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Initial Acquisition of Vermiwash from Vermicompost of Eudrilus Eugeniae

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The uses of chemical fertilizers have caused soil degradation alarmingly, threatening the use of soil resource in the future. Among the efforts that were studied to replace chemical fertilizers, there's the vermiwash, rich nutrition, simple to produce, and environment-friendly solution of plants. This attempt deals with the initial acquisition of vermiwash from vermicomposting two organic materials, coconut fiber and rice husk, using the earthworm species *Eudrilus eugeniae*. The result showed that the content of amino acid (0.496 mg/100 g), protein (737.607 mg/100 g) in vermiwash from rice husk was higher compared with the vermiwash from coconut fiber (amino acid: 0.393 mg/100 g, protein: 537.257 mg/100 g). The biochemical analysis of both same indicated the presence of DPPH free radical scavenging activity, IC_{50} = 274.72 mg/mL (vermiwash from rice husk) and IC_{50} = 665.76 mg/mL (vermiwash from coconut fiber). This study also showed the effectiveness of vermiwash on the germination of *Vigna unguiculata subsp* seeds. Maximum germination percentage (100 %), seedlings shoot length, and root length was obtained when seeds were treated with 1 % vermiwash from rice husk in 60 min.

1. Introduction

According to Aristotle, "earthworms are the intestines of the soil". Indeed, they are responsible for the development of soil fertility for millions of years before the green revolution. Nowadays, earthworms still play the main role in the branch of farming by being used to develop new eco-friendly fertilizers, many products from earthworms such as ground earthworm juice, must kill earthworms, vermicompost is difficult to harvest because it has to be separated from earthworms, and used only once. Vermiwash can overcome the above disadvantages: used throughout the farming period, fertilized many times, no need to preserve and have no bad smell like juice from earthworms. Vermiwash is the excretory product of earthworms, along with some trace nutrients leached from soil organic molecules (Sahoo et al., 2015). This extract is enriched with enzymes, micronutrients and plant growth promoter i.e. cytokinin, indole acetic acid, gibbrellin along with mucus secretion of earthworms, humic acid etc (Nayak et al., 2019). Vermiwash also contains nitrogen in various forms like nitrates, ammonia, mucus, nitrogenous excretory substances and organic nitrogenous compounds, which are released by the dead earthworm's tissues (Meena et al., 2021). Vermiwash is produced by different methods. It can be collected as a byproduct liquid extract from the vermicompost unit. There are some evidences proving that qualities of vermiwash depend on the used organic bed producing vermicompost (Zarei et al., 2018). Vermiwash plays an important role in plant growth and development. Esteban (2019) reported that 100% vermiwash improved the root length, grain yeild per hectare of mungbean. The other study stated that vermiwash can also increase the soil organic matter and nutrient content which are easily available for plants, causing higher crop yields (Elbanna et al., 2021). There was a report that the nutrients and growth promoting substances present in the vermiwash from Eisenia foetida showed its potentiality in seed germination of Lady Finger (*Abelmoschus esculentus*) (Senthilmurugan et al., 2018).
Hence this present study focused to gain information on properties of vermiwash produced by *Eudrilus eugeniae* from vermicomposting two organic substrates (coconut fiber & rice husk) that are agricultural waste materials in Vietnam, which are abundant and easy to find. The purpose of use in the model is to increase aeration for the

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earthworm rearing process. The study encourages the development of worm culture models to produce vermiwash and evaluates the effectiveness of vermiwash application on plants to gradually replace chemical fertilizers that are currently being implemented.

2. Material and methods

2.1 Procurement experimental animal

The popular composting earthworm species *Eudrilus eugeniae* was selected for the present study (Figure 1). They were supplied by An Phu earthworm farm (Provincial Road 15, Phu Hoa Dong ward, Cu Chi district, Ho Chi Minh City, Vietnam). The grown earthworms were transported safely along with native soil and kept moist.

Figure 1: Morphology of Eudrilus eugeniae

Soil (Lavamix), cow dung (Lavamix), and organic substrates are the products manufactured by industrial standards. They are the basic bedding material in vermicomposting.

2.2 Fabrication of vermiwash collection unit and experimental set-up

The vermiwash processing was modified from the model of coconut leaf vermicompost production technology (Gopal et al., 2018). This model uses waste plastic bottles, which are easy to find and could raise earthworms and use vermiwash in households, farms, or gardens. In detail, recycling the 5 L water bottle as a vermireactor (Figure 2).

