# Evaluation of Substrates for Optimizing Vermicomposting Products

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The high global market value of fresh mushrooms implies high importance of spent mushroom substrate (SMS) as a by-product that poses environmental challenges, if not properly treated. Reported studies demonstrate the positive effects of SMS on earthworm growth and reproduction, particularly when combined with other substrates such as cow dung (CD). This study utilized SMS and CD as substrates in varying ratios. A total of 375 young non-clitellated *Eisenia fetida* were randomly assigned to plastic containers, ensuring similar average individual worm weight, and maintained at 50% to 60% moisture. Weekly observations were made on earthworm average weight, cocoon production, and hatchling count. The growth and reproduction of earthworms differed significantly among treatments, with Treatment 3 (T3) (50:50 SMS and CD) showing the highest growth and Treatment 5 (T5) (0:100 SMS and CD) the lowest. Changes in pH, EC, total Kjeldahl nitrogen (TKN), total organic carbon (TOC), C/N ratio, and total available phosphorus (TAP) were observed over the experimental period, indicating dynamic nutrient dynamics within the substrates. The exchangeable potassium decreased with increasing proportions of CD, while the exchangeable sodium content showed variation across treatments. The observed variations can be attributed to differences in initial substrate composition and microbial activity during vermicomposting.

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## INTRODUCTION

The agricultural industry is one of the main sectors generating the largest quantities of agricultural solid wastes, such as cow dung, which may be allowed to accumulate indiscriminately and constitute a nuisance to global health and threat to food security, or be used as raw materials for the bio-economy (Bracco *et al*. 2018). The benefits of recycling of agricultural solid wastes include reduction of greenhouse gas emissions and use as fossil fuel as well as contributing greatly to the development of new green markets, creation of jobs, production of bio-energy, and bio-conversion of agricultural solid wastes to animal feed (McCormick 2013; Scarlat *et al*. 2015).

Spent mushroom substrate (SMS) represents the organic soil-like waste or by-product of the mushroom industry, which mostly consists of a blend of lignocellulosic material (*e.g.*, wheat, sawdust, rice straw, and corncobs), organic materials (*e.g.*, proteins, carbohydrates), residual mushroom mycelia, and nutrients (*e.g.*, nitrogen, phosphorus, and potassium) (Lou *et al*. 2017; Meng *et al*. 2018). The SMS also contains heavy metal compounds (*e.g.*, zinc, cadmium, and copper) (Gong *et al*. 2019) that may pollute the environment if no proper disposal method is implemented. The SMS contains a high content of nutrient (*e.g.*, protein, polysaccharide) and hydrocarbon (hydrogen and carbon), thus showing potential uses in many applications such as soil bioremediation and promoting root growth (Koo *et al*. 2011; García-Delgado *et al*. 2015; Liu *et al*. 2019).

In addition to soil bioremediation and promoting root growth, Abu Bakar *et al*. (2014) noted that spent mushroom compost (SMC) brings the highest growth and reproduction in earthworms when used solely as a vermicomposting substrate compared to other possible substrates such as cow dung, vegetable waste, and their combination. Similarly, studies (Gong *et al*. 2019; Ruangjanda *et al*. 2022) reported a positive relationship between worm growth and reproduction with increasing rate of SMC when it is combined with other substrates. Although some findings concluded that sole usage of SMC showed reduced worm biomass than SMC merged with other substrates (Bakar *et al*. 2011), they also suggested that higher portion of the substrate being SMC have a positive effect on worm biomass. Evidence implies that SMC can be a major feed for earthworms. However, there is need to explore the feed additives to enhance its suitability.

An earthworm has the ability of accelerating waste conversion 2 to 5 times (Atiyeh *et al.* 2000). Vermicomposting can offer several advantages beyond just the speed of conversion, which can make it appealing even when space is not a primary concern. The compost produced through vermicomposting (vermicast) is often richer in nutrients and beneficial microbes, and it has a better texture compared to regular compost. This can be advantageous for improving soil health and plant growth. Vermicomposting tends to produce less odor compared to conventional composting, which can be important in urban or indoor settings (Thakur *et al.* 2021).

