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Antimicrobial activity of vermiwash of earthworm *(Eisenia fetida)* on certain plant bacterial pathogens

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Abstract

Pesticides are well known to be toxic to most organisms when present in high concentration in the environment. The demand for products and technologies based on plants to control plant pathogens has increased in recent years due to hazardous pesticides. The present study was carried out during the year 2019 at VHNSNC (Autonomous), with objective of using vermiwash against selected plant bacterial pathogens. The best inhibitory effect of vermiwash of *E.fetida* on the growth of *Xanthomonas oryzae* and *Bacillus cereus* was seen to be 21mm to 19mm zone of inhibition. This research may show the way to formulation of novel natural antimicrobial agent.

Keywords: Vermiwash, Antimicrobial, *Eisenia fetida*

Introduction

Pesticides are a fundamental part and an essential element of modern agriculture (Damalas *et al*., 2011). Over the last 60 years, farmers have achieved major progress in foodstuff production via the application of pesticides. Reduction of pest activities and agricultural losses at a reasonable cost improved crop yields and promoted food availability during all seasons (Boxall *et al*., 2001). Exposure to pesticides normally occurs while preparing the spray solutions, loading in the spray tank and while applying the pesticide. Continued exposure to sub lethal quantities of pesticides for a prolonged period of time, may result in chronic illnesses in humans (Fleming *et al*., 2003). About three million cases are

reported worldwide every year that occur due to acute pesticides poisoning (Singh and Mandal, 2013). In particular, inappropriate use of pesticide has been linked with adverse effects on non-targeted organisms (Eleftherohorinos, 2008).

Earthworms are popularly known as the "farmer's friend". Earthworm influences microbial community, physical and chemical properties of soil (Esakkiammal *et al*., 2015). They have been used in medicine for various remedies since 1340 AD. Earthworm has been recognized in medicine as anti-inflammatory, analgesic and antipyretic agent (Abhishek *et al*., 2010). Vermiwash is basically a clear, transparent and pale yellow colored fluid. It consists of various excretory products, secretions of earthworms and beneficial micronutrients from soil organic molecule (Ismail, 1997). Freshly collected vermiwash contains many beneficial microbes that promotes the plant growth and prevents infections. The hormones that promote the plant growth like indole acetic acid, gibberellic acid, etc are also present. The vermiwash must be diluted before its application on the plants. It is one of the most cost efficient and environmentally friendly methods of waste disposal (Jayashree, 2006).

Nevertheless, the widely used methods for the determination of antimicrobial activity were confined to inhibition-zone assays with a modification of the two-layer radial diffusion method (Lehrer *et al.*, 1991) and the colony forming unit technique (Lassègues *et al*., 1989). Phytopathogenic bacteria can cause great crop damages which may lead to famine in several countries. It is important to know the principle of plant diseases to minimize the loss of crop yield. Plant pathogenic bacteria have negative effect on plant health and threat food security in the world. Another important fact is that in day to day life, humans and animals spread bacterial disease by cultivating, consuming, and excreting low-quality plant-based foods (Shymaa Bashandy, 2017).

Nowadays, advent of pesticides is increasing in agriculture day by day which ultimately destroys the fertility of soil upon long term use. Organic manure may be used as alternative source to control the hazardous effect of chemical fertilizer. In this way, vermiwash may be used for better yielding of crops. Vermiwash protect the environment from various chemical fertilizers. Hence the present investigation exhibited "Antimicrobial activity of vermiwash of earthworm (*Eisenia fetida*) on certain plant bacterial pathogens" has been carried out.

Materials and Methods

Procurement of *Eisenia fetida* vermiwash

Eisenia fetida vermiwash was procured from Jeepe Biotech Farm in Kullursandai, Virudhunagar distict, Tamil Nadu, India.

Purchase of Plant Bacterial Pathogen Cultures

Pure cultures of *Xanthomonas oryzae* and *Agarobacterium rhizogenes* were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, and India. Additional one plant pathogen *Bacillus cereus* was obtained from Department of Biotechnology, Mepco Schlenk Engineering College, Sivakasi, Tamil Nadu, and India. The pure cultures were stored in the refrigerator and were sub cultured often so as to maintain purity and viability of cells. Bacteria were sub cultured once in 3-4 days by streak plate method.

