Evaluation of vermicompost quality: Efficacy in seed germination and reproduction of *Eudrilus eugeniae*

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ABSTRACT

This study aimed to evaluate the quality of vermicompost produced through vermicomposting, using the germination index of two seed species and the reproduction of earthworms as indicators to ensure the absence of toxicity and promote the safe use of vermicompost. The experiment was conducted in 20-liter vermireactors, using 25 adult earthworms of the species *Eudrilus eugeniae* per reactor. Three treatments were applied, varying the proportions of fruit, vegetable, and grass clippings residues. The phytotoxicity of the vermicompost was assessed through the Germination Index (GI%) of lettuce and cucumber seeds. The GI was calculated by comparing the germination and root elongation of seeds treated with vermicompost extracts to the control. Earthworm reproduction was evaluated at the end of the experiment by comparing the initial and final number of earthworms and cocoons to calculate the reproduction rate. The data were analyzed by Analysis of Variance (ANOVA), followed by Tukey's test to identify significant differences between treatments. The results indicated that Treatment T3, which combined different proportions of residues, was the most effective in promoting both the growth and reproduction of earthworms and the germination of seeds, suggesting that the combination of organic residues is crucial for producing high-quality, non-toxic vermicompost. These findings highlight the importance of an integrated approach in treatment formulation to optimize sustainable vermicompost production.

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INTRODUCTION

According to a FAO study, approximately one-third of the food intended for human consumption is wasted globally, amounting to around 1.3 billion tons, raising urgent concerns about the sustainability of food practices (FAO, 2022). This issue becomes even more critical considering that in 2021, between 702 and 828 million people faced food insecurity (FAO, 2022). In Brazil, Amarante et al. (2022) highlight that in 2020, 79 million tons of solid waste were generated, of which 45.3% were organic waste, including food waste.

In response to this wasteful scenario, the United Nations set targets within the Sustainable Development Goals (SDGs), with target 12.3 focused on halving global food waste by 2030 (UN, 2015). This emphasis underscores the need to rethink food practices and waste management on a global scale.

Food waste, divided into animal and plant origins, represents a significant challenge. Dang et al. (2023) emphasize the variety of products derived from the food industry, while Galanakis (2022) points to the diversity of waste from plant sources. This wide range of waste highlights the complexity of adequately managing these materials.

As cities face increasing challenges in generating fruit and vegetable waste, the proper approach to disposing of these materials becomes crucial, as noted by Bhardwaj et al. (2022) and Jun et al. (2022). One of the primary sources of such waste is supermarkets, whose management practices still lack effective collaboration and stringent legislation, as evidenced by Moraes et al. (2022).

Even before the COVID-19 pandemic, the waste of fruits and vegetables had already reached significant levels, with 5.3 million tons associated with fruits and 5.6 million tons with vegetables (ABRAS, 2019). In 2020, supermarkets in Brazil contributed a significant percentage of this waste, highlighting the need for effective actions in this sector (Gustavsson et al., 2011; ABRAS, 2021; Moraes et al., 2022). In light of this wasteful context, the implementation of sustainable waste treatment technologies becomes essential.

Vermicomposting, a low-cost and highly efficient technique, stands out as an environmentally friendly approach. This process involves the degradation of waste by earthworms and microorganisms in a controlled environment, resulting in a high-quality final product known as vermicompost (Lim et al., 2011).

During the maturation phase of vermicomposting, microorganisms such as bacteria, fungi, and actinomycetes play a predominant role, continuing the transformation of the organic compounds digested by earthworms (Lim et al., 2011). As a result, the vermicompost produced contains high levels of organic matter, nutrients, and plant growth-promoting substances produced by the enteric bacteria of the earthworms, making it an excellent fertilizer (Ratnasari et al., 2023).

