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Farming Model Design and Evaluation Biological Activity of Vermiwash Worm (*Eudrilus Eugeniae*)

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An automated vermiwash farming system was developed to regulate soil moisture and facilitate efficient vermiwash collection. The system's performance was evaluated under both stable and disturbed conditions, maintaining soil moisture setpoints at 63 % and 66 %, respectively. Results demonstrated the system's reliable responsiveness and stability, confirming its feasibility for automated vermiwash production. Chemical analysis of the vermiwash indicated the following compositions: organic carbon at 12.56 %, total organic matter at 21.00 %, total nitrogen at 2.61 %, and total phosphorus at 2.07 %. Biochemical assays further revealed the presence of peptides (0.64 ± 0.04 mg/mL) and proteins (0.44 ± 0.00 mg/mL). Enzymatic activities measured in the vermiwash included amylase (0.039 ± 0.001 U/mL), cellulase (0.0013 ± 0.0001 U/mL), and protease (0.486 ± 0.044 U/mL). The antioxidant potential peaked on day 45, with a maximum scavenging activity of 47.97 ± 0.21 %. However, no antimicrobial activity was detected against the tested microorganisms. These findings highlight the system's potential for sustainable vermiwash production with beneficial biochemical properties, though further exploration is needed for its antimicrobial applications.

1. Introduction

Earthworms have long been recognized as vital allies in agriculture due to their significant contributions to soil fertility and organic matter decomposition. Among them, the African Nightcrawler **(***Eudrilus eugeniae***)**, a tropical species native to Western Africa, stands out for its high reproductive capacity and exceptional efficiency in decomposing organic waste. As a member of the epigeic group, *E. eugeniae* thrives in surface organic layers, making it particularly well-suited for vermicomposting processes (Blakemore, 2015) Vermiwash**,** a nutrient-rich liquid by-product, is produced when water percolates through vermicompost, absorbing a variety of bioactive substances excreted by earthworms (Bhagat et al., 2022). This eco-friendly and non-toxic liquid is rich in vitamins, plant growth hormones, enzymes, proteins, amino acids, humic substances, macronutrients, micronutrients, and a diverse microbial community. (Suthar, 2010). As earthworms burrow through soil and compost, they create mucus-lined channels that serve as microhabitats for beneficial microorganisms. The mucus not only aids in nutrient retention but also facilitates the transfer of bioavailable compounds into the percolating water. According to Kaur and Bhat (2024), vermiwash extracted using hot water contains multiple functional enzymes including protease, amylase, urease, and phosphatase, enhancing its role as a biofertilizer. Functioning similarly to hydroponic nutrient solutions, vermiwash provides essential compounds that stimulate plant growth while remaining environmentally sustainable. Beyond its nutritional attributes, vermiwash exhibits properties that may suppress plant diseases and pests. Its liquid form allows for easy uptake by plant roots and foliage, making it a potent natural fertilizer. Given its multifunctionality, vermiwash has attracted growing research interest for its applications in organic farming and sustainable agriculture.

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2. Material and method

2.1 Source of Earthworms

The African nightcrawler (*Eudrilus eugeniae*) used in this study was sourced from Phu Thanh Organic Agriculture Company Limited, located in Trung An Commune, Củ Chi District, Ho Chi Minh City, Vietnam.

2.2 Vermiwash worm farming model design

An automated vermiwash farming system was designed to maintain optimal soil moisture and enable passive vermiwash collection (Figure 1a). The setup includes a worm cultivation tank with layered drainage, water pump, reservoir, collection tank, nozzle, soil moisture sensor (MT), microcontroller (MIC), and level transmitter (LT). The sensor monitors bedding moisture and triggers irrigation when levels drop below setpoints (63 % or 66 %). Excess water percolates through the substrate, producing vermiwash in the collection tank. Two trials assessed system performance, tracking moisture regulation and stability under normal and disturbed conditions.