Vermicomposting requires less turning and maintenance compared to conventional composting, which involves regular aeration and turning. Vermicomposting can be more suitable in environments where maintaining the high temperatures needed for traditional composting is difficult. Vermicomposting is particularly advantageous in urban settings where space is limited and odor control is essential. Those who are interested in producing high-quality compost quickly might prefer vermicomposting regardless of space constraints. The choice between vermicomposting and traditional composting depends on specific needs, goals, and available resources (Tamanreet 2020).

Different studies (Adi and Noor 2009; Bakar *et al*. 2011; Abu Bakar *et al*. 2014; Vodounnou *et al.* 2016; Gong *et al*. 2019; Ruangjanda *et al*. 2022) revealed varying results regarding the usage of different substrate on the growth and reproduction of earthworm. Addition of SMC and cow dung (CD) has been found to increase important enzymatic activities including cellulase in vermicomposting (Gong *et al*. 2019). Therefore, this study tries to investigate the effects different rates of SMC and CD on the growth and reproduction of *Eisenia fetida.*

## EXPERIMENTAL

### *Eisenia fetida*, SMS, and CD

The experimental study on earthworms was performed on 375 young non-clitellated *Eisenia fetida* (5 treatments and 3 replicates, 25 worms in each experimental group). Worms were obtained from stock culture maintained at Hawassa University College of Agriculture, and the initial average weight of individual worm was

126.2±8.25mg. Cotton seed meal based spent mushroom substrate was purchased from local commercial mushroom producers. Additionally, fresh cow dung was collected from local cattle farms. The SMS was first sun dried for 2 to 3 days to prevent the infestation of insects. The CD was also air dried for 10 to 15 days until moisture level reached approximately 75%. The treatment groups consisted of different levels of SMS and CD. The percentages of SMS and CD are stated in Table 1.

**Table 1.** Percentage Combination of Substrates Across Treatments

| Treatment | SMS (%) | CD (%) |
|---|---|---|
| Treatment 1 (T1) | 100 | 0 |
| Treatment 2 (T2) | 75 | 25 |
| Treatment 3 (T3) | 50 | 50 |
| Treatment 4 (T4) | 25 | 75 |
| Treatment 5 (T5) | 0 | 100 |

### Experimental Procedures and Observations

Before the vermicomposting process began the samples were analyzed for their physico-chemical properties. Moisture, total organic carbon (TOC), nitrogen (N), carbon nitrogen ratio (C/N), and pH were analyzed.

The earthworms were randomly assigned to 15 plastic containers of 40 cm diameter and 3 L volume, assuring that worms in each plastic container had an average similar individual worm weight. The moisture content was maintained to 50% to 60% throughout the study period by regularly sprinkling of adequate quantity of distilled water. Earthworm average weight, cocoons, and hatchlings were weighed, sorted, and counted manually at the interval of a week. At the end of the experiment (5 weeks), the vermicompost was sieved, air dried, and physico-chemical parameters were analyzed.

### Data Management and Analysis

The data collected during the study was analyzed using SAS (Statistical Analysis System) statistical software. Effects of different rates of SMC and CD on growth, reproduction, nutrient content of *Eisenia fetida* and the quality of vermicompost was subjected to an analysis of variance (ANOVA) test. A *post hoc* analysis Tukey’s test was used to compare between means.

## RESULTS AND DISCUSSION

### Earthworm Growth

Earthworm growth in various mixtures of SMS and CD was significantly different ($P < 0.05$). Starting from week 1, growth T3 was significantly ($P < 0.05$) higher in growth and T5 was significantly ($P < 0.05$) lowest in growth. There was no significant difference in growth among T1, T2, T3, and T4. Further, no significant difference was shown between T4 and T5. This trend continued up to the end of the experiment (5 weeks) as shown in Fig. 1. The final average body of T3 was 763 mg and the lowest (T5) was 644 mg.