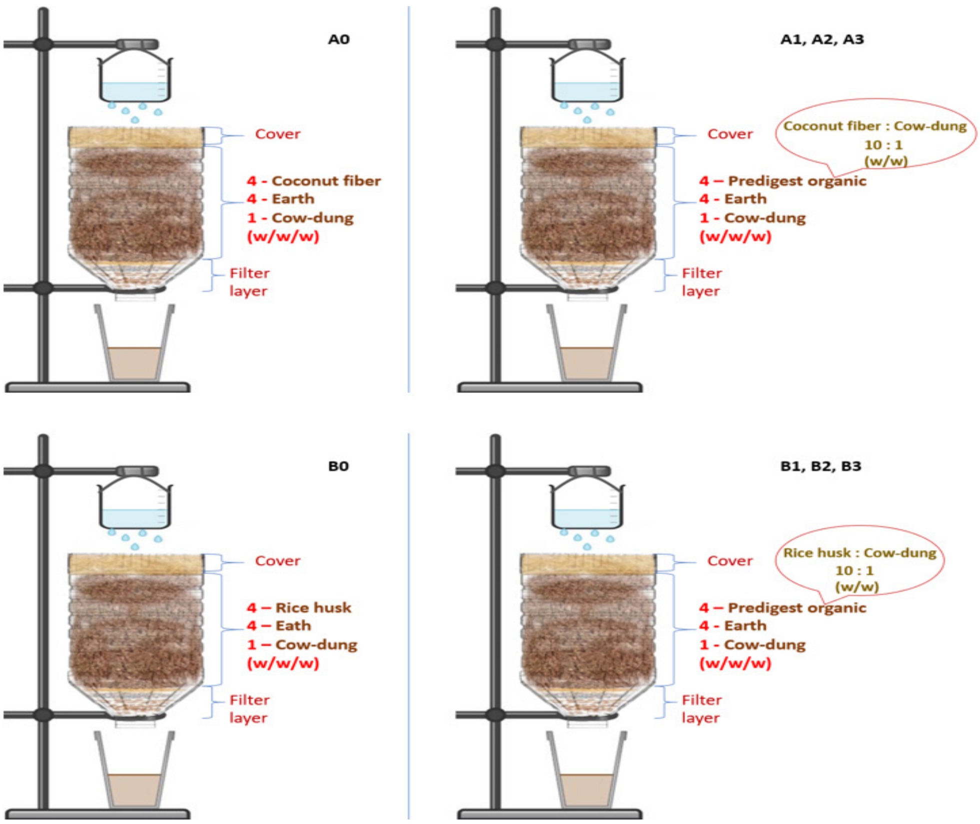

Figure 2: Experimental setup: (A0) control of coconut fiber; (A1, A2, A3) coconut fiber; (B0) control of rice husk; and (B1, B2, B3) rice husk.

The bore facing inwards the bottle was covered with a filter layer (contains broken brick, gravel, and sand) high up to 7.5 cm to filter the vermiwash liquid from the soil particles. The vermin was prepared: (a) organic substrates (coconut fiber/ rice husk + cow dung at ratio 10:1 w/w basic, composting before 15 day, (b) earth, (c) cow dung layered one over the other (a:b:c = 4:4:1 w/w). After all, those steps introduced *Eudrilus eugeniae* into the bottle separately for the attempt of experimentation.
The bottles A1, A2, A3 were for coconut fiber leaf litter. Excluding the A0 bottle (worked as the control), introducing 15 g of *Eudrilus eugeniae* into each bottle. Prepared the experimental bottles for rice husk leaf litter similarly following: B0 was the control. The B1, B2, B3 bottles were introduced 15 g of *Eudrilus eugeniae* into each. After the experimental setup, let 100 mL water sprinkle with the regular intervals in all bottles at room temperature everyday to keep the bed moist. The water percolating through the vermicompost model was collected amidst the filter layer, called vermiwash. The vermiwash was collected on the 5th, 10th, and 15th day.

2.3 Amino acid and protein content

The following biochemical parameters were studied for the present study. The total soluble protein was estimated using bicinchoninic acid followed by Smith et al. (1987). In the quantitative estimation of amino acid using Ninhydrin reagent, the absorbance of the Ruhemann's purple formed by the reaction at 570nm is measured.