Bacterial culture suspension

Nutrient broth was used for the preparation of bacterial culture suspension. Using a sterile loop, the bacterial cells were transferred from the stock culture plate into a small quantity of nutrient broth taken in a sterile test tube. It was used for studies only after 18 hours of incubation at room temperature.

Agar well diffusion method

The Muller Hinton Agar medium was poured into sterile petridishes; the plates were allowed to set. The organisms were uniformly swabbed on the plates and then well were bored in to the agar medium using a sterile 6mm cork borer. The wells were later filled up with the extract at quantity of 40µl, 60µl and 80µl. The plates were allowed to stand on for 1hour to allow proper diffusion of the extract and to prevent spillage onto the surface of the agar medium and then incubated at 37ºC for 24 hours.

Determination of Minimal Inhibitory Concentration (MIC) by turbidity method

The basic principle behind the test is the diffusion of particular extract with various concentrations into agar. 10ml of nutrient broth was taken in test tubes and sterilized at 121°C for 15 minutes. MIC was used to study the lowest inhibitory concentration of eluted fractions against the selected pathogens. The quantities of extract used were 20µl, 40µl, 60µl,

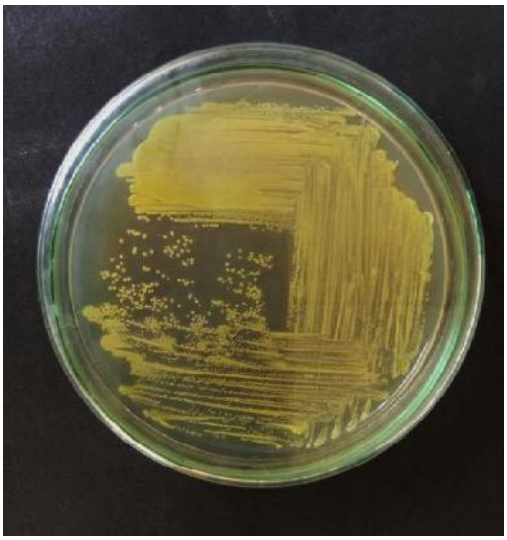
80µl, 100µl, 120µl. The pathogens were inoculated into the broth and incubated at 37°C for 24 hours. After 24 hours the turbidity were read at 490nm using UV- Spectrophotometer.

Result

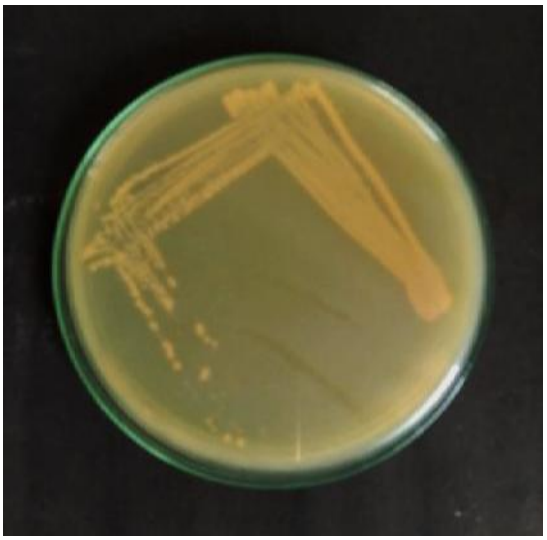
Table 1: Antibacterial activity of *Eisenia fetida* vermiwash on plant bacterial pathogens

Organisms	Zone of inhibition (mm) for the different concentration of vermiwash		
	40µl	60µl	80µl
Xanthomonas oryzae	15	18	21
Agarobacterium rhizogenes	12	15	18
Bacillus cereus	14	17	19