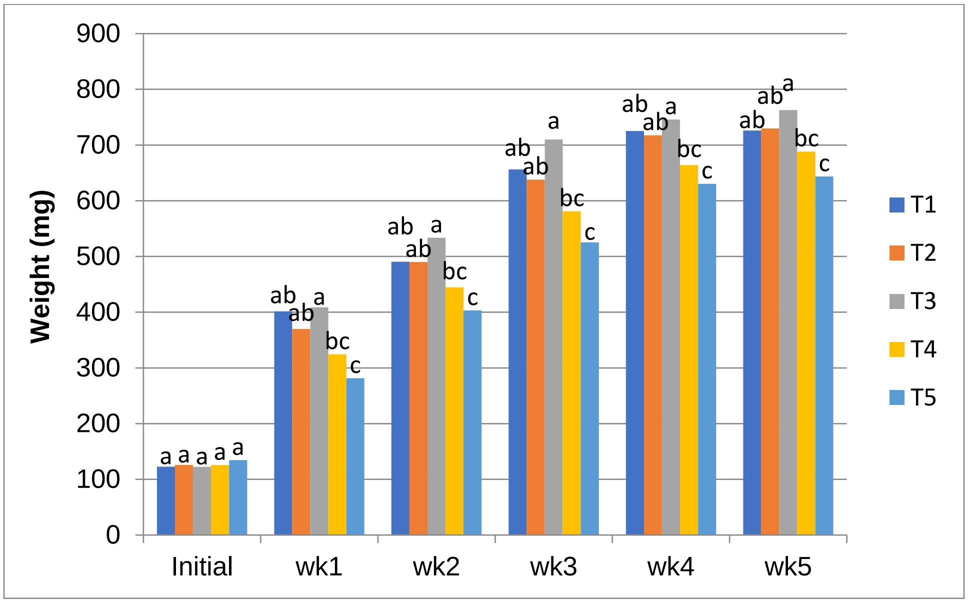

**Fig. 1.** Growth patterns of earthworms

## Cocoon Production

The number of cocoons in different mixtures of SMS and CD was significantly different ($P < 0.05$). Cocoon formation started in week-1 for T3 and T2 and in week-3 for the rest of the group. The maximum number of cocoons were observed in T3 (84.67 ± 14.33) on the 5th week and minimum in T5 (7.67 ± 33) on the 5th week (Fig. 2). The quality of the feed mixture determines the growth of earthworms and onset as well as the rate of cocoon formation.

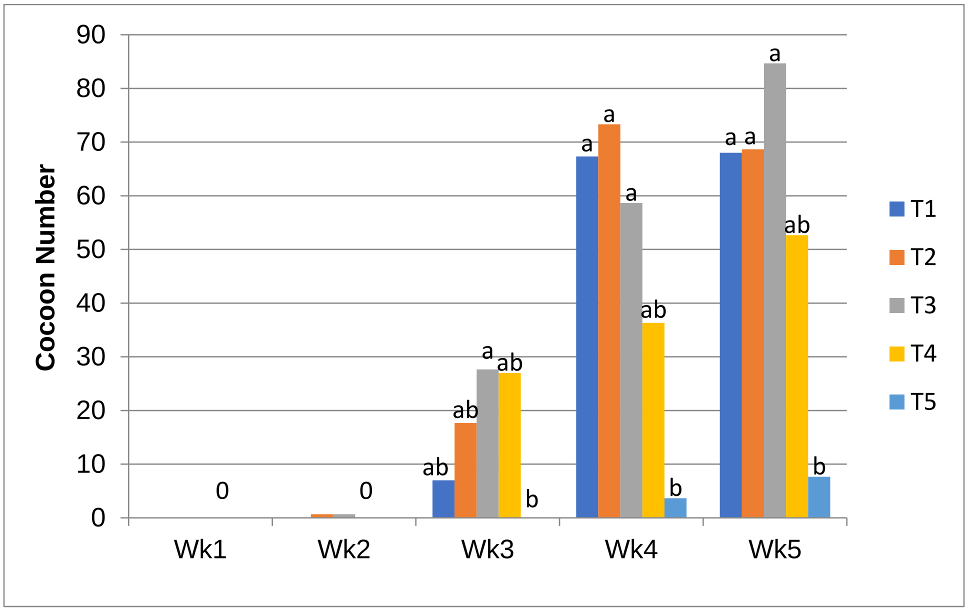

**Fig. 2.** Patterns of cocoon production during experimental periods

## Hatchling Formation

Hatchling formation was significantly different ($P < 0.05$). Hatchlings were observed for the first time in week-4 in all treatments except in T5. The maximum number of hatchlings were observed in T3 (140 ± 35) on 5th week and minimum in T5 (2 ± 2) on the 5th week of experiment (Fig. 3). The number of hatchlings was smaller in feed mixture T5 as compared to other treatment groups due to low production of cocoons.