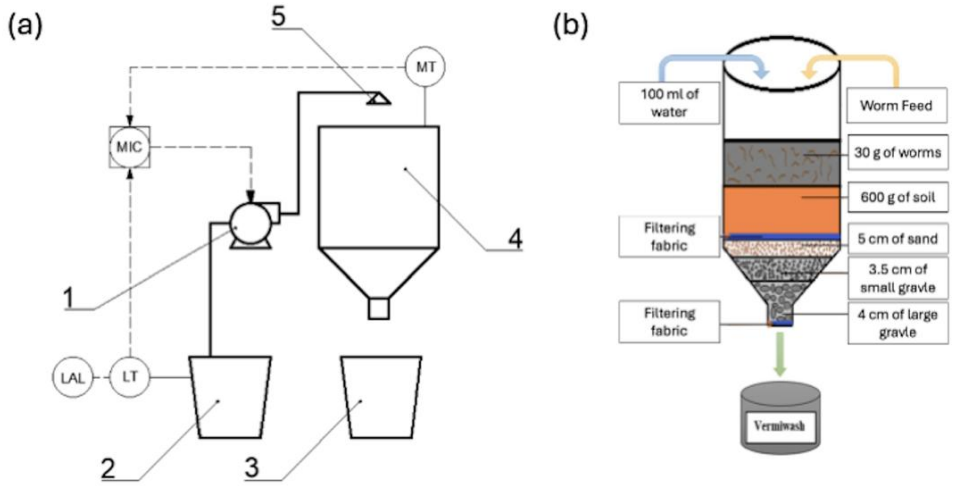

Figure 1: Design and structure of the vermiwash worm farming system, consisting of (a) P&ID diagram of the model; (b) filter material layers used in the vermiwash system

The vermiwash system's filter bed was built in layers: 4 cm large gravel, 3.5 cm small gravel, 5 cm quartz sand, and a filter cloth to prevent clogging. Above this, 600 g of soil was added, followed by 30 g of *Eudrilus eugeniae* worms (Figure 1b). Vermiwash was collected as nutrient-rich leachate percolating through worm-processed soil and the filter bed, based on a standard vermicomposting model

2.3 Analysis of chemical composition of vermiwash

The collected vermiwash was subjected to biochemical analysis to determine its protein content and enzymatic activity. Protein concentration was measured using the Lowry method, while amino acid content was quantified via a colorimetric reaction with ninhydrin reagent. The activities of amylase and cellulase were assessed based on the quantification of reducing sugars, employing the DNS (3,5-dinitrosalicylic acid) method as described by Miller. Additionally, protease activity was evaluated using the Anson method, which measures the release of amino acids from protein substrates.

2.4 Bioactive Properties of Vermiwash

The antioxidant activity of vermiwash was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, which measures the compound's ability to neutralize free radicals.
Vermiwash was precipitated with: solvents (acetone, ethanol) and $(NH_4)_2SO_4$: ratio of acetone: vermiwash = 3:1, 96% ethanol: vermiwash = 4:1, $(NH_4)_2SO_4$ 70 % saturated. The precipitate was collected by centrifugation at 15,000 rpm for 15 minutes. The precipitate was dissolved in PBS (Phosphate-buffered saline) pH 7.0
To assess the antimicrobial properties, vermiwash was tested against two bacterial strains (*Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 29213) and two fungal strains (*Ralstonia solanacearum* and *Pseudomonas cichorii*). The agar well diffusion method was employed to determine zones of inhibition, indicating antimicrobial activity.

3. Results and discussion

3.1 System Performance and Moisture Control

The automated irrigation system was evaluated through two tests to assess its moisture regulation capability. In the first test, the system was operated under stable conditions with a moisture setpoint of 63 %. As shown in Figure 2, the recorded soil moisture remained consistently close to the setpoint throughout the test period, indicating reliable baseline performance.

