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Research Article

INVITRO TOXICITY RESPONSES OF BLEPHARISMA INTERMEDIUM TO COMMONLY USED INSECTICIDE DELTAMETHRIN

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ABSTRACT

The pesticides are playing a major role in the contamination of the environment. Thepresent investigation was done on physiological changes and deltamethrin responses of the freshwater ciliate Blepharisma intermedium exposed to a selective pyrethroid insecticide. The obtained LC50 values of Blepharisma intermedium was found to be 57.54ppm respectively. The acute toxicity tests were done by using probitanalysis and worked out sublethal concentrations were found to be 10ppm, 13ppm, 16ppm and 19ppm respectively. The contractile vacuole activity was reduced when the organisms were exposed to different concentrations in time dependent manner. The nuclear changes leading to DNA damage was noted using Feulgen fast green technique (Rizzo and Nooden; 1973). The more abnormal forms were found to 67.2±0.79 percent at higher concentration 19ppm. The concentrations used for nuclear abnormality studies were 10ppm, 13ppm, 16ppm, 19ppm respectively.

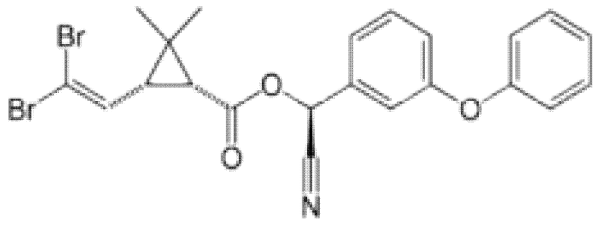
INTRODUCTION

The pesticides are playing a major role in the contamination of soils, aquatic ecosystem, inland and coastal waters and air (Chapalamadugu and Chaudry 1992). Pyrethroids were discovered in 1800's (Rose *et al*., 2000).Use of pyrethroids is increasing from last three decades (Meacham *et al*., 2008; Wolansky and Harrill, 2008). Deltamethrin is atype II synthetic pyrethroid(Barahona, 2005; Hossain 2005,Wolansky and Harrill, 2008). Synthetic pyrethroids are highly toxic to zooplanktonic communities (Tidou *et al*. 1992) and to fish (Mestres and Mestres 1992, Mittal *et al* 1994; Aydin 2004).

Protozoa are eukaryotic and unicellular microorganisms. Freshwater ciliates are being ubiquitous in nature, high reproductive rate, easy tomaintenancemakes them best suited models to screen toxicity effects of various pesticides (Morange, 2006). This organism involves in transformation of nutrients from one tropic level to another tropic level (Hynes, 1960; Carter & Cameron, 1973).Ciliate protozoa act as potential bioindicators of water quality and bio-monitors of ecosystem environment (Masood and Amanchi,2007, 2008). Tests on the acute toxicity of pollutants on ciliates have revealed that these microorganisms are useful bioindicators for evaluating the toxicity of water polluted by different pollutants (Madoni, 2011).

MATERIALS AND METHODOLOGY

Deltamethrin ($C_{22}H_{19}Br_2NO_3$)is an insecticideand its composition 2.8% of Deltamethrin + w/w Emulsifier: 8.00% w/w, Stabilizer (Butylated hydroxytoluene): 1.0% w/w+Solvent (naphtha) 88.20% w/w. manufactured by Bayer Crop science Limited. To carry out the experiments 0.5ml of pesticide solution was added to 4.5ml of culture medium to achieve desired concentration of each pesticide.



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Structure of Deltamethrin

Selected organisms: fresh water ciliate *Blepharisma Intermedium* was used in this study. Pure line stock culture of *Blepharisma intermedium* was supplied from Carolina Biological suppliers, NC, USA. Test organisms were sub cultured in the laboratory and maintained for further studies (Masood andAmanchi, 2008).

Test solutions: The test solutions were prepared according to recommendations given by APHA (2012). Stock solution of deltamethrin 1000ppm was prepared by using distilled water.