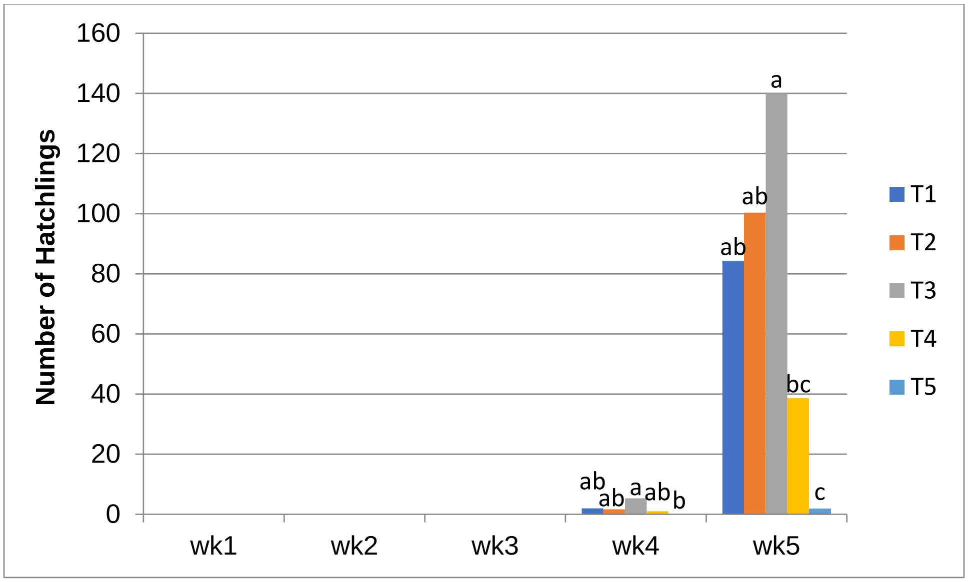

**Fig. 3.** Patterns of hatchling formations during the experimental periods

## Physico-chemical Characteristics of the Mixtures

Table 2 presents the comparison of two treatments, SMS, and CD, based on various physicochemical parameters. The SMS treatment exhibited a pH of 5.85, while the CD treatment had a higher pH of 7.44. The EC of the SMS treatment was measured at 3.02 mS/m, whereas the CD treatment had a higher EC of 6.25 mS/m. Nitrogen content (N %) was lower in the SMS treatment at 1.54% compared to the CD treatment, which had a higher N% of 2.52. Total organic carbon (TOC%) was higher in the SMS treatment at 55.0 compared to the CD treatment with TOC% of 43.9. The carbon to nitrogen (C/N) ratio was higher in the SMS treatment at 35.7 compared to the CD treatment with a C/N ratio of 17.4. Additionally, the total available phosphorus (TAP) content was lower in the SMS treatment at 2.66 g/kg, whereas the CD treatment had a higher TAP of 6.54 g/kg. The SMS showed higher exchangeable potassium (1.58 cmol/kg) compared to CD (1.15 cmol/kg). Conversely, CD exhibited slightly higher exchangeable sodium (1.58 cmol/kg) compared to SMS (1.60 cmol/kg).

The results indicate significant differences between the SMS and CD treatments in terms of their physicochemical properties. The pH of the SMS treatment was slightly acidic, whereas the CD treatment exhibited slightly alkaline conditions. These differences in pH can influence nutrient availability and microbial activity in the substrate, ultimately affecting plant growth and development (Pietri and Brookes 2009).

