Original Article

Effect of Cypermethrin on the Growth of Ciliate Protozoan *Paramecium caudatum*

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ABSTRACT

Objective: The objective of this study is to assess the effect of cypermethrin on the growth of ciliate protozoan *Paramecium caudatum*. **Materials and Methods:** Monoxenic culture of *P. caudatum*, were exposed to different doses (0.01, 0.05, 0.1, 0.15, and 0.2 µg/L) of cypermethrin along with control for 24, 48, 72, and 96 h time interval. The total numbers of live and dead cells were counted after trypan blue staining in Neubauer hemocytometer. **Results:** Marked decrease in the number of living cells with the increase in the concentration of cypermethrin and with increasing exposure time intervals was recorded. **Conclusion:** The results indicate that cypermethrin is toxic to *P. caudatum* even at low concentrations when it enters in the aquatic system through runoff.

Key words: Cypermethrin, *Paramecium*, population growth

INTRODUCTION

India is the second largest producer of fish and value of the output was about Rs. 91,541 crore during 2012–2013, which is about 4.36% of the value of agricultural and allied sector output at the current price.[1] Increased agriculture and agriculture allied services have solved the problem of food shortages by increasing the frequency of using chemical pesticides.[2] The indiscriminate use of synthetic chemical pesticides is often detected in the different water sources as contaminants,[2] which also enters in sediment, nutrient cycle, pathogens, and salts with runoff or leaching that increases during rainfall.[3]

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Access this article online	
Quick Response Code:	Website: www.toxicologyinternational.com
	DOI: 10.4103/0971-6580.172282

The use of modern synthetic chemical pesticides and due to their toxic properties there is an obvious risk that nontarget organisms are affected[4,5] either at the application site, or due to unintentional spreading, at nearby, or even distant areas.[6]

Pyrethroids are the widely used insecticides in agriculture and urban areas known to be used to control many pests, including moth pests of cotton, fruit, and vegetable crops.[7] Cypermethrin is a type of cyanophenoxybenzyl pyrethroid and is categorized as restricted use because of its high toxicity to fish[8] and is highly hydrophobic pyrethroids having a strong tendency to sorbs to sediment and accumulate in aquatic biota.[9] Invertebrates are even more sensitive to cypermethrin[10] but, lethality of cypermethrin to freshwater invertebrates is poorly documented.[8] In the

How to cite this article: Dutta J. Effect of cypermethrin on the growth of ciliate protozoan *Paramecium caudatum*. Toxicol Int 2015;22:100-3.

present study, *Paramecium caudatum* was used as a test organism to examine the effect of synthetic pyrethroids insecticide cypermethrin through an *in vitro* series of tests. The bioassays include the study of growth in number of organisms exposed to different concentration of the insecticide.

MATERIALS AND METHODS

Test compound

Commercial grade cypermethrin insecticide (cybergun - 25, 25% EC) used in this study was manufactured by Swastik Pesticide Ltd., India.

Experimental set up

The indoor microcosm (length and width: 80 cm; depth 40 cm) and the condition in the climate room (constant temperature 15°C ± 1°C; photoperiod 12 h) provided by cool fluorescent lamp. Unpolluted water from the deep well was used in the experiment, and the microcosms were replenished with the water from the same source at weekly intervals. Five of 10 microcosms were treated with cypermethrin while five of them were served as controls. The treatment concentrations (0.01, 0.05, 0.1, 0.15, and 0.2 μg/L of cypermethrin) were prepared by diluting the stock solution of cypermethrin in acetone and subsequently mixed into the water column.

Monoxenic culture of *Paramecium caudatum*

P. caudatum was selected as test species for present studies because of its ease to culture and maintain in the laboratory. Monoxenic culture of *P. caudatum* was prepared[5] and subsequently subcultured after every 5 days to ensure maintenance.

Cell population growth studies

For sampling 5 evenly distributed positions were chosen from each of the microcosm and depth-integrated samples were taken[11] using plastic tubes. Aliquots of 100 μL were taken from control and the exposed cultures at different time intervals. The samples were properly diluted in distilled water and fixed with Neutral Buffered Formalin containing, 10% (v/v) formalin in phosphate buffer saline pH 7.0 at a final concentration of 2–5% for 1 h. Total number of cells of paramecia from control and treated were recorded after 24, 48, 72, and 96 h time interval using optical microscope using ×40 magnification and Neubauer hemocytometer. The dead and live specimens were differentiated by using trypan blue staining technique.

Triplicates were maintained for all test concentrations. The results were analyzed statistically by applying Student's *t*-test to find the significance in comparison to control.

RESULTS

Study on population growth

The experiment was performed to observe the harmful effect of cypermethrin on the cell population growth of *P. caudatum*. The effects of cypermethrin on the population growth are shown in Figure 1. cypermethrin effect can be delineated from the control group as it has resulted in the increase in number of dead cells after the exposure of the organisms to 0.1 μg/L of the insecticide. The overall response is shown by a marked decrease in the number of living cells with the increase in dosage of cypermethrin and with increasing time intervals. The significant increase ($P < 0.05$) in the number of dead cells can be observed after 48 h of the treated tanks with the insecticide. In the tank containing 0.15 μg/L of the insecticide, the density of live cells reduced significantly after 24 h, however, the lethal effect can be observed after 48 h of exposure. The same trend is observed for the tanks containing 0.2 μg/L of the insecticide wherein the lethality is noted even after 24 h of the treatment. The observation for the number of live or dead cells shows that the tank containing 0.15 and 0.2 μg/L of cypermethrin has significant mortality ($P < 0.01$) from that of the control.

