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Effect of Neurotoxic Insecticide Dimethoate on Unicellular Freshwater Protozoan Ciliate *Paramecium sp.*

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Abstract

The freshwater protozoan ciliate Paramecium sp. was used to assess the effects of widely used insecticide in agricultural industries, dimethoate, which is a neurotoxin for insects and works by inhibiting the activity of acetyl cholinesterase enzyme. Since the ciliates like Paramecium are unicellular and do not possess any nervous system, an attempt has been made to check whether the ciliates can be considered as potential bioindicators for aquatic toxicity assessment and to evaluate the possible effects of this insecticide on them. In the present study, it was found that dimethoate affects the normal functioning of the contractile vacuole and locomotor movements at the concentration range of 0.5 mg/ml and 1 mg/ml (and above) if exposed to the insecticide for 20 minutes and 3 hours respectively. It also affects the growth rate of the ciliate, significantly, by increasing the generation time when subjected to the dimethoate (concentration 0.5mg/ml and above) for 24 hours and 96 hours exposure. Certain physiological deformities were also noted like irregular beating of cilia, blebbing and spinning movements.

Keywords: Ecotoxicology, contractile vacuole, generation time, cell lysis, growth rate.

Introduction

In a developing country like India the demand for food and shelter is ever increasing. In such case, where we have limited area of land for agricultural uses and population that is demanding high production of food and crops, we cannot put even a single crop-farm at the risk of destruction by pests such as insects or rodents. In order to be on safer side, the farmers are taught to use various types of pesticides – insecticide, herbicide, rodenticide, fungicide, and larvicide. Pesticides affect all members of an ecosystem and are responsible for the death of many aquatic animals especially fishes, amphibians and invertebrates and those that depend on them for food indicating that they not only affect target but also indirectly harm other non-target animal population when they enter into the environment and are of major concern in recent times[1-3]. Further, the level and speed of industrialization, especially textile industries, have contributed in the pollution of the environment[4].

According to Aktar *et al*, surface runoff is one of the major media for pesticides to enter into water body followed by leaching. Both of these processes are linked to earth hydrological cycle fundamentally. When the usage of urban water is included in the surface runoff, the pesticide residues in the municipal wastewater get along with the hydrological cycle model. Finally when the water enters an already existing water body or gets accumulated behind a barrier, it carries with it the dissolved materials and other contaminants that it picked up along its way to that particular point[5]. Previous studies indicate that pesticides are major toxicants which, if used extensively, pollutes aquatic environment[6]. Insecticides like dimethoate (belong to the class of organophosphorus insecticides) are neurotoxins that functions by inhibiting the nervous system and the Acetyl cholinesterase (AChE) therein causes the 'constant firing' of nerve impulses[7].

The studies of behavioural, morphological and physiological changes are becoming prominent in toxicity assessments in unicellular organisms, along with rotifers, insects, fish and rodents[8-12]. Free living fresh water protozoan ciliates are used as bioindicators of toxicity stress and chemical pollution mostly, especially in aquatic ecosystem[13]. Protozoan ciliates like *Paramecia* are at the bottom foundation of the heterotrophic eukaryotic food web and along with being an important bioindicator of environmental conditions, and also of changes in natural as well as anthropogenic activity influenced ecosystems, they also play a pivotal role as regulators of key ecosystem processes[14]. Many recent studies have shown that ciliates play a very important trophic role in periphytic communities and as the indication of pollution degree in rivers and lakes[15].

In the present work an attempt has been made to examine the possible effects of the neurotoxic insecticide dimethoate on the unicellular fresh water ciliate *Paramecium* which does not possess any sort of nervous system and to evaluate the possibilities of this organism to be considered as bioindicators for insecticide pollution of a water body.

Material and Methods

Selection and culture of experimental organisms: Protozoan ciliates like *Paramecium* occur widely in any stagnant

freshwater body making it easier to catch them and culture under laboratory conditions[16]. *Paramecia* were collected from the vicinity of Institute of Science, Mumbai, India. They were isolated and cultured separately in conical flasks containing sterilized hay infusion medium at room temperature to obtain a pure line stock culture. Six grams of hay (dried leaves of *Phleum pratense*) was boiled in one liter of distilled water followed by filtering on cooling. It was then autoclaved at 15 pounds for 15 minutes and preserved for further use. For culturing and sub-culturing, the hay infusion was diluted with distilled water (1:1 ratio) and poured into 150 ml conical flasks. Ciliates were inoculated into these flasks under sterile conditions. In order to provide food source for *Paramecia*, sterilized wheat grains were added in the culture media so as to trigger bacterial multiplication which in turn acted as a major source of food supply for the growth of ciliates [17]. Sub-culturing was done on every 5th day.