The EC values reflect the concentration of dissolved salts in the substrate, with the SMS treatment showing lower conductivity compared to the CD treatment. This difference may be attributed to variations in the organic matter content and mineral composition between the substrates. The lower cocoon production in T5 is due to higher salinity as studied by Jun *et al.* (2012). Nitrogen content was lower in the SMS treatment, indicating a potential limitation of nitrogen availability for plant uptake compared to the CD treatment. However, the SMS treatment had a higher TOC%, suggesting a richer source of organic carbon that can enhance soil fertility and microbial activity (Deenik *et al*. 2010).

The higher C/N ratio in the SMS treatment indicates a greater proportion of carbon relative to nitrogen, which could influence decomposition rates and nutrient cycling. Hou *et al.* (2008) found that the optimum C/N ratio for the highest growth of earthworms is 20. This could explain the reason for the highest growth of earthworms in T3. Additionally, the lower TAP content in the SMS treatment may necessitate supplemental phosphorus fertilization for optimal plant growth. Overall, the results highlight the importance of considering substrate characteristics when selecting materials for agricultural or horticultural applications. Further research is needed to explore the effects of these differences on plant performance and soil health over time.

**Table 2.** Initial Physico-Chemical Analysis of Substrates

| Treatments | SMS | CD |
|---|---|---|
| pH | 5.85 | 7.44 |
| EC (mS/m) | 3.02 | 6.25 |
| N (%) | 1.54 | 2.52 |
| TOC (%) | 54.99 | 43.88 |
| C/N ratio | 35.71 | 17.41 |
| TAP (g/kg) | 2.66 | 6.54 |
| Exchangeable K (cmol/kg) | 1.58 | 1.15 |
| Exchangeable Na (cmol/kg) | 1.60 | 1.58 |

Table 3 presents the results of a study comparing the effects of different treatments (T1, T2, T3, T4, and T5) on various physicochemical parameters including pH, electrical conductivity (EC), total Kjeldahl nitrogen (TKN), TOC, C/N ratio, and TAP. The initial values represent measurements taken at the beginning of the experiment, while the final values represent measurements taken after a certain period of time. The percentage change indicates the magnitude of change from the initial to the final values for each treatment.

All treatments showed an increase in pH from initial to final measurements, indicating an overall trend towards alkalinity. Treatment T1 exhibited the highest percentage increase in pH (21.0), while treatments T4 and T5 showed a slight decrease in percentage of pH (-0.28 and -0.94, respectively). The changes in pH could be attributed to various factors, such as microbial activity, nutrient availability, and substrate composition (López *et al*. 2021).

*Eisenia fetida* hosts a diverse microbial community in its gut, including bacteria, fungi, and other microorganisms. These microbes participate in the degradation process by enzymatic hydrolysis, oxidation, and fermentation of organic substrates. Studies have identified various bacterial genera, such as *Bacillus*, *Pseudomonas*, and *Enterobacter*, along with fungal species, like *Aspergillus* and *Penicillium*, contributing to decomposition within the earthworm's digestive system (Budroni *et al*. 2020).

The presence of *Eisenia fetida* accelerates the decomposition of organic matter in substrates. Through the process of ingestion, digestion, and excretion, earthworms fragment the organic materials into smaller particles, increasing the surface area for microbial colonization and enzymatic action. This enhanced surface area facilitates microbial degradation, leading to the release of nutrients and conversion of complex organic compounds into simpler forms (Pathma and Sakthivel 2012).

