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TOXICOLOGICAL AND PHYSIOLOGICAL EFFECTS OF DIMETHOATE ON THE FRESHWATER CILIATE *PARAMECIUM CAUDATUM*

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ABSTRACT

The freshwater ciliate protozoan *Paramecium caudatum* was utilized to investigate the potential toxic and functional impacts of the insecticide Dimethoate, an organophosphate and acaricide. During acute toxicity tests, lower concentrations slightly reduced cell motility, whereas higher doses caused abnormal rocking movements, eventually leading to complete immobility. The median lethal concentration (LC_{50}) after 3 hours of exposure was calculated as 0.1 ppm. Exposure to Dimethoate resulted in notable cellular deformities, including cell swelling, an oval-shaped appearance, and at higher concentrations, a reduction in body length accompanied by cytoplasmic darkening. Leakage of cellular contents further indicated damage to the cell membrane. Altered activity of the contractile vacuole was observed after 20 minute exposure to concentrations of 0.001, 0.005, 0.01, and 0.03 ppm, suggesting disruption of osmoregulatory functions. Observed disturbances in phagocytosis and vacuolar activity imply that Dimethoate may exert significant physiological effects on *P. caudatum*. Due to their ease of culture, short life cycles, rapid responses, and reproducibility in experiments, ciliates like *Paramecium* prove to be valuable model for assessing toxicity, physiological effects, and environmental risk.

KEY WORDS: Dimethoate, Cytotoxicity, *Paramecium caudatum*, Phagocytosis.

INTRODUCTION

In a developing nation like India, the need for food and housing is continually rising. Given the limited land available for agriculture and a growing population demanding higher food production, we cannot afford to jeopardize any crop fields due to pests like insects or rodents. To mitigate this risk, farmers are educated on the use of various pesticides, including insecticides, herbicides, rodenticides, fungicides, and larvicides. However, these pesticides can impact all members of an ecosystem, leading to the deaths of many aquatic organisms, such as fish, amphibians, and invertebrates, as well as those that rely on them for sustenance. This indicates that pesticides not only harm their intended targets but also indirectly threaten non-target animal populations when they enter the environment, making it a significant concern in recent times (Miyoshi, *et al.*, 2003). Protozoan ciliates such as Paramecia form the fundamental base of the heterotrophic eukaryotic food web. In addition to being significant bioindicators of environmental conditions and changes brought about by both natural occurrences and human activities, they also play a crucial role in regulating essential ecosystem processes (Finlay and Fenchel, 2004).

Numerous recent studies indicate that ciliates are crucial for the trophic dynamics within periphytic communities and serve as indicators of pollution levels in rivers and lakes (Amanchi and Bhagavathi, 2009). *Paramecium* sp. serves as a preferred organism for cytotoxicity research. Its relatively brief generation time in comparison to invertebrates, along with its rapid growth capacity in laboratory culture mediums, makes it particularly useful for investigating the effects of xenobiotics across multiple generations of cells. This ciliate has been utilized to assess the impact of various xenobiotics (Benbouzid *et al.*, 2015). Dimethoate can cause rapid, fatal poisoning to humans, insects, fish, mice. Dimethoate may cause mutations (genetic changes) (New Jersey Department, 1999). The use of non-target organisms in environmental toxicology is essential for comprehending the wide array of toxic effects that pesticides and other pollutants can have on various organisms. In recent years, there have been successful efforts to substitute fish and other aquatic organisms traditionally used as bioindicators of organic pollutants with ciliates (Venkateswara Rao *et al.*, 2006).

In the present work, *P. caudatum* was used as model organism to assess the effect of Dimethoate through an *in vitro* series of cytotoxicity tests. The bioassays planned in this study include simple and fast acute test that give information about the locomotor behaviour, morphology, phagocytosis, contractile vacuole activity of the cells when exposed to different concentrations of Dimethoate.

MATERIALS AND METHODS

Test compound

Commercial grade of Dimethoate 30% (Rogorin) insecticide used in this study was manufactured by Insecticides, India Ltd. It is a widely used organophosphate insecticide and Acaricide. It acts both by contact and through ingestion. It is readily absorbed and distributed throughout plant tissue.