2.4 Antioxidant Analysis

Scavenging of DPPH free radical is the basis of a common antioxidant assay. 0.5 mL of vermiwash was added in a test tube containing 0.5 mL of DPPH solution then incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Ascorbic acid equivalent antioxidant capacity (AEAC) was calculated by putting the value of absorbance in standard ascorbic acid curve and AEAC value was expressed in terms of mg/g. Where % S is the scavenging rate. Ac is the absorbance of the control. As is the absorbance of test extract solution. Ab is the absorbance of a blank sample. Then % of inhibition was plotted against concentration, and from the graph, IC_{50} was calculated using Eq(1).

$$\mathrm{S\%} = \left(\frac{\mathrm{Ac} - (\mathrm{As} - \mathrm{Ab})}{\mathrm{Ac}}\right) \times 100\ \% \qquad (1)$$

2.5 Germination Experiment

The germination assay was based on the experimental design established by Chithra et al., 2016. *Vigna unguiculata* subsp. seeds were purchased, then healthy seeds were selected. The seed (10 Nos) was soaked in sterile distilled water and treated with various concentrations (0.5 %, 1 %, 2 %) of two vermiwash liquids (from rice husk and coconut fiber) for various times intervals (30 min, 60 min, 120 min). The treatments can be labeled as Table 1. The treated seeds were placed in sterile petri dishes containing a thin layer of wet cotton. Seeds were treated with distilled water to be served as a control. The percentage of seed germination, root length, and shoot length were recorded after the 7th.

Table 1: Germination experiment

Vermiwash	Coconut fiber			Rice husk		
Concentration (%)	0.5	1	2	0.5	1	2
Treatment	T1	T2	T3	U1	U2	U3

3. Results and discussions

Vermiwash is a watery extract of vermicompost, the wash of earthworms present in the medium and has a honey brown color. This decoction contains the earthworm coelomic fluid, mucus, enzymes, microorganisms, plant nutrients, vitamins, and plant growth-promoting substances.

3.1 Amino acid content

The amino acid content in vermiwash from coconut fiber and its control were showed in Figure 3a. In the control, the amino acid value increased steadily due to the appearance of organisms that decomposed the organic matter and created bad smells. On the contrary, in vermiwash preparation earthworm joined early in the decomposition process and boosted amino acid production to gain the highest amino acid content (0.393 ± 0.086 mg/100 g) on the 5th as a result. In the production of vermiwash using rice husk, *Eudrilus eugeniae* showed their help to stabilize amino acid content that could be seen in Figure 3b. The amino acid level was comparatively more in all the experiments with rice husk, while the highest level was 0.496 ± 0.088 mg/100 g on the 15th d.

On the other hand, vermiwash produced from both coconut fiber and rice husk was brown and odorless during the experimental time. This might due to earthworm metabolism which accelerated the process of organic conversion. Therefore, it would be comparatively effective if the production of vermiwash was carried out with *Eudrilus eugeniae*.

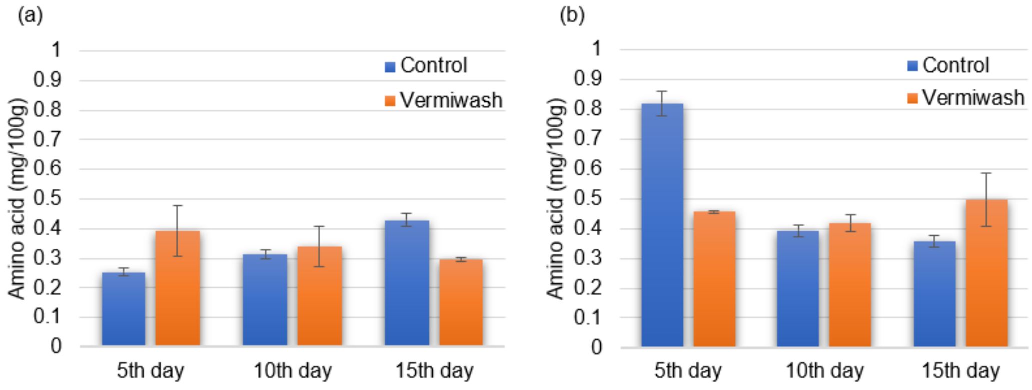

Figure 3: Amino acid content of vermiwash from (a) coconut fiber and (b) rice husk

3.2 Protein content

The protein level in both types of vermiwash was higher on the 5th and 10th compared to control samples (Figure 4a and 4b). This may indicate *Eudrilus eugeniae* helped to enrich compost (Khyade et al., 2016). That level in the control increased dramatically on the 15th up to 879.7 mg/100 g (Figure 4b) because anaerobic organisms had taken over the decomposition process and caused odor in bottles A0 and B0.

