delfin insecticide (*Bacillus thuringiensis*) to an unicellular ciliate protozoan, *Euplotes patella*

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Abstract

Ciliates have been exploited as useful and highly potential models for water quality fluctuations and toxicant influx. They have remained as models due to ubiquitous nature, speed of analysis, faster generation time, minimal epigenetic variability and genomic similarity to highest organism. These organisms have developed specialization of intracellular structures and functions, comparable what has occurred between the different cells of a multicellular organism. Depletion in the food vacuole formation and changes in the contractile vacuole activity highlighted the importance of Euplotes. The tests carried in this study are simple, fast and give overall information about the ecophysiological effects of delfin in response to toxicant influx.

Key words: Delfin, e*uplotes patella*, cytotoxicity, ecophysiology, food vacuole activity.

Introduction

A better understanding of indicator potential and physiological responses of ciliates to toxicant stress could provide more information for gauging ecosystem viability and stress recovery. Ciliates are also major concern as they are more important as nutrient regenerators particularly of nitrogen and phosphorus than bacteria. These unicellular organisms offer the possibility of direct study of independent cells possessing specific features of single cells and of whole organisms at the same time[1]. This study has immense use not any for assessing the quality of water bodies subjected to organic pollution and sewage influx, but it throws little light on the responses and toxic effects of chemical pollutants in the water bodies. Ciliates have many advantages as test organisms for investigating environmental pollution such as ubiquitous distribution, high reproductive rate, ease of culturing and accessibility of experimental manipulation render ciliates to use in laboratory experiments[2].

Ciliates showed high conservation of genes and better matches of coding sequences to those of humans and hence can be used as models in ecotoxicological studies alternative to eukaryotic organisms[3]. Ciliates also play a significant role in the ecosystem as they can provide an early warning indicator of changes in the environment and also perform key functions in energy flow and elementary recycling in ecosystems[4]. Microorganisms represent one of the links of which pesticides could be transmitted through the food chain to man[5].

In the present study attempt has been made to evaluate possible toxicity of delfin on an organism other than the target. Because of the absence of the relative data on the environmental toxicity of this pesticide the cellular model *Euplotes patella* is chosen for evaluation.

Material and Methods

Selection and culture of experimental organisms: Protozoan ciliates, being cosmopolitan in nature are found in ponds, streams and lakes were selected as test species for the present studies. *Euplotes patella* were collected and isolated from freshwater pond within the vicinity of Osmania University, Hyderabad, India. The organisms were cultured separately in sterilized hay infusion medium at room temperature in the laboratory to obtain a pure line stock culture. Six grams of dried hay was boiled in 1 liter distilled water, cooled and filtered. Then, it was sterilized in an autoclave for 15 minutes at 15 pounds and preserved for further use. Sterile precautions were taken in its use[6]. Hay infusion has been widely used as a basic and most appropriate culture medium for ciliates. It gives the ciliates an environment nearest to their own habitat. For culturing the organisms, hay infusion was diluted with distilled water in the ratio of 1:1 and poured into different petridishes. Ciliates were inoculated into it under sterile conditions to obtain a pure line stock culture. The essential part of the process was to provide the ciliate protozoa with a medium, rich in bacteria, so that they would continue to grow normally. In order to boost bacterial multiplication sterilized wheat grains were supplemented in the culture media, which in turn is the chief source of food supply for the growth of ciliates. Sub cultures on every 7th day was done throughout the study.

Test solutions: Delfin (*Bacillus thuringiensis* kurstaki serotype 3a, 3b) is used for the control of a wide range of caterpillars in vegetables, cabbage white butterfly, potato moth and ornamentals. It is composed of *Bacillus thuringiensis* kurstaki serotype 3a, 3b and dispersing agents. Commercial grade delfin was manufactured by CERTIS LLC, Columbia, USA, and

imported and packed by Margo biocontroles (P) Ltd, Bangalore, India. Stock solution and experimental concentrations of delfin was prepared as recommended by APHA[7]. Stock solution of 10,000 mg / lit of delfin was prepared. Distilled water was used to dissolve this insecticide. After preliminary range finding experiments, the appropriate stock solutions and the test concentrations were selected, prepared afresh and used for further studies.

Test Procedure: In acute experiments 0.5 ml of insecticide 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 concentrations and control was maintained deprived of test concentration. Log phase cultures were used for the experiments. The ciliates were picked up with the help of a micro pipette and resuspended into a cavity block (55mm and 55mm) containing 4.5ml of hay infusion and 0.5ml of the toxicant of known concentration. Several concentrations of delfin were tested. At each concentration 50 organisms were exposed. At the same time, control organisms, not exposed to any pesticide were maintained simultaneously. The cavity block, after adding pesticide was placed under binocular microscope for observing the ciliates. Direct manual counting was performed under a binocular microscope in a cavity block[8]. The ciliates normally swim around and are not easy to count. To reduce the error and increase the accuracy of counting, the cavity block has been graduated into approximately 50 squares. Under toxic conditions, the organisms slow down their movement and occurrence of death was easily determined visually.