Random testing: Different grade solutions of dimethoate were prepared using distilled water. The organisms were tested randomly for the effects on their morphology and behavior. Few concentrations were selected randomly for further tests of contractile vacuole (CV) activity and Growth Rate (GR) and Generation Time (GT) tests.

Contractile Vacuole Activity Test: One set of organisms were kept in the solutions of selected concentration for 20 minutes and that of another set were kept for 3 hours. Triplicates were maintained along with control for each concentration of both the sets. After the stipulated time the cells were transferred to protamine coated slides so as to immobilize those [18]. The cells were observed individually and rate of pulsations (from the beginning of one pulsation to the beginning of the next) of posterior CV was noted.

Growth Rate and Generation Time Test: The ciliates were again divided into 2 types of sets with triplicates for each concentration of both the sets along with control set. 5 cells were introduced in each cavity block having desired concentration and were exposed for 24 hours. On the other hand, the other set had 3 cells in each cavity block with the desired concentration and were kept for 96 hours. The total number of cells was counted after the stipulated time and Number of Generations and Generation Time were calculated using the following formulae:

$$Number of generations\,(n) = \frac{\log N_1 - \log N_0}{\log 2}$$

$$Generation\ Time(g) = \frac{Time\ of growth}{Number\ of generations}$$

where, N_1 = number of cells at 24 hour and 96 hour, N_0 = number of cells at time T_0, *Time of growth* = 24 hours and 96 hours.

Results and Discussion

Contractile vacuole activity: Dimethoate had maximum inhibitory effect on CV activity at the concentration of 2.5 mg/ml which is just 5.45 pulsations per minute as compared to control having 10 pulsations per minute for 20 minutes exposure to the chemical. It was observed that the CV activity changes in concentration dependent manner. Though there was no significant decrease seen at 0.5 mg/ml concentration. However, the CV activity (pulsations per minute) tends to increase when the ciliates were kept in the same concentrations respectively for 3 hours. In fact, there was no difference in the value of pulsations per minute of the control and that of the 0.5 mg/ml concentration of dimethoate in the case of 3 hours exposure. Kitching had emphasized on the fact that the pressure built at the CV membrane or the tension in that area may be responsible for the pressure needed to discharge or expel the fluid content of the vacuole via the CV pore[19]. The CV activity or the expulsion frequency or the pulsation rate can be modified or affected by array of external factors[20]. Stock *et al*. discovered that the change in the ionic factor of the surrounding environment is directly or indirectly responsible for the change in ionic composition of the cytosol as well as that of the CV fluid [21]. They came to the conclusion that osmolarity of the CV fluid is always hypertonic to the cytosol, and that the osmolarity of the cytosol is always hypertonic to the external osmolarity. The reduction in the rate of water segregation was seen as soon as there was an increase in the external osmolarity[22]. According to Masaki *et al*, when the monoclonal antibody (DS-1) was injected into the cell, the frequency of water discharge through the CV in *Paramecia* was reduced with respect to dose, time and site[23].

Table-1
Contractile vacuole activity for 20 minutes exposure

Concentration of dimethoate (mg/ml)	Time for one pulsation (seconds)	Pulsations per minute
(Control)	06.00	10.00
0.5	06.33	09.47
1.0	07.33	08.18
1.5	08.00	07.50
2.0	10.33	05.81
2.5	11.00	05.45

Table-2
Contractile vacuole activity for 3 hours exposure

Concentration of dimethoate (mg/ml)	Time for one pulsation (seconds)	Pulsations per minute
(Control)	06.00	10.00
0.5	06.00	10.00
1.0	06.33	09.47
1.5	06.67	09.13
2.0	08.00	07.50
2.5	08.00	07.50