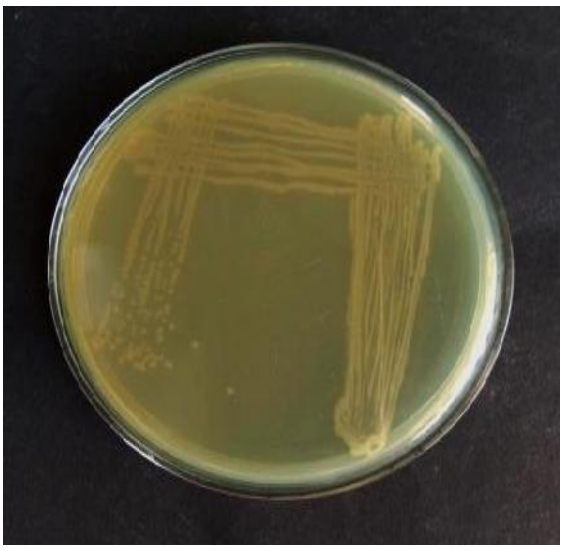
Plate 1: Vermiwash



Xanthomonas oryzae



Agarobacterium rhizogen



Bacillus cereus

Plate 2: Pure culture of Plant Bacterial Pathogens

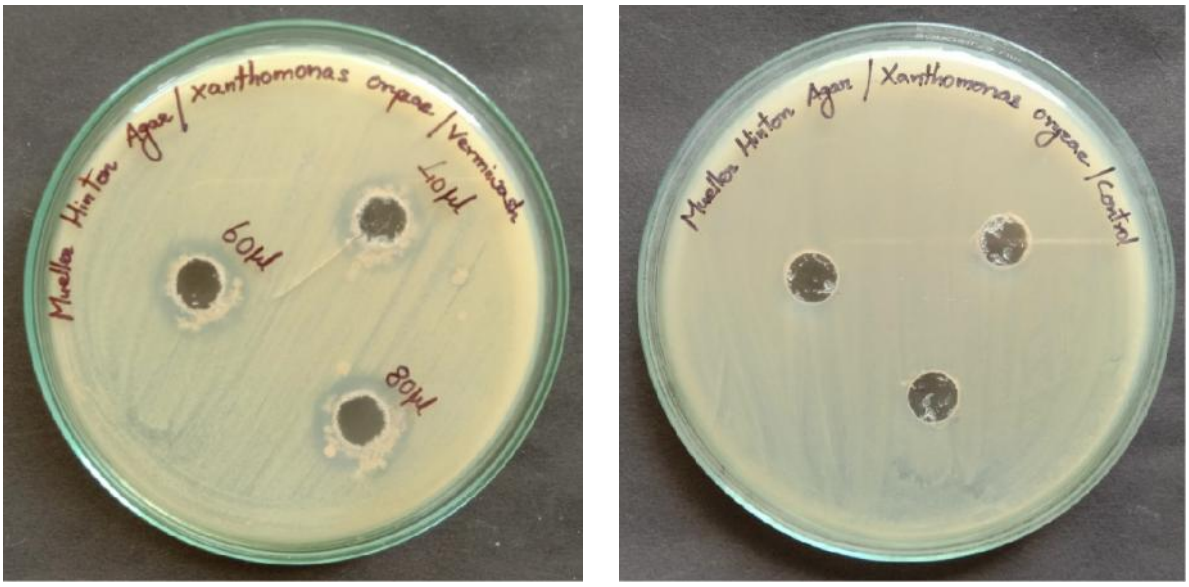


Plate 3: Antimicrobial activity of *Eisenia fetida* Vermiwash against *Xanthomonas oryzae*