***Preparation of Culturing Medium*:** Hay infusion media was used as culture media forgrowing the ciliates (Kirby, 1950). 6 – 8 grams of hay was added to 1 liter boiling distilled water in a beaker and boiled for 30 minutes, cooled at room temperature and filtered using watt men filter paper. Then the filtrate was sterilized in an auto clave for 15 minutes for 15 ponds. Later the auto calved hey filtrate was kept in the lab for 24 hours without any lid. For culturing ciliates, Hey media was diluted in the ratio of 1:1. Cultures were maintained at room temperature25 $\pm 2^0$C. In order to induce ciliate multiplication boiled wheat grains were added to culture media. Log phase cultures were used throughout the study.

***Acute toxicity studies*:** Acute toxicity tests were conducted for 3hours to measure immediate cell response, such as swimming pattern, cell motility and cytopathological changes under pesticidal stress as suggested by Apostol, (1973). The tests were repeated thrice.0.5 ml of known concentration of deltamethrin solutionwas added to 4.5 ml of culture medium containing about 50 organisms. Observation were made and counting was done for every 10min during the first hour and after 20 min interval for next two hours. Total disintegration of the cell was used as the lethal point. Immediate cytopathological responses were recorded at various concentrations to determine the LC_{50} values by plotting a graph (Finney 1964).

***Contractile VacuoleActivity*:** Contractile Vacuole discharges its contents through a permanent pore (Patterson 1981). Immobilization of the *Blepharisma intermedium* was done by using protamine coated slides,As the test organisms appeared not to be harmed by this procedure (Marsot and Couillard, 1973). The organisms were exposed to sub-lethal concentrations10ppm,13ppm, 16ppm and 19ppm of deltamethrinfor10 and 20 minutes, single individualorganism was be picked and the rate of pulsation of contractile vacuole i.e. time required for one complete pulsation was determined. The rate of pulsation for each individual organism was calculated separately. Equal number of observations were done in controls.

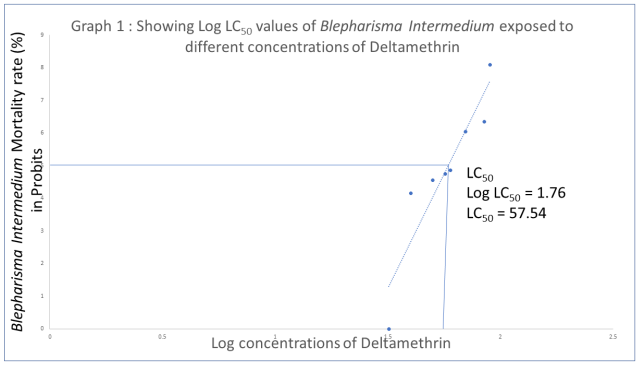
***Food vacuole activity*:** About25 organisms were exposed to sub-lethal concentrations 10ppm,13ppm,16ppm and 19ppmof deltamethrin for one hour respectively,to identify the number of food vacuoles formed. The test organismswere be divided into two groups, 1) Treated organisms, 2) control organisms. Treated organism from each concentration were picked with the help of micropipette, mixed with India ink and kept for 10 minutes.10 organisms from each concentration were taken, immobilised and number of food vacuoles formed was recorded. Control cells with same molar concentrations of India inkwas run simultaneously. A few organisms are collected from both the experimental groups on protamine coated slides for immobilization and the counting is done. Preparation of carmine suspension and counting of food vacuoles was done as method suggested by Nilsson (2003).

Macro Nuclear Aberration study

Feulgen fast green technique Feulgen fast green (Rizzo and Nooden ; 1973) staining technique was used to study the macro nuclear changes in *Blepharisma intermedium* exposed to deltamethrin. Macro nuclear studies gives an immediate qualitative picture of the nuclear changes that have been induced by thepesticides (Nageswara Rao Amanchi, 2009). The organisms were treated withsub lethal concentrations of 10ppm, 13ppm, 16ppm and 19ppm for one-hour exposure to deltamethrin.Carnoy'sfixactive was used for cell fixation. The treated organisms werehydrolysed in 1N HCL at 27^0C temperature and washed in distilled water,slides were transferred into Schiff's reagent and incubated for 1 hr.Schiff's reagent wasprepared as suggested by De Tomasi(1930).The organisms were immersed in three changes of sodium bisulphate solutions, again rinsed with water, dehydrated in graded alcohol, cleared in xylene and mounted with DPX. The exposed *Blepharisma intermedium* wasshown various macronuclear changes like rod shape, vacuolated, unevenly divided, fragmented macronucleus and karyolitic forms were observed.