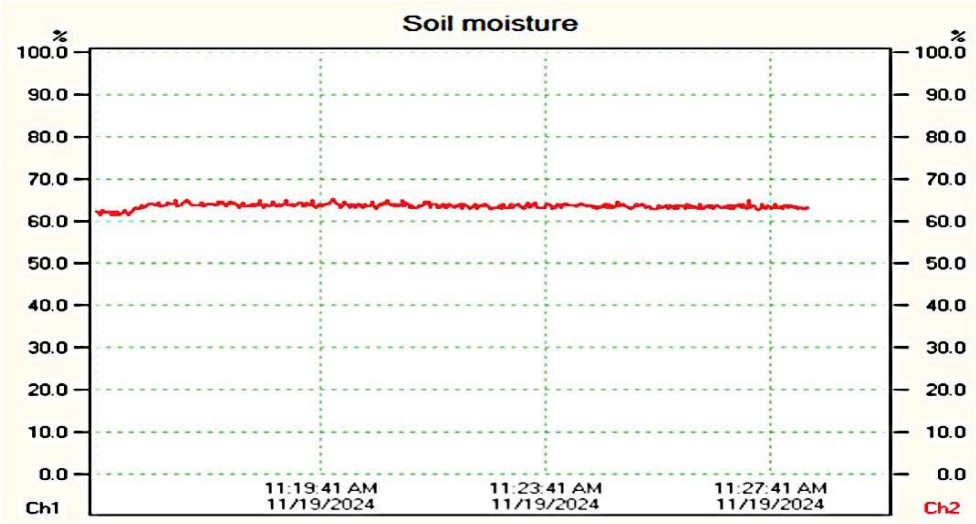

Figure 2: Baseline moisture control at 63 % setpoint

In the second test, a manual water disturbance was introduced to simulate fluctuating environmental conditions, followed by a tight-band control test with a moisture setpoint of 66 %. The system responded promptly to the disturbance and effectively maintained soil moisture within a narrow tolerance range. As shown in Figure 3, the system demonstrated both rapid recovery and high precision, confirming its capability to regulate moisture under more challenging conditions.

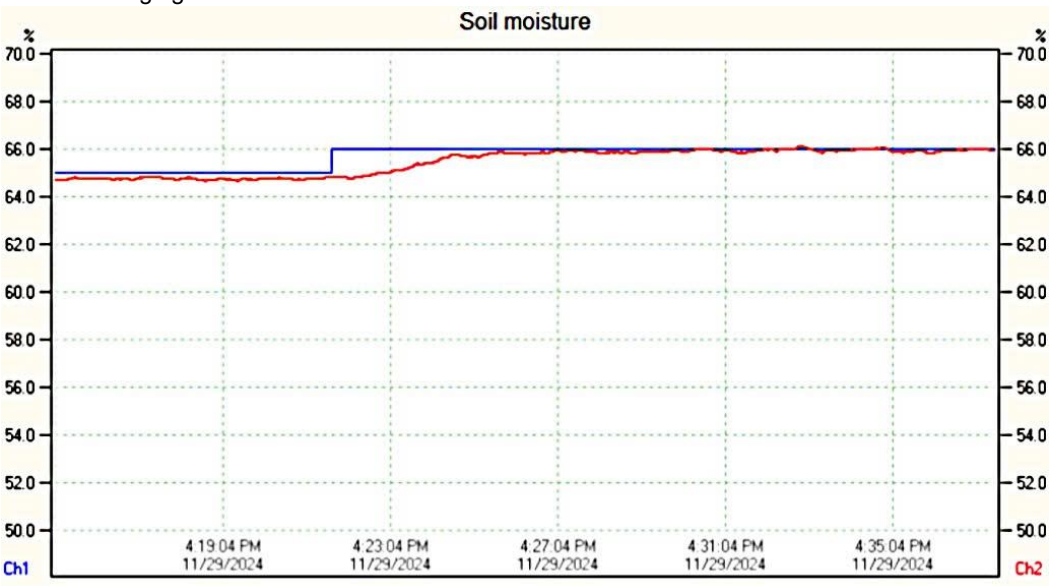

Figure 3: Moisture regulation at 66% after disturbance

These results confirm the system's capability to accurately and autonomously regulate soil moisture, thereby maintaining optimal conditions for vermiwash production without the need for manual intervention.