DISCUSSION

When pollutants are released into aquatic habitats, direct (toxic) effects on aquatic biota are possible. Important contribution of microorganisms in the present era is their usage as gauging agents of toxicosis stress, bioremediation, and as biomonitors in the aquatic bodies, which are getting polluted by pesticide residues, domestic sewage and industrial effluents.[5] Protozoans such as *Tetrahymena pyriformis, Spirostomum ambiguum, P. caudatum, Oxytricha fallax* are mostly used for laboratory research. The cytotoxicity of many different xenobiotic compounds was evaluated using ciliates as these are sensitive to environmental alterations and have been proposed as the biological indicators of environmental pollution.[12] Among the various end points recommended to evaluate the cytotoxic effects,[5,13] population growth rate have been used extensively.[14-17]

Cypermethrin is a type of cyanophenoxybenzyl pyrethroid and is categorized as a restricted use pesticide by US Environmental Protection Agency because of its high toxicity to fish.[8] The present work was focused on to study the effects of cypermethrin on the population growth; it clearly depicts that the insecticide is lethal. The trend of increase in the number of organisms at 0.01 μg/L of cypermethrin at 24 and 48 h time interval and then the decline in growth of paramecium at higher doses (0.1, 0.15, 0.2 μg/L) and

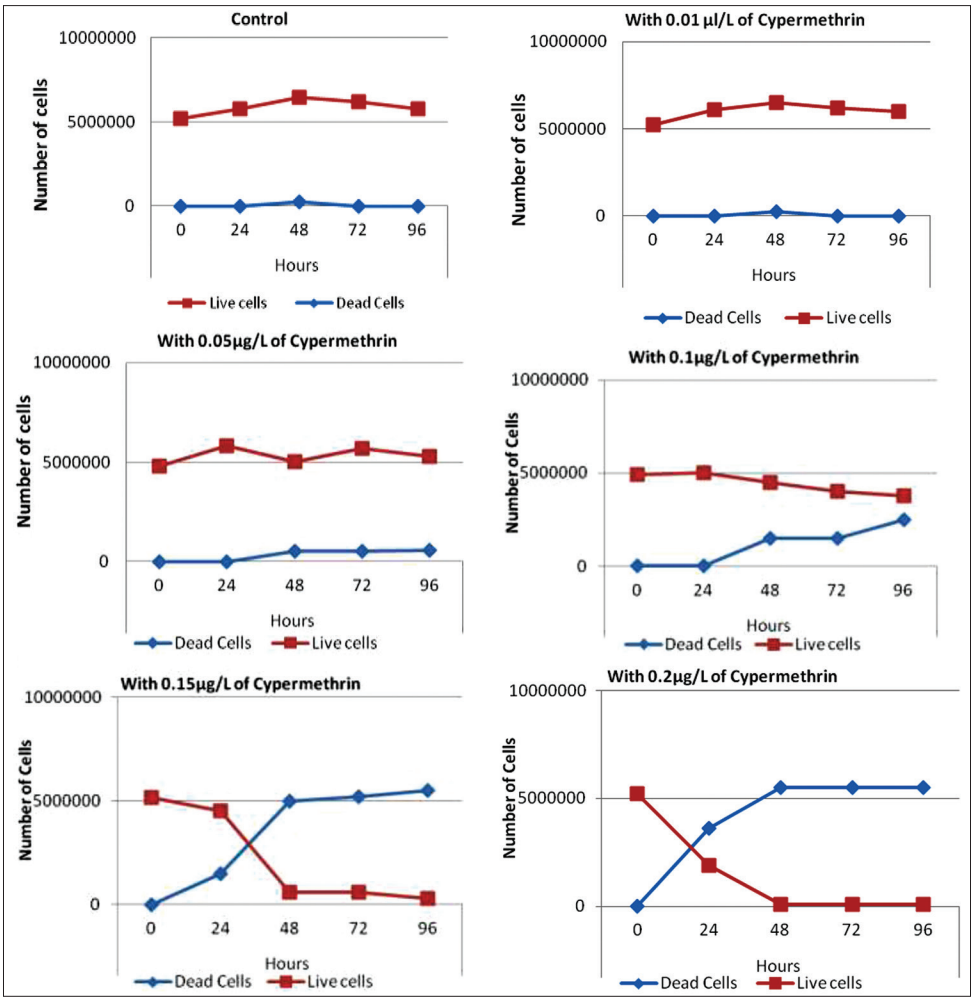

Figure 1: Effect of cypermethrin on the cell population growth at different concentration and time intervals

time interval (48, 72, and 96 h) showing the toxic effect of insecticide.[18] Results from the study agree with the previous studies[19,20] as lower concentration of cypermethrin, does not have any adverse effect of the population growth of paramecium as the result obtained is nearly same as that of the control values.

CONCLUSION

The present study clearly emphasize that the cypermethrin insecticide, which naturally come along with the agricultural runoff in the aquatic system in high concentrations are lethal to *P. caudatum*. The reduction in population growth indicates that these pesticides in high concentration, are toxic to protozoans. Since aquatic environments serve as sinks for numerous environmental pollutants, the effects of these substances on the resident aquatic organisms can be quite serious.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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