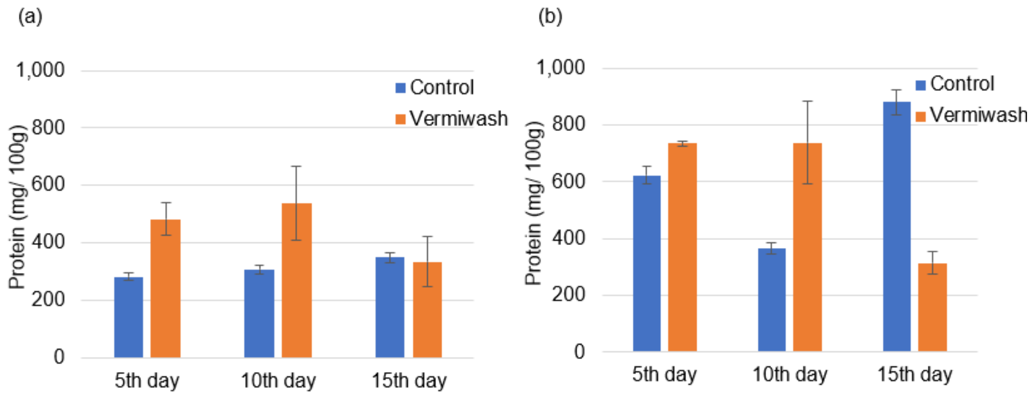

Figure 4: Protein content of vermiwash from (a) coconut fiber and (b) rice husk

It can be seen in Figure 4, the protein content of vermiwash produced with the use of rice husk was comparatively more in the 5th sample (734.0 ± 10.55 mg vs 482 ± 55.67 mg/100 g) and 15th sample (737.6 ± 146.48 mg vs 537.2 ± 128.02 mg/100 g). Consequently, the nutrient status of vermiwash produced in the experiment was found to be greater with rice husk. The organic degradable refuse of plants had been shown to provide a good source of nutrients to improve productivity. The amino acid and protein content from rice husk was higher than from coconut fiber suggested the effeciency of worm farming model with rice husk supplement is better than that of coconut fiber. Rice husk retains the moisture more than in coconut fiber, harbored compounds (chloride and ligin) that inhibit the activity of extracellular enzymes secreted by earthworms, reducing the ability to decompose substances and metabolism of earthworms.

3.3 Antioxidant assay

According to Sahoo et al. (2015) DPPH free radical scavenging activity was recorded in vermiwash (68.8 ± 4.58 %). While, in this study, (72.15 ± 3.72) % activity exhibited in coconut fiber sample whereas in rice husk sample it is (84.41±1.16) % (Figure 5). Therefore, IC_{50} was calculated as 274.72 mg/mL (vermiwash from rice husk) and 665.776 mg/mL (vermiwash from coconut fiber). The antioxidant activity of vermiwash from rice husk was 3 -times higher than from coconut fiber.

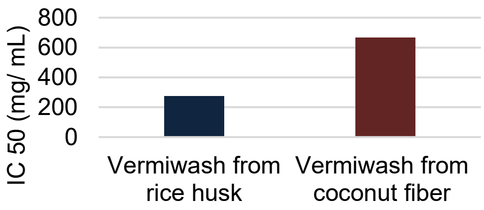

Figure 5: IC_{50} of Vermiwash on 15th

3.4 Germination experiment

Vermiwash was a potent organic fertilizer that plays a vital role in plant growth and development; contributed to increasing initiation of the root, root growth, plant development, promoting growth rate, and improving crop production (Sinha et al., 2010). The data from Figure 6 indicated both treatment T1 (coconut fiber vermiwash 0.5 %) and treatment U2 (rice husk vermiwash 1 %) obtained the maximum germination percentage (100 %) without depending on the time of treating; while minimum percentage (70 %) was observed in seeds with rice husk vermiwash 1 % in 30 min.