Acute Toxicity Test: To measure immediate response under pesticidal stress acute toxicity test was conducted as suggested by Apostol[9]. About 50 organisms were picked from the pure line stock culture in hay infusion and added separately into different cavity blocks containing 0.5 ml of toxicant of known concentration and 4.5 ml of diluted hay infusion. The exposure time was three hours. Immediately, the cavity block was kept under the microscope and counting was done at intervals of 10 min during first one hr and thereafter 20 min interval during the next two hrs. Immediate cytopathological responses were recorded. Total cell disintegration was used as the point of lethality. Several concentrations of each pesticide were tested to determine the lethal, tolerated and safe concentrations. LC_{50} value was calculated against the mortality curve for three hrs. Controls devoid of pesticide, with same number of organisms were run simultaneously.

Food Vacuole Activity: Food vacuole formation was studied after exposure to sub-lethal concentrations of delfin. All the cultures were maintained at room temperature. All observations were carried out on individuals, which grew well and displayed normal behavior. Test organisms were exposed to 100ppm delfin for 30 min and 1 hr. For experimental studies the ciliates were divided into two groups. i. Treated cells: Treated cells from each concentration after known exposure time were picked with the help of micropipette, mixed with 1 molar carmine suspension and kept for 10 min. Ten organisms from each concentration were taken, immobilized and numbers of food vacuoles formed were recorded. ii. Control cells: These were mixed with same molar concentration of carmine suspension but devoid of pesticides. Immobilization and counting of number of food vacuoles formed were done similar to treated cells. Preparation of carmine suspension and counting of food vacuoles was done by the method suggested by Brutkowska[10].

Contractile Vacuole Activity: The most common problem faced by several workers is the observation of contractile vacuole, as the ciliates move too fast thereby making the observation of contractile vacuoles very difficult. Protamine coated slides used in this study to immobilize *Euplotes patella* as the ciliates appeared not to be harmed by this procedure as demonstrated by Marsot and Couillard[11]. After the animals were exposed to the sub lethal concentrations for 20 min, single individuals were picked and the rate of pulsation of posterior contractile vacuole i.e., the time required for one complete pulsation was determined (from the beginning of one contraction to the beginning of next). Observations were made on the ciliates in each concentration. The rate of pulsation for each individual is calculated separately as suggested by Masood and Khan[12]. Equal number of observations was done in controls.

Results and Discussion

Acute toxicity test and cytopathological responses: The acute effects can be mortality, behavioural changes and cytopathological responses to toxicants manifested in the form of changes in body size and shape, ultra structural deformities as reported in various ciliates[13-15]. There was instant mortality in concentrations of 900ppm and above. 150ppm markedly increased the motility of cells within two minutes of exposure and cells aggregated to corners of the cavity block. An angular and irregular deformation of body, damage to pellicle, enlargement of contractile vacuole, stress egestion of food vacuoles and cytoplasmic vacuoles culminating in the destruction of cell body were observed at higher concentrations. Significant changes in morphology and motility of the organisms were observed. The calculated LC_{50} value was found 355.99 ± 21.30ppm. Inhibition in oxygen uptake, ciliary abnormalities and disruption of surface structure were reported in Paremecia with increased concentration and exposure time of Aldicarb, carbaryl, and mexacarbate pesticides[16]. Similarly the effects of three insecticides, dieldrin, dimethoate and permethrin, on the growth of *Tetrahymena pyriformis* were studied for 5 days. Inhibition of growth rate, rounding of cells, mucocyst discharge and cell lysis were common cytopathological changes observed by Kumar et al[17]. Effects of arginine - vasopressin and its functional analogues on the contractile vacuole of the *Amoeba proteus* was studied by Bagrov et al[18]. The marine ciliate *Euplotes crassus* was exposed to different sub lethal concentrations of mercury, cadmium and

chromium and its behaviour was video-recorded and analysed. It was found that the rhythmic changes in creeping velocity were affected by each heavy metal, inducing an increase in their duration and frequency[19]. *Paramecium caudatum* samples treated with nano-particles of Ag (17 nm across) and Co-ferrite (300 nm across) were showed that the inner vacuoles increase in number and in volume in Co-ferrite treated cells as compared with Ag treated ones. But then, cilia-less areas increase on the surface of the body of Ag treated cells. In the case of Co-ferrite treated cells, cilia-less areas are not clearly detected[20]. The accumulation and bioconcentration factor of aquatic ciliate protozoan *Tetrahymena pyriformis* exposed to 0.1ppm, 0.5 ppm and 1.0ppm parathion for 2-12 hrs was reported by Solanki and Paliwal[21]. The ciliate protozoan took 10 hrs to accumulate maximum 923.43ppm amount of parathion from a medium containing 1.0ppm insecticide. Maximum bioconcentration factor of 973.80 was obtained 0.1ppm parathion affect after 8 hrs of treatment.