One of the most commonly used species in the vermicomposting process is the earthworm *Eudrilus eugeniae*, which can reach up to 35 cm in length and is geographically distributed across specific regions of the continent, although it is now found in various parts of the world. This species exhibits distinct anatomical and behavioral characteristics that differentiate it from other species. Its reproductive system is efficient, with specialized organs for producing and depositing cocoons containing fertilized eggs. This process is influenced by environmental factors such as temperature and soil moisture, which play a crucial role in its reproduction and population success (Nattudurai et al., 2014).

Additionally, *Eudrilus eugeniae* is widely used in the biostabilization of organic materials, as highlighted by Santos et al. (2021), and demonstrates a remarkable ability to digest and assimilate organic matter, as evidenced by Khaldoon et al. (2022). Native to the African continent, this species is commonly found in the soils of Nigeria and neighboring countries. Its adaptability to captive environments is particularly notable in tropical regions (Malheiros, Maia, and Campos, 2019).

Vermicompost, a nutrient-rich product, emerges as an invaluable alternative. However, its potential toxicity represents a significant concern, especially due to the possibility of contamination by harmful substances during the process. To ensure the safety of vermicompost intended for food cultivation, phytotoxicity analysis becomes essential, utilizing germination indices and bioassays (Bhat et al., 2017). These tests not only determine the maturity of the vermicompost but also indicate its impact on cultivated plants (Mendes et al., 2021).

Another crucial aspect is the consideration of earthworm reproduction during the vermicomposting process. This biomass, intrinsically related to earthworm reproduction, not only indicates the quality of the vermicomposting system but also contributes to the efficient degradation of waste. Additionally, it plays an important role in pathogen suppression, significantly contributing to the production of a safer, more stable, sanitized, and non-toxic vermicompost (Blouin et al., 2019; Mendes et al., 2021; Oyege et al., 2023). In light of this, the objective of this study is to evaluate the quality of vermicompost produced through vermicomposting, using the germination index of three seed species and earthworm reproduction as indicators, aiming to ensure the absence of toxicity and promote the safe use of this vermicompost.

MATERIALS AND METHODS

VERMICOMPOSTING PROCESS

The residues used in the vermicomposting process were derived from fruits and vegetables donated and collected from a produce market located in the city of Pelotas, Brazil. Fresh green grass was used as a structuring material. The experiment was conducted in experimental units consisting of three treatments, following a 3x2 factorial design, with each treatment replicated twice.

The experiment was carried out in vermireactors, each with a working capacity of 20 liters. In each vermireactor, 25 adult clitellated earthworms of the species *Eudrilus eugeniae*, sourced from the breeding facility of an ecotoxicology laboratory at the Federal University of Pelotas, were introduced. The experimental setup and the proportions of residues and structuring material are detailed in Tables 1 and 2.

Table 1: Vermicomposting Experiment with Three Treatments

Treatment	Number of Earthworms	Species of Earthworm	vermireactor (L)
1	25	*Eudrillus eugenia*	20
2	25	*Eudrillus eugenia*	20
3	25	*Eudrillus eugenia*	20

Table 2: Proportions of Residues and Structuring Material

Treatment	Fruit (%)	Vegetable (%)	Grass Clippings (%)
1	80	-	20
2	-	20	80
3	40	40	20

EARTHWORM REPRODUCTION

After the vermicomposting process, the earthworms were carefully removed manually from each reactor. During this stage, a precise count of the number of earthworms and cocoons in each reactor at the end of the experiment was conducted. A comparison between the initial and final number of earthworms was performed, and the reproduction rate (%) of the earthworms was then calculated using the following equation (2):

$$\text{Reproduction Rate} = (\text{final number of earthworms} / \text{initial number of earthworms}) \times 100 \qquad (2)$$