Growth Rate and Generation Time Test: The growth rate tests revealed that increasing concentrations does have negative effect on number of generations by increasing the generation time. For 24 hours exposure, the results gave clear and gradual decrease in the number of cells (or number of generations) as the concentration was increased. But, however, in the case of 96 hours exposure period the difference among the values of number of generations and generation time of first three concentrations was not so significant, though the generation number and generation time of 2.5 mg/ml concentration showed a significant difference. This might have happened that the *Paramecia* got adapted to their surrounding when exposed to it for longer duration of time. This concentration of dimethoate does not causes cell death due to the ability of ciliates to survive in water containing high concentration of organic matter and dissolved oxygen [24]. The number of species of ciliate present in the effluent indicates qualitatively the efficiency of removal of pollution from the waste water during treatment in the rotating biological reactor[25,26]. Adl and Gupta showed that 32 species of ciliate were found to be residing in forest soil containing high organic matter which may be used as the potential bioindicators of soil fertility[27]. Generally, the sudden and constant changes in the water body contents due to the industrial wastes and agricultural run offs seems to be the major factor responsible for all the variations occurred in the quality of water along with the zooplankton community in any water body[28].

Table-3
Growth rate test for 24 hours exposure

Concentration of dimethoate (mg/ml)	Mean number of cells at 24 hour	Number of generations	Generation time (hours)
(Control)	17.00	1.847	12.994
0.5	15.66	1.647	14.572
1.0	14.66	1.551	15.473
1.5	14.00	1.485	16.161
2.5	10.33	1.046	22.945

Table-4
Growth rate test for 96 hours exposure

Concentration of dimethoate (mg/ml)	Mean number of cells at 96 hour	Number of generations	Generation time (hours)
(Control)	27.00	3.169	30.293
0.5	20.33	2.760	34.782
1.0	20.33	2.760	34.782
1.5	19.00	2.662	36.063
2.5	09.33	1.637	58.644

Behavioural changes: Many behavioural changes were observed in *Paramecia* while carrying out various tests. First visible change to occur at lower concentrations of the test organism were irregularities in ciliary beating, that is, short interruptions and spinning movements, which often resulted in swimming away of the cell. At higher concentrations, the movement of the cilia became weaker and irregular, after a while deformity in the cell occurred and finally the organisms died. Cell lysis or exocytosis was seen prominently in many cells. Under pesticide stress, many cells could not move normally in a straight line but were spinning around themselves. The very first effect right after adding the toxicant can be seen is the movement of the cells towards the border of the cavity. As the time passes the cell shows slowing down movement or irregular spinning and turning. Velocity of *Paramecia* was noticed to be affected greatly with the environment. The velocity increases greatly at lower concentrations, as compared to control set, for the first 10 minutes and then decreases significantly. Reduction in the size of the cells was also observed at higher concentrations. Blebbing of the cells was commonly seen at all the concentration at some or the other point of time. At higher concentrations the blebbing was observed to occur in a short time while the lower concentrations created blebbing later. When *Paramecium caudatum* cells were exposed to Monocrotophos (>100 mg/L), affected cells showed alteration in their shape, by developing irregular blebbing of the cell membrane followed by cell lysis[29].

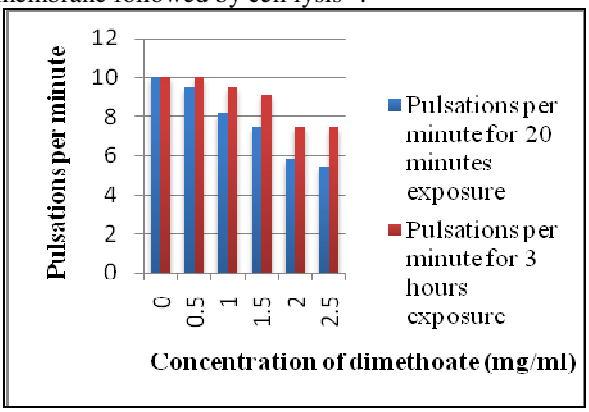

Figure-1
Effect of dimethoate on pulsation rate of posterior CV of *Paramecium sp.*