**Table 3.** Physico-chemical Analysis Vermicomposts

| Treatments | | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| pH | Initial | 5.85 | 6.25 | 6.65 | 7.04 | 7.44 |
| | Final | 7.08 | 7.01 | 6.97 | 7.02 | 7.37 |
| EC (mS/m) | Initial | 3.02 | 3.83 | 4.64 | 5.44 | 6.25 |
| | Final | 2.32$^d$ | 4.03$^c$ | 5.88$^b$ | 6.57$^b$ | 7.82$^a$ |
| TKN (%) | Initial | 1.54 | 1.79 | 2.03 | 2.28 | 2.52 |
| | Final | 2.10 | 2.25 | 2.39 | 2.41 | 2.37 |
| TOC (%) | Initial | 54.99 | 52.22 | 49.44 | 46.67 | 43.89 |
| | Final | 58.70$^a$ | 50.96$^b$ | 47.45$^{bc}$ | 47.45$^{bc}$ | 43.88$^c$ |
| C/N ratio | Initial | 35.71 | 29.17 | 24.35 | 20.47 | 17.42 |
| | Final | 28.66$^a$ | 22.61$^{ab}$ | 20.00$^b$ | 19.78$^b$ | 18.65$^b$ |
| TAP (g/kg) | Initial | 2.66 | 3.63 | 4.60 | 5.57 | 6.54 |
| | Final | 2.21$^d$ | 4.01$^c$ | 4.76$^b$ | 5.96$^a$ | 5.95$^a$ |

The activity of *Eisenia fetida* and its associated microbial community results in significant chemical transformations within substrates. These changes include alterations in pH, organic carbon content, nitrogen availability, and nutrient concentrations. For instance, studies have reported a decrease in pH levels due to microbial production of organic acids during decomposition. Furthermore, the conversion of organic nitrogen to ammonium by microbial activity increases nutrient availability for plant uptake (Garau *et al*. 2022). The decomposition facilitated by *E. fetida* and its microbial symbionts enriches the substrate with nutrients essential for plant growth. The release of nitrogen, phosphorus, potassium, and other micronutrients enhances soil fertility and improves nutrient cycling dynamics. Additionally, the microbial metabolites generated during decomposition, such as humic substances and microbial biomass, contribute to soil structure and water retention capacity, thereby promoting plant health and productivity (Dominguez *et al*. 2011).

The EC values varied among treatments, indicating substantial fluctuations in salt concentration over the experimental period. These changes may reflect alterations in nutrient leaching, ion exchange, and microbial decomposition processes within the substrates (Lim *et al*. 2015).

Initial TKN levels ranged from 1.54% to 2.52%, with treatment T5 having the highest initial TKN value. While all treatments showed an increase in TKN levels from initial to final measurements, the magnitude of change varied, ranging from -5.95% to 36.36%. These changes could be attributed to nitrogen mineralization, microbial activity, and organic matter decomposition within the substrates (Fog 1988).

Initial TOC levels ranged from 43.9% to 55.0%, with treatment T1 having the highest initial TOC value. Treatment T1 also exhibited the highest percentage increase in TOC (6.75%), while treatments T2 and T3 showed a decrease in TOC levels. These changes suggest variations in organic matter decomposition, microbial activity, and carbon sequestration within the substrates.

The C/N ratio decreased in all treatments from initial to final measurements, indicating a relative increase in nitrogen compared to carbon content. Treatment T1 had the highest initial C/N ratio, while treatment T5 had the lowest. The percentage change in the C/N ratio ranged from -22.5% to 7.1%, reflecting alterations in organic matter decomposition and nutrient cycling dynamics. Initial TAP levels ranged from 2.66 g/kg to 6.54 g/kg, with treatment T5 having the highest initial TAP. The percentage change in TAP varied among treatments, ranging from -16.9% to 10.5%. These changes may be influenced by phosphorus mineralization, microbial activity, and soil pH dynamics (Arenberg and

Arai 2019). Overall, the results highlight the dynamic nature of substrate physicochemical properties and the influence of different treatments on nutrient dynamics and microbial processes. Further analysis and experimentation are necessary to elucidate the underlying mechanisms driving these observed changes and their implications for soil health and plant growth.

Table 4 presents the exchangeable potassium (K) and exchangeable sodium (Na) content measured in centimoles per kilogram (cmol/kg) for different treatments. Treatment T1, consisting of 100% SMS and 0% CD, exhibited the highest exchangeable potassium content at 1.59 cmol/kg. As the proportion of cow dung in the treatments increased, there was a general trend of decreasing exchangeable potassium content. This is evidenced by the decreasing values observed from T2 to T5. T2, T3, T4, and T5, with increasing proportions of cow dung, showed decreasing exchangeable potassium values of 1.09, 1.06, 1.27, and 1.42 cmol/kg, respectively.