STATISTICAL ANALYSIS

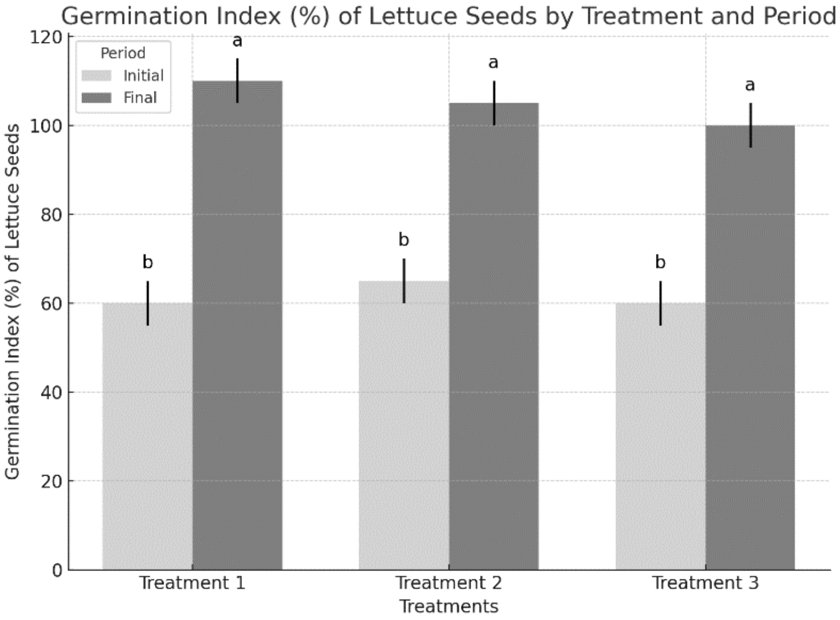
The data were analyzed using Analysis of Variance (ANOVA) to determine significant differences between treatments, with a significance level of 5% ($p < 0.05$). Tukey's multiple comparison test was applied to identify specific differences between treatments, indicated by distinct letters in the graphs.

RESULTS AND DISCUSSION

GERMINATION INDEX

Figures 1 and 2 present the Germination Index (GI) results for lettuce and cucumber seeds, respectively. Figure 3 shows the GI% over time for both lettuce and cucumber seeds. In Treatment 1, the GI of lettuce seeds was significantly higher in the final period, reaching about 110%, compared to the initial period, which was around 60%. For Treatment 2, a similar pattern was observed, with the GI in the final period being significantly higher, reaching approximately 105%, compared to the initial period, which was around 55%. In Treatment 3, the GI in the final period was about 110%, while in the initial period, it was approximately 55%. As in the previous treatments, there was a significant difference between the periods.

Figure 1: Comparison of Lettuce Seed GI (%) Between Treatments Throughout the Experimental Period



Regarding Figure 2, in Treatment 1, a significantly higher GI of cucumber seeds is observed in the final period, reaching approximately 120%, compared to the initial period, which recorded around 60%. In Treatment 2, the GI also significantly increased from the initial to the final period. Initially, the GI was around 55%, while in the final period, it rose to approximately 110%. Treatment 3 follows the same pattern as the other two. In the initial period, the GI was about 55%, and in the final period, it increased to approximately 115%.

Figure 2: Comparison of Cucumber Seed GI (%) Between Treatments Throughout the Experimental Period