3.2 Evaluation of Chemical Composition of Vermiwash

Vermiwash was a liquid by-product derived from the vermiculture process, commonly utilized as a nutrient-rich organic foliar fertilizer. Its chemical composition is notably diverse, containing a wide range of macronutrients (e.g., nitrogen, phosphorus, potassium), micronutrients (e.g., zinc, iron, copper), as well as a robust population of beneficial microorganisms. These components collectively contribute to enhanced plant growth, nutrient uptake, and overall soil health, making vermiwash a valuable input in sustainable and organic agriculture.

Table 1: Chemical composition analysis results of vermiwash

No.	Parameter	Unit	Standard (*)	Sample value
1	pH	-	≥ 5	8.17 ± 0.02
2	Total Dissolved Solids (TDS)	mg/L	-	6.6 ± 1.11
3	Electrical Conductivity (EC)	mS/cm	-	38.21 ± 0.03
4	Gravity	g/cm^3	-	1.01 ± 0.01
5	Organic carbon	%	-	12.56 ± 0.11
6	Organic content	%	≥ 20	21.00
7	Total nitrogen	%	≥ 2	2.61 ± 0.12
8	C/N ratio	-	≤ 12	4.81
9	Total phosphorus	%	≥ 2	2.07 ± 0.1
10	Total Aerobic Microorganisms	CFU/ml	≥ $1x10^6$	8.53 x 10^7
11	Protein content	mg/mL	-	0.44 ± 0
12	Peptide content	mg/mL	-	0.64 ± 0.04
13	Amylase activities	U/mL	-	0.039 ± 0.001
14	Cellulase activities	U/mL	-	0.013 ± 0.001
15	Protease activities	U/mL	-	0.486 ± 0.044

(*): According to QCVN 01-189:2019/BNNPTNT – National Technical Regulation on the quality of fertilizers, organic fertilizer group.

The chemical composition analysis of vermiwash revealed a notably high content of organic nutrients. The organic carbon concentration reached 12.56 %, while total organic matter was measured at 21.00 %, both of which exceed the minimum thresholds specified in the Vietnamese National Technical Regulation QCVN 01-189:2019/BNNPTNT for organic fertilizers. These values indicate that vermiwash offers a rich nutrient source for both soil enrichment and plant development. In addition, the vermiwash sample exhibited high levels of essential macronutrients, including total nitrogen at 2.61 % and total phosphorus at 2.07 %, meeting or surpassing national standards. These nutrients are critical for vegetative growth, particularly in leaf development and root formation. Microbiological analysis further demonstrated a high density of aerobic microorganisms, with counts reaching 8.53×10^7 CFU/mL. This microbial load significantly exceeds the minimum requirement for organic foliar fertilizers, emphasizing vermiwash's role not only as a nutrient carrier but also as a potential biological control agent. The beneficial microbes present in vermiwash may contribute to plant health by competing with pathogens for resources, producing antimicrobial compounds, and inducing systemic resistance mechanisms that suppress the activity of harmful organisms (Mehta et al., 2014). Biochemical analysis identified the presence of peptides (0.64 ± 0.04 mg/mL) and proteins (0.44 ± 0.00 mg/mL), suggesting the existence of bioactive peptides naturally derived from earthworms. These compounds are known for their antimicrobial potential and may play a role in enhancing vermiwash's protective effects against plant pathogens, thereby supporting its application in sustainable and organic agriculture.

3.3 Bioactive Properties of Vermiwash

3.3.1 Antioxidant activity of vermiwash

The antioxidant activity of vermiwash, assessed using the DPPH free radical scavenging assay, exhibited a progressive increase over time. The highest scavenging capacity was observed on day 45, reaching 47.97 ± 0.21 %, indicating a significant enhancement in antioxidant potential as the vermiculture process advanced. This trend suggests that prolonged vermicomposting may contribute to the accumulation of bioactive compounds with free radical-neutralizing properties, further enriching the functional value of vermiwash (Huynh et al., 2021)

