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Effect of Dimethoate on Earthworms and its Bacterial Mediated Detoxification

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Abstract: Biodegradation has received an increased attention as a safe, eco-friendly, and cost-effective technique to decontaminate soil and water. According to WHO, the health threat faced by human beings in the 21st century is drug resistant microbes. Here an attempt is made to evaluate the effect of Dimethoate upon *Pheritima posthuma* through toxicity testing. To determine the effect of pesticide detoxification by bacterial isolates from soil dwelling organisms, a comparative histopathology of normal earthworm, earthworm with untreated dimethoate, and earthworm with bacterial treated dimethoate was also conducted.

Keywords: Antimicrobial agents, Earthworm, Dimethoate, Bacterial isolates, Detoxification

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Introduction

Dimethoate, causes severe pollution of soil and groundwater and affects aquatic organisms, bees, plants, birds, earthworms, humans, and other animals by inhibiting largely acetylcholinesterase (AChE) enzymes. It is an organophosphorus pesticide that accumulates in soil after every use in agricultural fields., widely used in our crops to protect from different kinds of insects. Its half-life is around 200 days in soil depending on temperature, pH, microorganisms, and organic contents. The primary metabolite of this pesticide, omethoate, is highly toxic than dimethoate and hence the pesticide needs to be degraded to protect our environment from its harmful effects.

Biodegradation has received increased attention as a safe, eco-friendly, and cost-effective technique to decontaminate soil and water. Bacteria such as *Kocuria turfanensis*, *Lactobacillus plantarum*, *Pseudomonas aeruginosa*, *Lysinibacillus sphaericus*, *Brucella sp.*, and *Bacillus licheniformis*, and fungi such as *Aspergillus niger*, *Trichoderma koningii*, and *Penicillium notatum*, and plants such as *Typha latifolia*, *Nasturtium officinale*, *Amaranthus caudate*, *Phaseolus vulgari*, and *Lactuca sativa*, are used to degrade dimethoate (Ferdaus *et al*., 2024) .

Insects that reside in contaminated habitats have robust immune systems that enable them to fend off microbial diseases. The gut-dwelling bacteria in such insects create antimicrobial substances that could serve as a source for new antibacterial substances. Numerous antagonistic bacteria found in insects produce bioactive substances that are being extracted and studied for their potential to cure a number of human ailments. Some microorganisms produce specialised toxins that support the insect immune system in order to defend their insect hosts from diseases, parasitosis, and other parasites (Zhang *et al*., 2004). As the bio-accumulation and bio-magnification of lethal pesticides are the main cause of the loss of plants, microbes and animal biodiversity, microbial based bioremediation of toxic pollutants has been proposed to be a safe and sustainable mean of decontaminating the environment.

Earthworms are known as ecosystem engineers due to their burrowing activities and interactions with soil microorganisms by enhancing soil structure, nutrient cycling, and organic matter decomposition. As essential inhabitants of soil ecosystems, they also play a crucial role in the degradation of dimethoate, a common pesticide found in contaminated environments. This is achieved through their gut microbiota and enzymatic activities, which facilitate the degradation process. Consequently, it is vital to understand the intricate interactions between earthworms, microorganisms, and dimethoate degradation in order to develop comprehensive strategies for mitigating pesticide pollution in agricultural systems. However, the use of pesticides and insecticides poses significant threats to earthworm populations and their beneficial activities. These chemical compounds can directly harm earthworms, leading to toxicity, mortality, and behavioral changes, while also impacting their reproductive capabilities and the vital process of soil nutrient cycling. Research has demonstrated the negative impacts of pesticides on earthworm populations, revealing reduced abundance, altered community structure, and decreased rates of soil organic matter decomposition following exposure to commonly used pesticides such as organophosphates and pyrethroids. These findings highlight the urgent need to consider the ecological implications of pesticide use and emphasize the importance of adopting sustainable pest management practices that minimize harm to earthworms and other beneficial soil organisms (Miglani and Bisht, 2019). In the present study an attempt is made to evaluate the effect of Dimethoate upon *Pheritima posthuma* through toxicity testing.

Materials and Methods

Pheritima posthuma were maintained in moisture soil in the laboratory. Varying concentrations of dimetoate were prepared ranging from 0, 50, 100, 150 and 200 mg/ml to perform mortality assay and to detect the time of paralysis. To prepare the treated pesticides, the gut isolation of *Periplaneta americana* was employed ensuring a sterile environment and cultured in a nutrient broth to facilitate the growth of microorganisms within it. For the isolation of pesticide resistant bacteria, 1 g of sample was inoculated into 250 ml Erlenmeyer's flask containing 100 ml sterilized nutrient broth enriched with 1 ml of pesticide (Dimethoate) solution to make the concentration of 200 mg l^{-1} and were incubated at 37°C for 48 h. Following incubation, 10 ml of the broth culture was transferred to the freshly prepared nutrient medium having same composition. The same procedure was successively repeated for 4 times to achieve the complete enrichment of pesticide resistant bacteria culture. The bacterial strain that could tolerate maximum concentration of pesticide was considered and selected as superior strain which was taken for further studies. (Mulligan *et al*., 2016). The strain was sub cultured in pure plates to avoid further contamination.