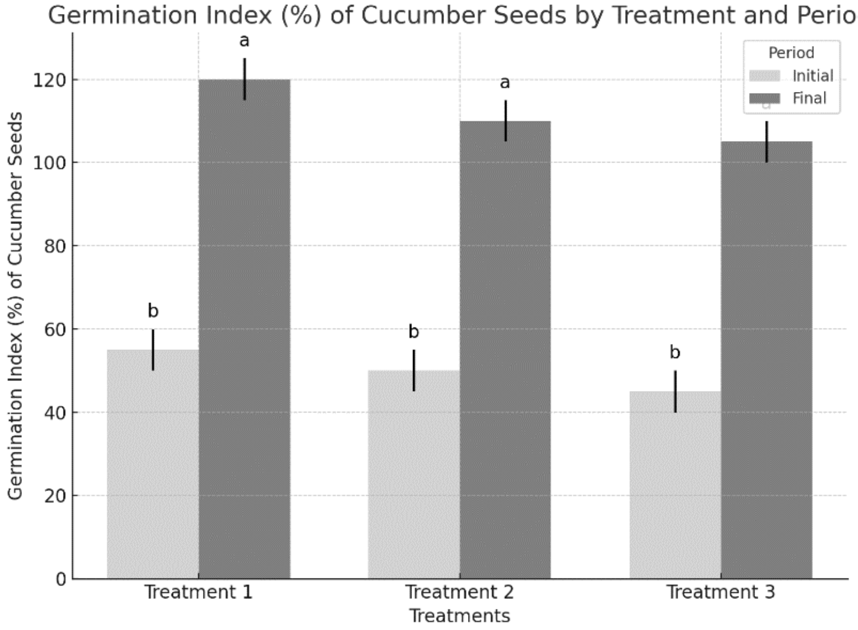


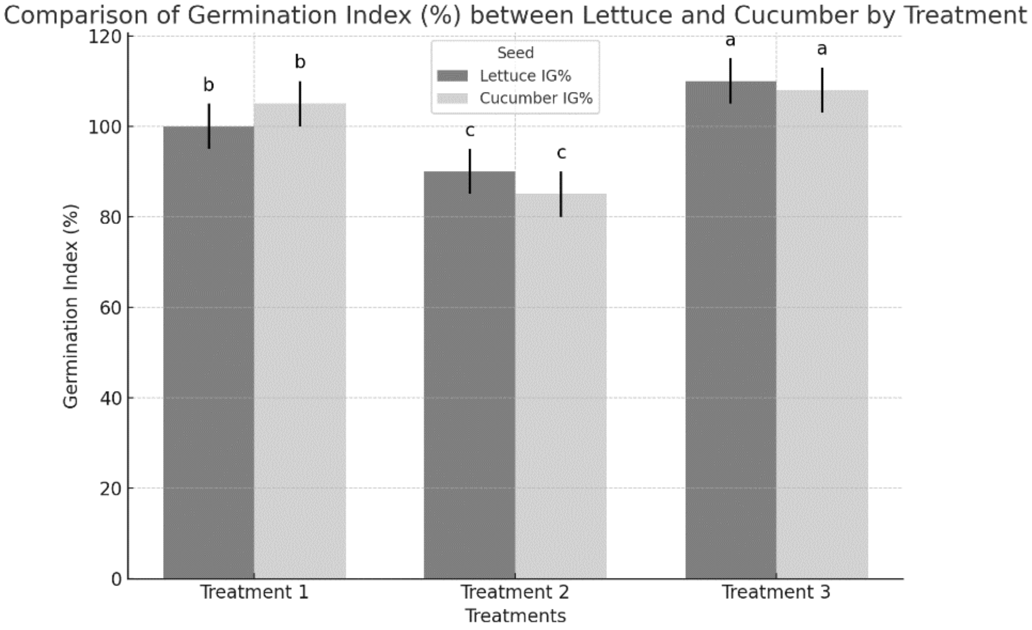
Figure 3 compares the Germination Index (GI) between lettuce and cucumber seeds across three different treatments. The figure highlights the differences in GI between the two seed species within each treatment, indicating statistical significance.

In Treatment 1, both lettuce and cucumber seeds showed similar GIs, around 100%, with no statistically significant differences.

In Treatment 2, there was a significant difference between the GI of lettuce and cucumber seeds. The GI of lettuce seeds was lower, about 90%, while the GI of cucumber seeds was close to 100%. In Treatment 3, the GI was again high for both seeds, with values close to 115% for lettuce and cucumber. There was no statistically significant difference between the two seeds in this treatment.

These results suggest that while Treatments 1 and 3 promoted high and similar levels of germination in both seeds, Treatment 2 was less effective for lettuce seeds, resulting in a significant difference in GI compared to cucumber seeds.