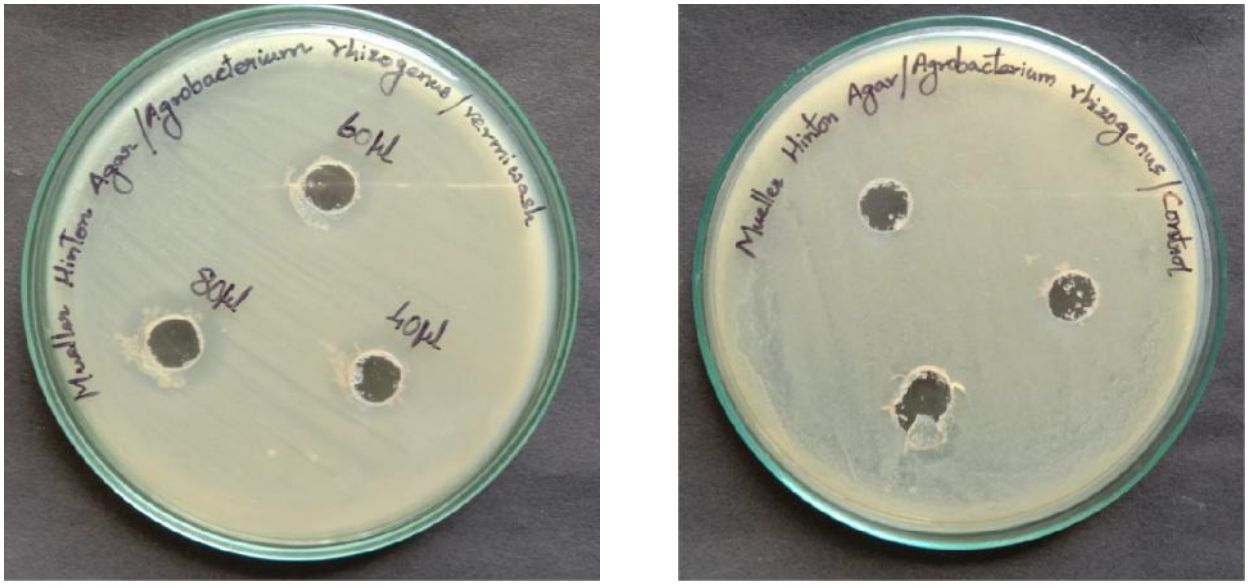


Plate 4: Antimicrobial activity of *Eisenia fetida* Vermiwash against

Agarobacterium rhizogenes.

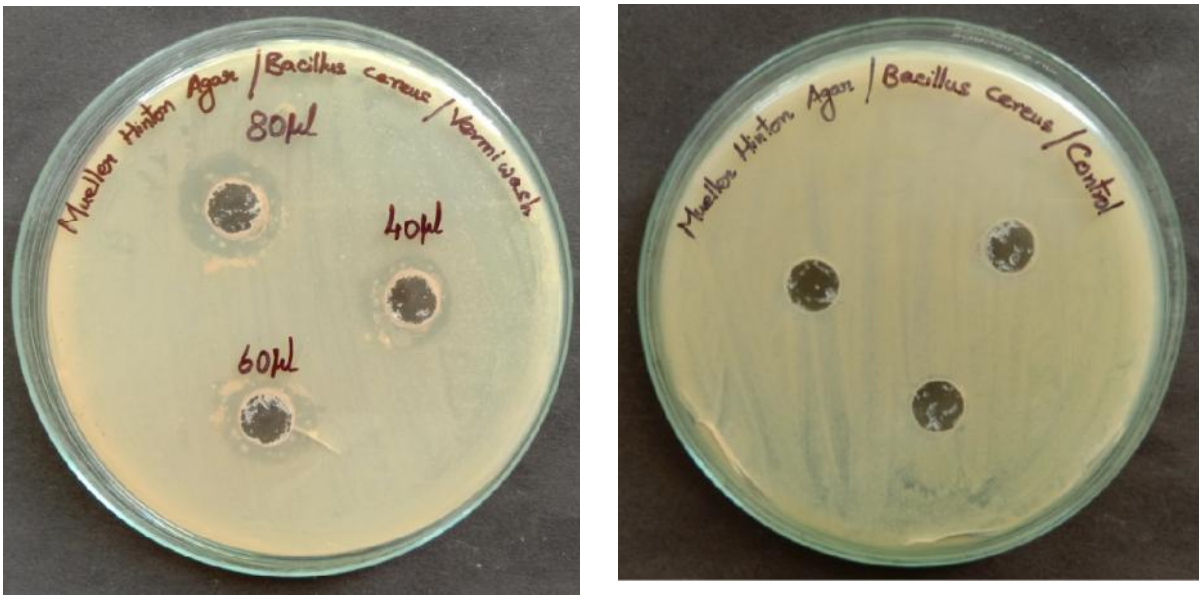


Plate 5: Antimicrobial activity of *Eisenia fetida* Vermiwash against *Bacillus cereus*

Table 2: Turbidity method of *Eisenia fetida* vermiwash on Plant Bacterial Pathogens

Organism	Extract	OD Value						
		Control	20µl	40µl	60µl	80µl	100µl	120µl
Xanthomonas oryzae	Vermiwash	0.27	0.23	0.20	0.17	0.13	0.09	0.05
Agarobacterium rhizogenes		0.23	0.19	0.16	0.14	0.11	0.07	0.03
Bacillus cereus		0.21	0.18	0.15	0.12	0.09	0.06	0.02