Phagocytosis in *Euplotes patella* exposed to 100ppm of delfin: The average number of food vacuoles formed in Euplotes was 7.0 in control cells. After 30 min exposure to 100ppm concentration, the mean number of food vacuoles was reduced to 5.4 causing 22.86% inhibition. The highest exposure time that is 1 hr for the same concentration the mean number of food vacuoles was decreased to 4.8 with the retardation of 31.43%, represented in figure 1. Food vacuoles are formed through phagocytosis and are comparable to the digestive tract of metazoan organisms[22]. The duration of the food vacuole formation depend both on the physiological conditions of the cell and also on the external factors that are responsible to influence them[23]. Formation and movement of the food vacuoles depend on ciliary motility because cilia are responsible for both locomotion and moving food vacuoles towards the cytostome. Factors effecting the ciliary action should also affect the rate at which food vacuole form. The present observation suggested that there could be a damage to cell menbrane and also cilaia structure so that phagocytosis was depleted.

Contractile Vacuole activity in *Euplotes patella* exposed to delfin for 20 min: *Euplotes patella* was greatly affected as manifested by its contractile vacuole activity. The average time for one pulsation in cells treated with 100ppm of delfin at 20 min was reported as 40 seconds, whereas in control cells it was 17.14 seconds. The contractile vacuole activity was changed concentration dependent manner. The lowest vacuolar retardation recorded was 3.0 pulsations in one min, which is little higher than the control value (3.5 pulsations in one min) (figure 2). The cytosol of the fresh water protozoan *Paramecium caudatum* is always hypertonic to the external solution[24]. According to Masaki et al[25]. the water expulsion frequency of the contractile vacuoles in Paramecia was reduced in dose, time and site dependent manner, when the monoclonal antibody DS-1 was injected into the cell. The contractile vacuole is dynamic and highly responsive to changes in the cell's environment. In all the concentrations, Euplotes showed clear relationship between the vacuolar output and length of exposure. The present investigation was carried out with the hope of adding information in the existing knowledge of protozoan studies in toxicity assessment, which may help us to come out with a solution to reduce pollutional stress in the cells' environment.

Conclusions

In conclusion, it is to be stated that there are all grounds to consider the Euplotes as a sensitive bioindicator to freshwater pollution and this experiment reveals how Euplotes respond to the delfin induced pollution stress. Further, the results reported here seem to justify our attempt to carry on experimental biomonitoring because the sensitivity of test is good and this approach is economical and simple.

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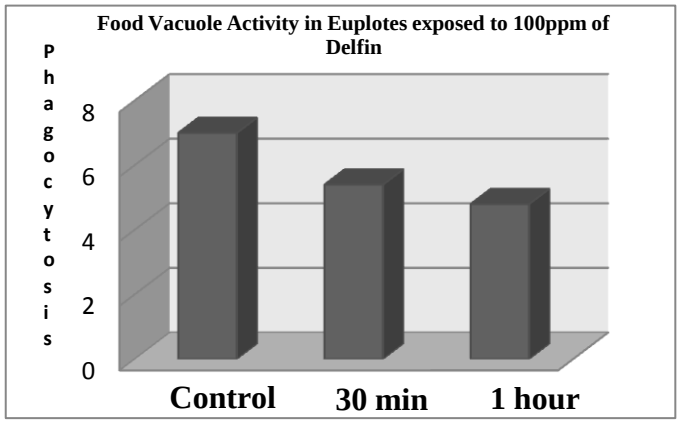

Figure-1
Food Vacuole activity in *Euplotes patella* exposed to 100ppm of delfin for 30 min and 1 hr.

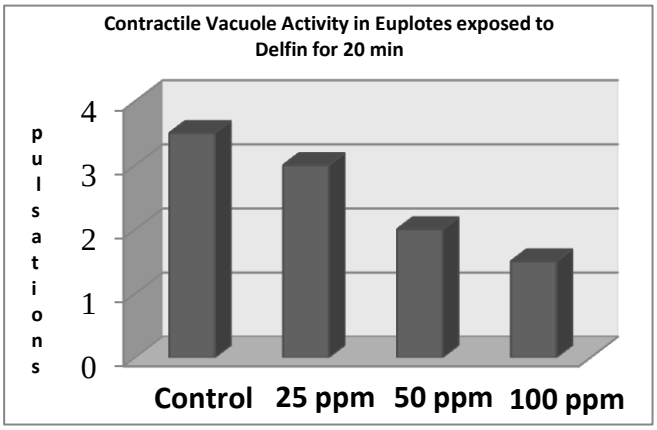

Figure-2
Contractile Vacuole Activity in *Euplotes patella* exposed to delfin for 20 min.

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