Figure 3: Comparison of Germination Index (GI%) Between Lettuce and Cucumber Seeds by Treatment



The Germination Index (GI) of seeds is a commonly used biological indicator to determine the phytotoxicity and maturity of compost (Luo et al., 2018). The latest Chinese agricultural industry standard, titled "Technical Specifications for the Composting of Livestock and Poultry Manure," released in 2019, requires the GI of mature compost to be at least 70%. This standard aligns with the recently revised Chinese standard for organic fertilizers (NY525–2021), which also requires a GI ≥ 70% (Yang et al., 2021).

The results indicate that the treatments were effective in improving the GI of both lettuce and cucumber, with specific variations depending on the treatment and species. Treatment 2 showed a notable difference between the two species, suggesting that specific factors in this treatment may have been more suitable for cucumber than for lettuce. These factors may include variations in nutrient availability, moisture, or other soil parameters that directly influence the germination process.

The absence of significant differences in GI between lettuce and cucumber in Treatments 1 and 3 suggests that the conditions in these treatments were suitable for both species, resulting in good germination performance. This implies that these treatments may have broader potential for application across different crops, being less dependent on the specific needs of a single species.

Based on the observed results, it is possible to relate the differences in the Germination Index (GI) to the composition of the applied treatments. According to Alromian (2020), nitrogen (N) is an essential component for lettuce plants, integrating into proteins, chloroplasts, and phospholipids, and playing a fundamental role in plant growth and development. In this context, Treatments 1 and 3, which contain fruit residues, may provide more readily assimilable forms of nitrogen, creating more favorable conditions for lettuce seed germination. This superior nitrogen availability may explain the potentially higher germination rate observed in these treatments compared to Treatment 2, which is composed exclusively of vegetable residues.

The lower availability of nitrogen in readily assimilable forms in Treatment 2 may have limited the initial development of lettuce seeds, resulting in a lower GI. Furthermore, according to Domínguez et al. (2021), the fibrous structure of vegetable residues, such as those used in Treatment 2, tends to cause slower decomposition and gradual nutrient release. This slower decomposition process may have resulted in a lower germination rate for cucumber seeds in this treatment compared to the treatments containing fruit residues, where faster decomposition may have made nutrients available more immediately and efficiently to the seeds. This difference in nutrient release may have contributed to the inferior performance observed in the GI of cucumber seeds in Treatment 2 compared to the other treatments.

These interpretations reinforce the importance of considering the composition of organic residues used in treatments, as they directly influence the availability of essential nutrients for seeds, affecting germination rates. Therefore, the strategic use of organic residues that offer faster decomposition and nutrient release, such as fruit residues, may be more advantageous in promoting effective germination in different types of seeds (Mendes et al., 2021).

EARTHWORM REPRODUCTION

Figure 4 presents the number of earthworms and cocoons per treatment, categorized into cocoons, juveniles, and adult earthworms, with treatments indicated as T1, T2, and T3. The error bars represent the variability within each group. For the "Cocoons" category, treatments T2 and T3 showed similar numbers, while treatment T1 had a significantly lower number ($p < 0.05$). Treatment T3 stood out with the highest number of cocoons, being significantly superior to T1 ($p < 0.05$).

In the "Juveniles" category, treatment T3 again had the highest number, being significantly superior to treatments T1 and T2 ($p < 0.05$). T1 and T2, on the other hand, did not show significant differences between them, both with lower numbers of juveniles ($p > 0.05$). Regarding adult earthworms, treatment T3 presented the highest number, with a significant difference compared to treatments T1 and T2 ($p < 0.05$). Although T2 was superior to T1, it still had a significantly lower number of adult earthworms compared to T3 ($p < 0.05$). Treatment T1 had the lowest number of adult earthworms, significantly lower than T2 and T3 ($p < 0.05$).