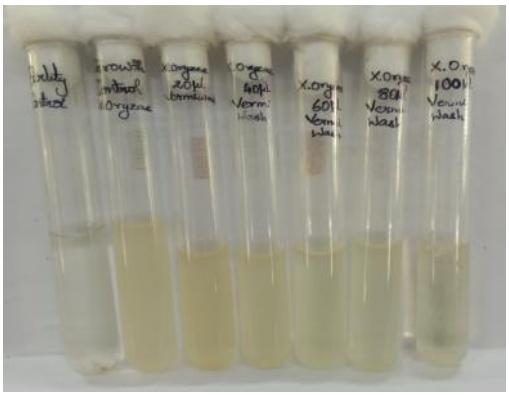


Plate 6. Minimal inhibitory concentration of *Eisenia fetida* vermiwash against *Xanthomonas oryzae.*

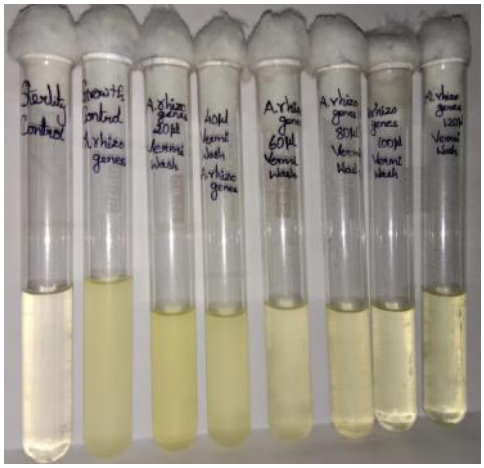


Plate 7. Minimal inhibitory concentration of vermiwash against *Agarobacterium rhizogenes.*

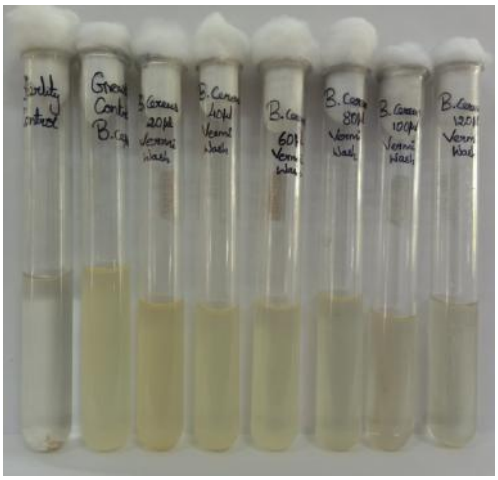


Plate 8. Minimal inhibitory concentration of vermiwash against *Bacillus cereus*

Discussion

The vermiwash of *E.fetida* was tested against three bacterial strains *Xanthomonas oryzae, Agarobacterium rhizogenes* and *Bacillus cereus*. The observed results are summarized in Table 1. The influence of vermiwash of earthworm on the growth of bacterial cultures was evaluated against selected plant pathogenic strains. The best inhibitory effect of vermiwash of *E.fetida* on the growth of *Xanthomonas oryzae* and *Bacillus cereus* was seen to be 21mm to 19mm zone of inhibition. The inhibitory effect is comparatively less against *Agarobacterium rhizogenes* exhibiting 18mm inhibitory zones.

From the study it is evident the earthworm vermiwash possess antimicrobial activity. The suppressive effect is highly concentration specific and the study on plant bacterial pathogens indicates that the vermiwash can be effectively used to control the plant bacterial pathogens. Several studies have also reported the inhibition of plant bacterial pathogens by vermiwash. Reiter and Salter (2002) have reported strong inhibition for *Xanthomonas oryzae*. *Bacillus cereus*, culture plate method using vermiwash. Their studies indicate the vermiwash can be used to control *Xanthomonas oryzae, Bacillus cereus* both the laboratory studies as well as in the field. Our studies have proved inhibition of *Xanthomonas oryzae, Agarobacterium rhizogenes,* and *Bacillus cereus* by the vermiwash. But vermiwash were tested on the plant bacterial pathogens directly and at laboratory level.

From the experiment it is evident that vermiwash have direct influence on the plant bacterial pathogens. Through this study was carried out at laboratory level, it has given promising

result that can be used to carry out more detailed research in the field of plant bacterial pathogens control in vermiwash.

The present study reports that earthworm's can be used only environmental monitoring but also in the acquisition of novel molecules for agricultural therapeutic purposes. This study may lead to formulation of new natural antimicrobial agent and thus may found to be beneficial in future prospects for mankind.

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