Potassium and sodium are essential nutrients for plant growth, with K playing a crucial role in various physiological processes and Na influencing osmotic balance in plants. The differences in exchangeable K and Na between SMS and CD suggest their varying contributions to soil nutrient dynamics and plant health (Wakeel 2013).

**Table 4.** Values of Exchangeable K and Na Contents in Different Treatment Groups

| Treatments | Exchangeable K (cmol/kg) | Exchangeable Na (cmol/kg) |
|---|---|---|
| T1 | 1.59 | 1.47 |
| T2 | 1.09 | 1.08 |
| T3 | 1.06 | 1.14 |
| T4 | 1.27 | 1.08 |
| T5 | 1.42 | 1.09 |

The decrease in exchangeable K-content with increasing proportions of CD could be attributed to several factors. Cow dung is known to have lower K-content compared to SMS. Additionally, the microbial activity during vermicomposting might have led to the conversion of K into forms less available for exchange, thereby reducing the measured exchangeable K-content in the final product (Uzoma *et al*. 2011).

The treatment T1 showed an exchangeable Na-content of 1.47 cmol/kg. There was not a clear trend in the exchangeable Na-content across the treatments. Treatments T2, T4, and T5 showed similar values, while T3 had a slightly higher value of 1.14 cmol/kg. The variation in exchangeable Na content across treatments might be influenced by the initial composition of SMS and CD, as well as the dynamics of Na during vermicomposting. Sodium content in CD was higher compared to SMS. Its behavior during composting can be complex, influenced by factors such as microbial activity and organic matter decomposition (Yu *et al*. 2022).

### Economic and Ecological Advantages of Optimized Vermicomposting Methods

Vermicomposting provides a sustainable solution for organic waste management, significantly reducing the costs associated with waste disposal. This method turns waste into valuable compost, reducing the need for commercial fertilizers and minimizing landfill usage. Mixing SMS with cow dung produces a nutrient-rich organic fertilizer, reducing the need for commercial chemical fertilizers. This can significantly lower production costs for farmers.

Both SMS and cow dung are agricultural by-products that often pose disposal challenges. By combining them, farmers can convert waste into valuable fertilizer, enhancing the economic value of these otherwise discarded materials. Studies (Gong *et al.* 2019; Yu *et al.* 2022) have shown that the addition of SMS and CD increased enzyme activities and organic matter decomposition. This leads to better crop yields through decomposing and exposing nutrients to plants and, consequently, higher economic returns.

The mixture promotes the growth of beneficial soil microorganisms, which play a crucial role in nutrient cycling and disease suppression. The combination provides a more balanced nutrient supply to plants, incorporating both the fast-releasing nutrients from cow dung and the slower-releasing nutrients from SMS (Vincze *et al.* 2024). The experiment conducted in this paper research demonstrated that crops fertilized with a 50:50 mix of SMS and cow dung gave a significant increase in earthworm growth and reproduction. This in turn facilitated faster waste conversion into organic fertilizer.

## CONCLUSIONS

1. A 50:50 mixture of spent mushroom substrate (SMS) and cow dung (CD) exhibited significantly higher earthworm growth potential as a substrate. Treatments T1, T2, and T4 showed comparable earthworm growth.
2. Treatment T3 also resulted in the highest number of cocoons produced, indicating favorable conditions for reproductive activity. In contrast, Treatment T5 exhibited the lowest cocoon production, which can be attributed to the lower quality of the feed mixture, leading to reduced earthworm reproductive output.
3. Treatment T3 showed the highest number of hatchlings formed, further indicating the superior suitability of the 50:50 SMS and CD mixture for earthworm reproduction. Treatment T5 exhibited the lowest number of hatchlings formed, consistent with the trend observed in cocoon production.
4. Initial physicochemical analysis revealed significant differences between SMS and CD treatments in terms of pH, EC, N content, TOC, C/N ratio, TAP, and exchangeable K and Na contents. The changes observed in the physicochemical properties of vermicomposts over time indicate dynamic transformations influenced by microbial activity, nutrient availability, and decomposition processes.

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