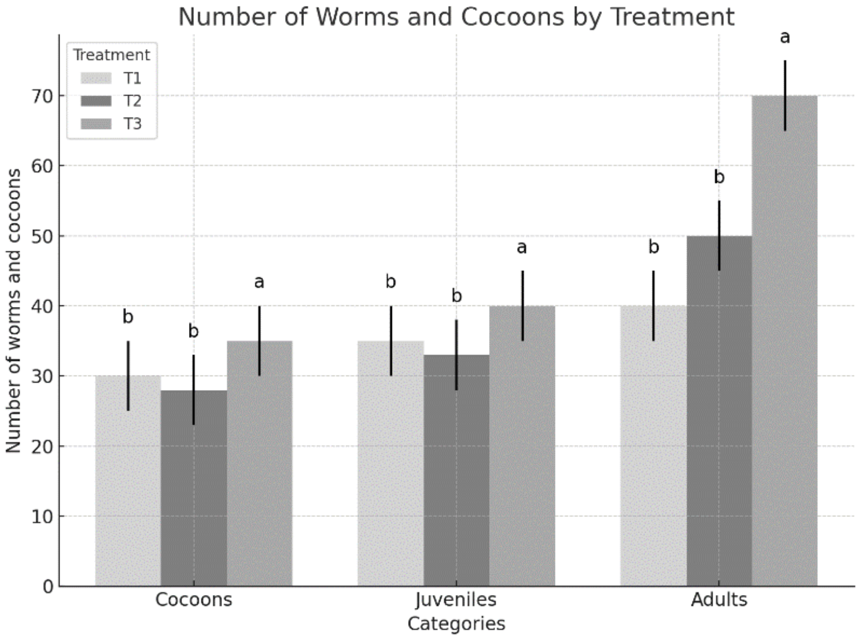
As observed by Nweke et al. (2020) in their study with the earthworm *Eudrilus eugeniae*, the results differed from those observed in treatment T2. In the study, the use of a diet composed of vegetable residues proved to be the most effective in promoting earthworm reproduction, evidenced by the increase in the number of survivors, cocoons produced, and biomass weight at the end of the experiment. However, Suthar (2009) found that a combination of residues promotes both growth and reproduction rates of earthworms, provided they are mixed with bulking materials in appropriate proportions. This possibly explains the satisfactory results observed in treatment T3.

These observations suggest that treatment T3 was the most effective in promoting earthworm reproduction and growth, resulting in higher numbers of cocoons, juveniles, and adult earthworms. Treatment T1, on the other hand, was the least effective, particularly in the production of adult earthworms, which may indicate lower nutrient availability or suboptimal conditions for earthworm growth. Treatment T2 had an intermediate performance, better than T1 but still inferior to T3 in all analyzed categories.

The microbial community plays a fundamental role in the degradation and transformation of organic matter during composting, directly affecting the production of water-soluble substances and influencing seed germination (Wang et al., 2021). The quality of the resulting compost can also impact earthworm reproduction, as they depend on an adequate environment and balanced nutrients to reproduce effectively. This effect was evident in the results obtained in the phytotoxicity analysis and earthworm reproduction in Treatment T3.

These differences may be related to the composition of the materials used in each treatment, directly affecting the reproduction and development of earthworms. Treatment T3 may have offered a more nutritious environment or more favorable conditions for reproduction, resulting in greater reproductive success and a higher number of adult earthworms. These results are crucial for optimizing earthworm cultivation conditions, depending on the experiment's objective, whether to increase the production of cocoons, juveniles, or adults.

Figure 4: Distribution of Earthworms and Cocoons by Categories and Treatments



CONCLUSION

This study highlighted the importance of the composition of organic residues for seed germination and earthworm reproduction. Treatments that combine different organic residues have proven to be more effective in creating favorable conditions for the release of essential nutrients.

The variability in results underscores the need to carefully select the materials used in vermicomposting to maximize the efficiency of the process. An integrated approach that considers the needs of both plants and earthworms is essential for optimizing composting and promoting sustainability in agricultural production.

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