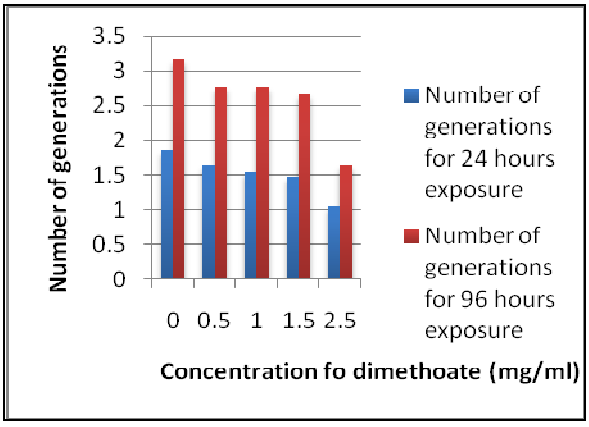

Figure-2
Effect of dimethoate on number of generations of *Paramecium sp.*

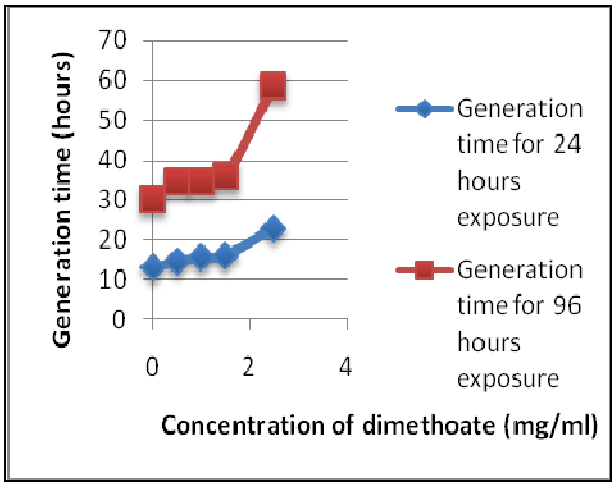

Figure-3
Effect of dimethoate on generation time of *Paramecium sp.*

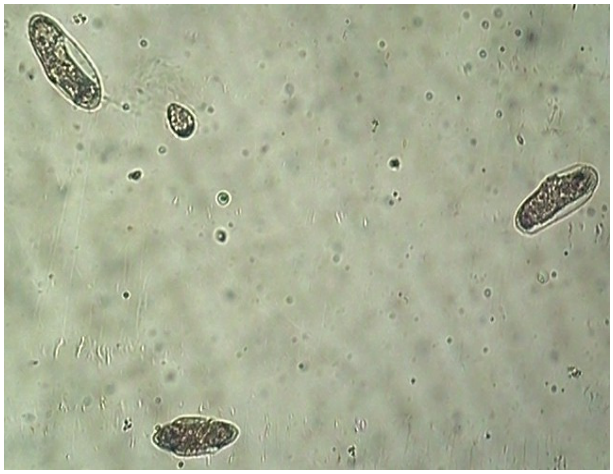

Figure-4
Deformities in the normal cell structure

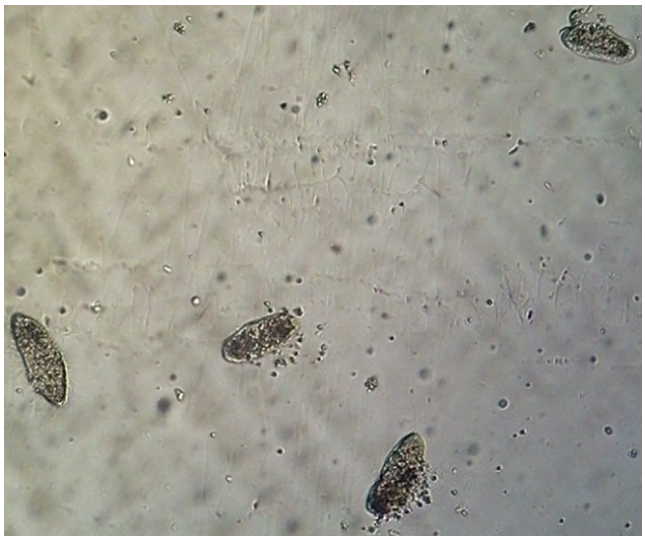

Figure-5
Cells underwent lyses showing exocytosis

Figure-6
***Blebbing of a Paramecium* cell**

Conclusion

After considering the results of the present study, it is to be concluded that the *Paramecium* does get affected by the neurotoxic insecticide dimethoate, irrespective of lacking any sort of nervous system, and can prove to be a potential and responsive bioindicator for the pesticide stress in the water body. The methods used were cheap and easy to perform. The sensitivity of the experimental organism has made it an alternative model organism in the place of eukaryotes for carrying out bioassay of toxicity having economic and effective approach.

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