Test Organism

P. caudatum, were collected from a freshwater body, Osmania University, Hyderabad, India. To establish a pure line culture, the organisms were cultured in a sterilized hay infusion medium and the log phase cultures were utilized for further studies. Six g of dried hay was boiled in one liter of distilled water, then cooled and filtered. After that, it was sterilized in an autoclave for 15 minutes at 15 pounds of pressure and stored for future use. Hay infusion medium has been extensively recognized as a suitable culture medium for ciliates. It provides an environment for the ciliates that closely resembles their habitat to culture the microorganisms, hay infusion medium was mixed with distilled water at a 1:1 ratio and distributed into various cavity blocks. Under sterile conditions, ciliates were introduced into the medium to develop a pure stock culture. Sub-culturing was performed every sixth day (Bick, 1972).

Acute toxicity studies

The stock solution and experimental concentrations of Dimethoate were prepared according to the APHA (2002). A stock solution of 1000 ppm of Dimethoate was prepared using distilled water as the diluent. Following initial rough dose-finding trials, the relevant stock solutions and test concentrations were identified, freshly prepared and utilized for the toxicity assessments. The test protocols used were those recommended by Apostol (1973) and Masood (1986). An acute toxicity test was carried out over a duration of 3 hours. In these acute experiments, 0.5 ml of the pesticide solution was mixed with 4.5 ml of culture medium to reach the desired concentration of each pesticide. One Hundred organisms were added to each cavity block. All test concentrations were conducted in triplicate. After adding the pesticide, each cavity block was placed under a binocular microscope, and counts were conducted at 10-minute intervals during the first hour and then at 20-minute intervals for the remaining two hours. The LC_{50} value and lethal concentration were determined from the mortality curve over the three-hour period. Simultaneously, control groups containing the same number of organisms but no pesticide was run.

Phagocytosis

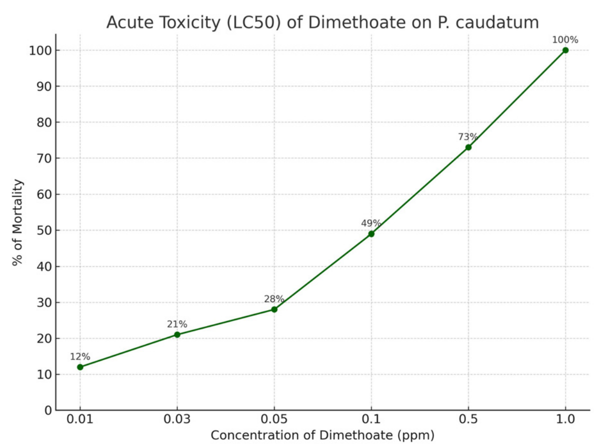
Phagocytosis was examined following exposure to a sub-lethal concentration of Dimethoate such as 0.001, 0.005, 0.01, 0.03. The test organisms were subjected to different concentrations for one hour. Ten treated cells from the same concentration after 1 hour of exposure to the toxicant were collected using a micropipette, combined with a one molar carmine suspension, and allowed to sit for 20 minutes. Ten organisms from each exposed group were selected, immobilized on slides using Methyl cellulose and the number of food vacuoles that formed was quantified. Likewise, control paramecia were mixed with the carmine suspension, immobilized, and the formation of food vacuoles was counted. The preparation of the carmine suspension and the counting of food vacuoles were conducted using the method proposed by Nilsson (2003a); Nageswara Rao *et al.* (2012)

Contractile vacuole activity

The activity of the contractile vacuole was assessed in the cells after they were immobilized on slides, following the procedure outlined by Marsot and Couillard (1973). For cells subjected to sub-lethal concentrations for 20 minutes, a single individual cell that appeared normal in every visible aspect was selected using a micropipette and the rate of pulsation in one vacuole. Observations were conducted on the cells at each concentration, and an equal number of observations were performed on controls at room temperature.

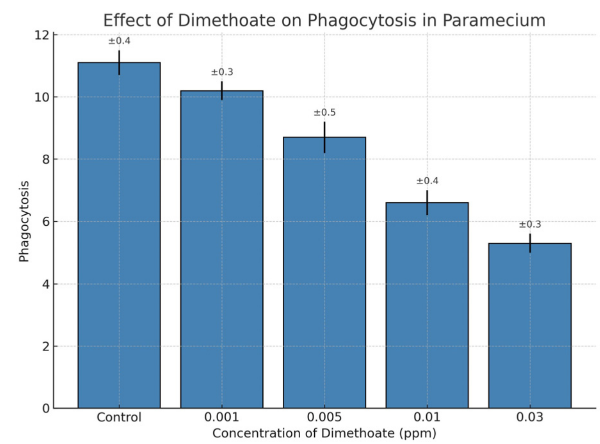
RESULTS AND DISCUSSION

A lethal concentration of 1.5 ppm of Dimethoate resulted in immediate cellular death, completely halting ciliary movements, contractile vacuole activity, and food vacuole activity. The determined LC_{50} value for a 3-hour exposure was 0.1 ppm and the LC_{100} was 1ppm (Graph 1). At a concentration of 0.5 ppm, ciliates exhibited abnormal and erratic movements. When exposed to higher concentrations, notable changes included shortening of the longitudinal axis, darkening of the cytoplasm and enlargement of the contractile vacuole were clearly noted. No observable cytotoxic effects were found at concentrations of 0.03, 0.01, 0.005, and 0.001 ppm after 3 hours of exposure.