RESULTS

Acute toxicity studies: *Blepharisma Intermedium*was exposed different concentrations of deltamethrin for 3hrs, various changes in the body size, shape, ultrastructural deformities were observed. The lethal point was found at the concentration of 90ppm for *Blepharisma Intermedium* (graph: 1)and the calculated LC_{50}value against mortality curve was found to be 57.54ppm. The worked out sublethal concentrations for *Blepharisma Intermedium* were 10ppm, 13ppm, 16ppm and 19ppm respectively.



Graph 1 showingAcute toxicity effect of deltamethrin on *Blepharisma Intermedium.*

Contractile Vacuole Activity: Contractile vacuole activity was performed for each individual organism after exposing 10ppm,13ppm, 16ppm and 19ppm concentrations for 10 & 20 minutes of exposer time. Observations were made for individual cellby comparing with control. There was maximum reduction in number of pulsations of *Blepharisma Intermedium*(table 1) was found to be 1.00 ± 0.63, 0.80±0.40 and 1.40 ± 0.48, 0.80±0.74 at 19ppm and 16ppm

concentrations of Deltamethrin for 10 & 20 minutes. compared to control organism 3.80±0.40. The minimum reduction 2.20±0.40 and 1.60±0.48 observed at the concentration of 10ppm in treated cell of the *Blepharisma Intermedium.* (One-way ANOVA was done $F_{4,20} = 22.296$; $P<0.05$).

Table 1 Contractile activity in *Blepharisma Intermedium* exposed to Deltamethrin

Concentration in ppm	Exposure time	Pulsations per minute
10	10	2.20±0.40
	20	1.60±0.48
13	10	2.00±0.63
	20	1.20±0.40
16	10	1.40±0.48
	20	0.80±0.74
19	10	1.00±0.63
	20	0.80±0.40
Control		3.80±0.40

One way ANOVA was done $F_{4,20}$= 22.296%; $P < 0.05$

***Food vacuole activity**: Blepharisma intermedium* showsreduction in number of food vacuoles (fig: 1) against different concentrations of Deltamethrin. The gradual decrease in the phagocytic activity was recorded by increasing the concentration. Minimum inhibition of food vacuole was for one exposure 34% at concentration of 10ppm. Maximum inhibition was found 76% at concentration. (one-way ANOVA showing $F_{4, 45} = 93.603$; $P< 0.05$).

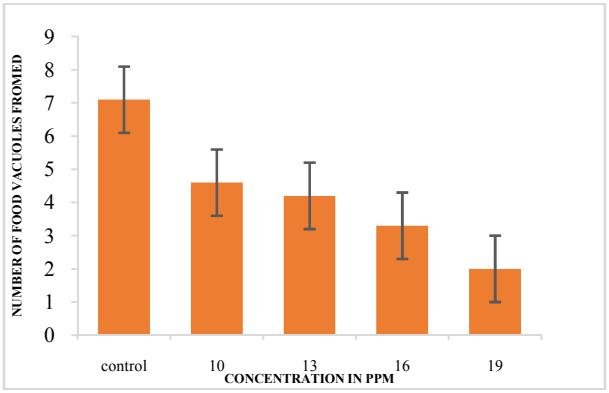


Fig 1 Food vacuole activity in *Blepharisma intermedium* exposed to different concentrations of Deltamethrin for 1hr Cytochemical changes of nucleus

The treated groups of *Blepharisma Intermedium* **(**Table 2) has shown various macronuclear changes includingvacuolated, fragmented, unevenly divided, karyolysis.Percent abnormal forms reported were 23.2±0.63, 34.1±0.74, 51.6±0.7 and 67.2±0.79 at each concentration of 10ppm, 13ppm, 16ppm and 19ppm. Presence of maximum number of vacuolated forms were recorded were 20.7±0.67 at 19ppm and the minimum values recorded were6.6±0.7 at concentration 10ppm.