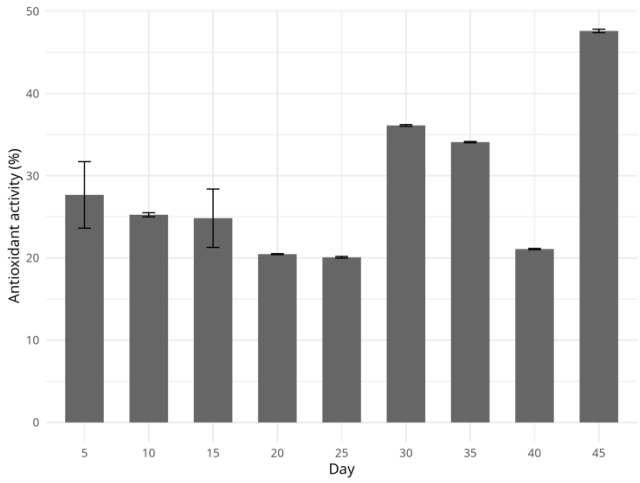

Figure 3: DPPH Free Radical Scavenging Activity of Vermiwash Over Time in vermicomposting model

3.3.2 Antibacterial activity of vermiwash

The antibacterial potential of vermiwash is closely associated with its bioactive components, particularly proteins and peptides secreted by earthworms. To enhance this biological effect, protein and peptide fractions were concentrated to increase their activity levels. This concentration step is essential for improving the efficacy of vermiwash as a natural antimicrobial agent. To obtain higher yields of bioactive proteins and peptides, various precipitating agents, including salts and organic solvents, were investigated. These agents facilitate the selective precipitation of proteinaceous compounds from vermiwash, thereby enriching fractions with potential antibacterial properties. The concentrated protein and peptide extracts are expected to exhibit enhanced inhibitory effects against plant pathogens, due to their ability to disrupt microbial cell membranes, inhibit enzyme systems, or interfere with pathogen metabolism.

The experimental results, summarized in Table 2, indicate that ammonium sulfate precipitation produced the most favorable outcomes among the tested precipitating agents. This method yielded the highest concentrations of bioactive compounds, with protein content reaching 1.03 ± 0.02 mg/mL and peptide content at 1.12 ± 0.04 mg/mL. Compared to the original vermiwash, these values represent a 4.29-fold increase in protein and a 2.55-fold increase in peptide concentration.

Table 2: Antibacterial zone diameter of vermiwash and precipitated fractions (mm)

Sample	Protein Content (mg/mL)	Peptide Content (mg/mL)	Antibacterial zone diameter of bacterial strains (mm)			
			E. coli ATCC 35218	*S. aureus* ATCC 29213	*Ralstonia solanacearum*	*Pseudomonas cichorii*
Negative control: PBS buffer	(-)	(-)	-	-	-	-
Positive control: chloramphenicol 0.2 ppm	(+)	(+)	32.2 ± 0.6	34.0 ± 1.6	30.0 ± 0.8	42.7 ± 0.5
Vermiwash	0.24 ± 0	0.44 ± 0.04	-	-	-	-
Acetone precipitate	0.96 ± 0.05	0.89 ± 0.01	-	-	-	-
96% Ethanol precipitate	0.87 ± 0.01	0.91 ± 0.02	-	-	-	-
$(NH_4)_2SO_4$ precipitate	1.03 ± 0.02	1.12 ± 0.04	-	-	-	-

Note: "(+)" indicates the presence of antibacterial components (used in the positive control); "(-)" indicates absence (used in the negative control); "–" indicates no inhibition zone was observed for that bacterial strain.

Results from the antibacterial assay showed that neither the crude vermiwash derived from Eudrilus eugeniae nor its concentrated protein fractions exhibited any measurable zones of inhibition against the tested pathogenic bacterial strains. This can be explained by the peptide components in vermiwash. Peptide concentration may increase, vermiwash did not contain sufficient amounts of potent antibacterial peptides such as lysenin, cytolytic eiseniapore, fetidine, and Lumbricin I. These peptides are located in the body cavity fluid of earthworms and

have a significant impact on bacteria that protect worms from pathogens (Gupta and Yadav, 2016). Coelomic fluid contains many proteins and peptides with high biological activity that can disrupt bacterial cell membranes, create pores, and inhibit bacterial growth (Cho et al., 1998). The process of collecting vermiwash typically involves washing or stimulating the surface of earthworms to release fluid. As a result, vermiwash contains little coelomic fluid and, therefore, lacks the potent antibacterial peptides found within the worm's body. Consequently, directly collected coelomic fluid contains a higher concentration of antibacterial peptides than vermiwash. Current research on vermiwash often focuses on its ability to support plant nutrition, stimulate the growth of beneficial microorganisms, and improve soil quality rather than its antibacterial properties.