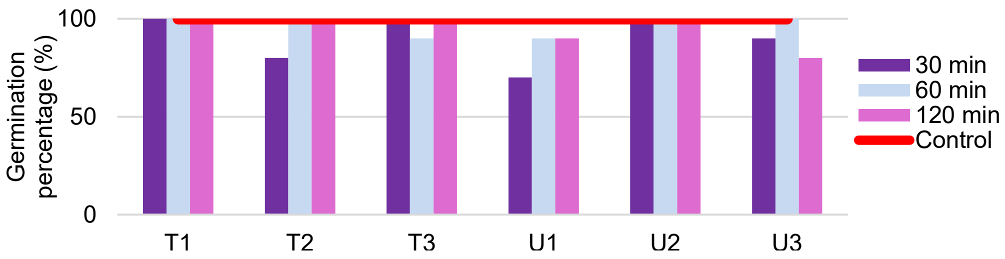

Figure 6: Germination percentage (%)

It is shown in Figure 7a that the seeds were treated with U2 (rice husk vermiwash 1 %) in 60 min had a maximum length for shoot 1.31 cm ± 0.48 cm. On the contrary, the minimum shoot length (0.69 cm ± 0.20 cm) was recorded in U3 (rice husk vermiwash 2 %) in 120 min. Figure 7b showed the maximum root length (2.24 cm ± 1.34 cm) was recorded in seeds with treatment U1 (rice husk vermiwash 0.5 %) in 30 min and minium length for root in seedling (0.51 cm ± 0.42 cm) were observed with U1 (rice husk vermiwash 0.5 %) in 120 min.

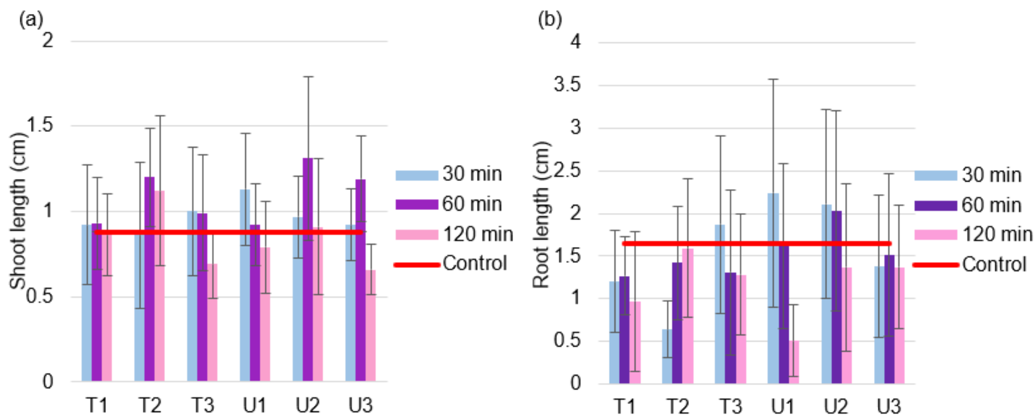

Figure 7: (a) Shoot length and (b) Root length

From the data of Figure 6 and 7, the maximum germination percentage (100%), maximum length of shoot (1.31 ± 0.48 cm) and maximum length of root (2.24 ± 1.34 cm) were all higher compared with respective values in control sample: 100% seed germination, 0.88 ± 0.18 cm shoots length and 1.64 ± 0.23 cm in root length. This could due to the presence of IAA in vermiwash that supported the growth of root hair (Gopa et al., 2012) or the presence of protease of vermiwash that played role in seed germination. Furthermore, the vermiwash amylase could help for the availability of a simple carbon source for enhancement of plant growth and productivity (Zambare et al., 2008).

4. Conclusion

The role of earthworms in vermiwash production depends on the nature of organic input. In this study, the quality parameters such as antimicrobial activity, antioxidant activity, amino acid, protein level were found to be higher in the experiment with vermiwash from rice husk. Also, the result showed the effectiveness of vermiwash on the germination of *Vigna unguiculata subsp* seeds. Thus, the result from the germination experiment suggested vermiwash could be an effective fertilizer at the right concentration and time treatment. Vermiwash is an environmentally friendly product by using grown earthworms in a simple and inexpensive model that can be implemented in different climatic conditions and replicated for all households and farms contributing to soil improvement and pollution reduction.

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