Table 2 Deltamethrin induced nuclear changes (%) in *Blepharisma Intermedium*exposed for one hour.

Conc/ppm	Percent abnormal forms	Vacuolated	Fragmented	Unevenly divided	karyolysis	Other deformities
10	23.2±0.63	6.6±0.7	8.3±0.82	3.5±0.71	10.4±0.52	4.4±0.7
13	34.1±0.74	7.3±0.67	9.4±0.52	3.8±0.63	13.4±0.7	7.3±0.67
16	51.6±0.7	12.2±0.63	13.8±0.79	6.3±0.67	12.4±0.52	10.8±0.79
19	67.2±0.79	20.7±0.67	18.6±0.52	11.1±0.88	18.4±0.52	11±0.67
Control	2.7±0.67	---	2±0.47	---	---	1.4±0.521

P values are significant $P < 0.05$

DISCUSSION

The use of insecticides has increased due to pest control management in agriculture and household applications. Pyrethroids are neurotoxic not only to insects but also to the mammals and zooplankton including fresh water ciliates.Pyrethroids interact with sodium channels of nervous system and inactivates sodium current following by membrane depolarisation (Narahashi, 1992). Low concentrations stimulate the nerve membrane and at higher concentrations block the nerve entirely (Rose *et al*.,2001).

Acute toxicity studies were carried out to observe theimmediate cytopathological responses.Changes in the body size, shape and ultrastructural deformities were observed in ciliates, when exposed to different concentrations deltamethrin. The data obtained from the acute toxicity studies was important in establishing the relative toxicity of pesticides, as well as in providing information to carry out further experiments. Studies revels that the action of deltamethrin on both calcium uptake and depolarization-evoked neurotransmitter release in rat brain (Symington *et al*. 2007b; Symington *et al*. 2008). Similar results were also reported by various authors (komala 1982, Schultz et.al 1981, Masood, Amanchi 2008) in different experimental organisms.

Blepharisma Intermedium showed maximum reduction in the contractile vacuole activity when the cells were exposed to higher concentrations 18ppm and 19ppm of deltamethrin. The recorded results were found to be 1.00 ± 0.63 ,0.80 ± 0.74 and2.20 ± 0.40, 1.60 ± 0.80for 10 & 20 minutes, due to the effect deltamethrin. Minimum reduction was observed at lower concentrations of deltamethrin. The effect toxicant ofwas studied by (Rossbach 1872; Hartog 1888). If the osmotic pressure of the medium is increased, expulsion of the contractile vacuole decreases If the added toxicant is diluted the expulsion will increase. (Stoner & Dunham, 1970; Hampton & Schwartz, 1976). The ion physiological studies influence the functions of contractile vacuoleDunham & Child (1961). Amoeba and Tetrahymenahave shown reduced contractile vacuole activity when the cells are subject to a hyperosmotic shockPrusch (1977) and Dunham & Kropp (1973).

Damage to cilia and ultra-structure of plasma membrane causes reduction in food vacuole formation. The cilia present in cytostome may get damaged under the insecticidal influx in to cytoplasm from outside. Under stress conditions ciliates exhibit starvation to avoid un favourable conditions (khan and Masood; 1983).More number of Nuclear abnormal forms wereobserved when organisms were exposed to various sublethal concentrations of deltamethrin. Reduction in size shape, rod shape, vacuolated, blebbing and blacking of cytoplasm were observed at higher concentrations. *Blepharisma Intermedium* exposed to the concentration of 19ppm the percent abnormal forms were found to be (67.2±0.79).Higher concentrations the ciliary movement was arrested and lysis of the organism was observed

CONCLUSSION

Commercial grade deltamethrin was toxic even at very low concentrations like 10ppm,13ppm, 16ppm and 19ppm. Changes in osmatic media influenced both contractile vacuole

activity and food vacuole activity in *Blepharisma Intermedium.* A beaded nucleus of *Blepharisma Intermedium*is highly useful in studying genotoxicity effects of deltamethrin. Hence it is concluded that *Blepharisma Intermedium* is a suitable model for basic bioassay studies as well mutagenic and carcinogenic studies.

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