4. Conclusion

This study aimed to develop an automated vermiwash production system to facilitate the broader adoption of vermiwash as an organic fertilizer in sustainable agricultural practices. Chemical analysis of the vermiwash revealed high levels of key nutrients, including organic carbon (12.56 %), total organic matter (21.00 %), total nitrogen (2.61 %), and total phosphorus (2.07 %), in addition to measurable amounts of peptides (0.64 ± 0.04 mg/mL) and proteins (0.44 ± 0.00 mg/mL). These compounds contribute not only to improved soil fertility but also to the proliferation of beneficial microbial communities, which support the ongoing decomposition of organic matter and the sustained release of nutrients essential for plant growth. According to Kaur et al. (2015), vermiwash also contains nitrogenous excretory products, plant growth-promoting hormones, and key enzymes that may further enhance plant resistance to environmental stress. In the present study, the DPPH free radical scavenging activity of vermiwash increased steadily over the 45-day vermicomposting period, ranging from 20.12 % to 47.97 %, indicating a growing antioxidant potential as the compost matured. Despite its nutritional and antioxidant richness, no antimicrobial activity was detected against the tested microorganisms, even after protein enrichment. This finding underscores that, although vermiwash was highly effective in enhancing plant nutrition, supporting soil microbiota, and improving soil quality, its direct antimicrobial activity was limited. The results highlight the importance of distinguishing between vermiwash—which primarily consists of surface secretions—and coelomic fluid extracts, which contain well-characterized antimicrobial peptides such as lysenin, eiseniapore, and fetidin. A clear understanding of the source and nature of earthworm-derived products is essential for accurately evaluating their biological functions and potential applications in agriculture

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References

Bhagat A., Singh S., Gudeta K., Bhardwaj S., Bhat S.A., 2022, Beneficial functions of vermiwash and vermicompost for sustainable agriculture, Environmental Management Technologies, 229-241.

Blakemore R.J., 2015, Eco-taxonomic profile of an iconic vermicomposter — the 'African Nightcrawler' earthworm, Eudrilus eugeniae, African Invertebrates, 56(3), 527-548.

Cho J.H., Park C.B., Yoon Y.G., Kim S.C., 1998, Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization, Biochimica et Biophysica Acta (BBA)-Molecular basis of disease, 1408(1), 67–76.

Demirkan E., Sevgi T., Aybey A., Kocakurt H., 2022, Investigation of some biological properties of coelomic fluid originating from the red worm Eisenia fetida, Karaelmas Fen ve Mühendislik Dergisi, 12(2), 183–193.

Gupta S., Yadav S., 2016, Immuno-defense strategy in earthworms: a review article, International Journal of Current Microbiology and Applied Sciences, 5, 1022–1035.

Huynh O.N., Bui C.-D.N., Tran T.T., 2021, Initial acquisition of vermiwash from vermicompost of Eudrilus eugeniae, Chemical Engineering Transactions, 89, 493-498.

Kaur M., Bhat R.A., 2024, Modulated vermibiotechnology for the management of solid waste, Zero Waste Management Technologies, 185–212.

Mehta C.M., Palni U., Franke-Whittle I.H., Sharma A.K., 2014, Compost: its role, mechanism and impact on reducing soil-borne plant diseases, Waste management, 34(3), 607–622.

Suthar S., 2010, Evidence of plant hormone like substances in vermiwash: an ecologically safe option of synthetic chemicals for sustainable farming, Ecological Engineering, 36(8), 1089-1092.