The earthworms were cultured in treated pesticides to determine the detoxification rate (Qi

Table 1: Concentration depended impact of dimethoate on paralysis and death of *Pheretima posthuma*

Concentration of Dimethoate (mg/ml)	Paralysis (min)	Death (min)
50	128	159
100	74	106
150	58	79
200	31	55

Fig. 1: Histopathological analysis of *Pheretima posthuma* to confirm detoxification.

and Wei, 2017). At each sampling period, earthworms were removed from the soil and washed with distilled water. After a depuration of 6 h, as recommended, earthworms were narcotized, placing them in 7% alcohol until relaxed and sequentially dehydrated in graded ethanol concentrations and embedded in paraffin wax with different melting points. Slides with cross sections of earthworms were stained using the hematoxylin–eosin for light microscope observation, and photographed with an Labomed microscope equipped with a digital colour camera Micaps pro HDMI (Pankaj *et al*., 2016).

Results and Discussion

Effect of pesticide up on Pheretima posthuma

The effect of dimethoate on terrestrial animals was analyzed by taking *Pheretima posthuma* as a model organism at varying concentrations i.e from 50 to 200 mg/ml. It was observed that it showed different morphological and behavioural changes in response to the treatment. Escaping tendency was observed in all concentrations whereas the higher concentration response was visible from 12th minute of administration. It remained peaceful in 50 mg/ml concentration up to 55th minute (Table 1) and showed paralysis in 128 minutes and death occurred at 159 minute. Higher degree of secretion of coelomic fluid was observed even in smaller concentrations. They were found to be getting paralyzed depending upon the concentration administrated. At higher concentration (200 mg/ml) the paralysis was observed at 31st minute after administration and found died at 55th minute. In all the four different concentrations body elongation is the major morphological feature (Huang *et al*., 2018; Jin *et al*., 2018). The effects of pesticide on earthworm varied depending on the dose and the duration of exposure.

Histopathological analysis

Histopathological study of normal earthworm, earthworm with untreated dimethoate (200 mg/ml) and earthworm with bacterial treated dimethoate was conducted. Drastic changes at the tissue level organization of earthworms was noticed when administrated with 200 mg/ml pesticide (Fig. 1). Aberrations such as vacuole formation between muscles and ectodermal spoilage were observed. However, the earthworms treated with bio treated dimethoate resembled the histology of normal animal suggests that the bacterial treatment detoxified the pesticide.

The increase in the use of the pesticides has increased both the hazards to human health as well as pollution of the environment. Pesticides can kill earthworms directly, either by disrupting their nervous system or by causing other physiological problems. Pesticides can reduce the number of eggs that earthworms produce, the hatching success of their eggs, the survival of their young ones and can disrupt the gut microbiota. When earthworm populations decline, the decomposition of organic matter slows down, nutrient cycling is disrupted, and soil aeration is reduced. This can lead to a variety of problems, including soil compaction, nutrient deficiencies, and the loss of biodiversity. It is important to follow the label instructions carefully before pesticide usage to avoid contamination of soil and destruction of soil dwelling organisms (Jiang *et al*., 2019; Tang *et al*., 2019).

Dimethoate is highly toxic to earthworms- the effects being dose-dependent. Though the earthworms have some ability to metabolize and detoxify organophosphate pesticides like dimethoate, their internal detoxification mechanisms are overwhelmed by high concentrations. However, soil-dwelling micro-organisms can also degrade dimethoate, contributing to its detoxification and potentially mitigating some of its harmful effects on earthworms in the environment. The use of pesticide resistant bacteria can possibly reduce the harm caused by pesticides on soil and water microbiota. The study conducted by Tinker and Ottesen (2016) and Monica *et al*. (2023) investigated the gut microbiome of *Periplaneta americana* and *Blattella germanica* and provided valuable insights into its role in health and social behaviour. Li *et al.* (2020) have conducted a study that focused on the gut bacterial community of the *Periplaneta americana* across different developmental stages. and explored the potential functions of these bacteria, revealing their involvement in crucial processes such as digestion, immunity, and even resistance to insecticides.

At higher concentrations, dimethoate causes a significant percentage of earthworm mortality, including juveniles, immatures, and adults. Dimethoate can cause disruptions at the cellular and physiological levels, affecting overall earthworm health. The results obtained in the present study indicate that dimethoate is highly toxic to earthworms (De Silva and Amarasinghe, 2008). Therefore the impact of continuous application of such pesticides in soil for higher crop production should be avoided.

Conclusion

Earthworms, renowned for their pivotal role in soil structure maintenance and nutrient cycling, thrive in an environment characterized by reduced pesticide contamination. The integration of pesticide-degrading bacteria into sustainable agricultural practices and comprehensive environmental management strategies holds immense potential in combating pesticide pollution and fostering overall ecological well-being. Further comprehensive investigations in this realm are imperative to unravel the intricate interactions among soil microorganisms, soil ecosystems, and the broader environment, thereby paving the way for efficacious and environmentally conscious approaches in pest control and agricultural practices.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, Vimala College (Affiliated to University of Calicut), Thrissur 680009, Kerala, India.

Author Contributions

Each author has contributed equally in designing the research, conducting thorough analysis, drafting the manuscript, and refining its content. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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