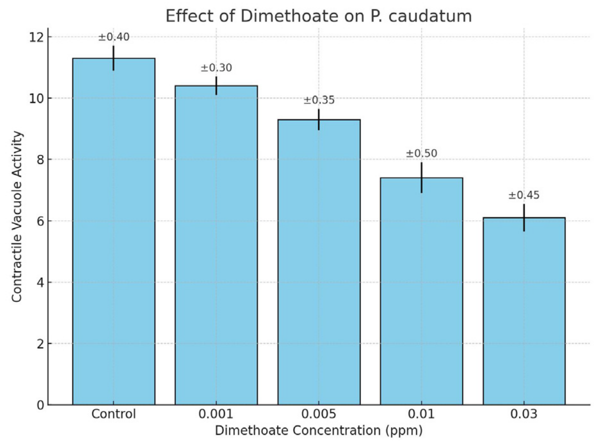
Graph 1. Acute toxicity effects of Dimethoate on *Paramecium caudatum*

Inhibition of food vacuole activity was noted after exposure to 0.03 ppm, with the experimental ciliates showing average food vacuole counts of 10.2, 8.7, 6.6, and 5.3 in concentrations of 0.001, 0.005, 0.01, and 0.03 ppm respectively after a 1 hour of exposure (Graph 2). The formation and movement of food vacuoles are reliant on ciliary motility since cilia facilitate both locomotion and the transport of food vacuoles to the cytostome. Any factors influencing ciliary action are likely to also impact the rate of food vacuole formation. Dimethoate induced a significant and sharp decline in food vacuole formation after one hour of exposure. This result implies potential damage to the cell membrane and the internal contents of the organisms. It is probable that Dimethoate disrupts cytoplasmic contractions, which are essential for the separation of food vacuoles. Additionally, the current studies indicate that while ciliates retain the ability for suspension feeding, this process is somewhat inhibited. This effect is dependent on time (Ouissem Moumeni, 2016).



Graph 2. Effect of Dimethoate on food vacuole of *Paramecium caudatum*

Paramecium is a commonly studied organism for understanding the structure and function of contractile vacuoles and has been the focus of research by many authors over the years. Stock *et al.* (2002) stressed on the point that the pressure developed at the pulsatory vacuole membrane might be responsible for the pressure required for the discharge of water content from vacuole through pulsatory vacuole pore. The expulsion frequency of pulsatory vacuole might be changed or influenced by exterior factors. It was further concluded that the contractile vacuole fluid osmolarity is constantly hypertonic to the cytoplasm, and the osmolarity of the cytoplasm is constantly hypertonic to the osmolarity of surroundings. When exposed to a concentration of 0.03 ppm Dimethoate for 20 minutes, the contractile vacuole activity was most significantly inhibited, with an average number of pulsation for 1 min both control and exposed (Graph 3). At all tested concentrations (0.001, 0.005, and 0.01 ppm), a marked reduction in vacuolar activity was observed (Graph 3).



Graph 3. Effect of Dimethoate on Contractile Vacuole of *Paramecium caudatum*

CONCLUSION

The present study demonstrates that Dimethoate exerts profound toxicological effects on *Paramecium* even at sub-lethal concentrations. Sub-lethal concentrations (0.001-0.03 ppm), while not resulting in immediate cell death, significantly disrupted physiological functions such as food vacuole formation and contractile vacuole pulsation frequency. These impairments suggest that Dimethoate interferes with fundamental processes such as osmoregulation, cytoplasmic streaming, and membrane integrity. The close relationship between ciliary function and food vacuole formation implies that the compound's inhibitory effects on motility may underlie broader physiological disruptions. The study highlights Paramecium as a sensitive bio indicator for pesticide toxicity and underscores the ecological risks posed by organophosphates like Dimethoate even at low environmental concentrations. Further research is recommended to explore the long-term effects of chronic exposure and to better understand the underlying mechanisms of toxicity.

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Conflict of Interest – None

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