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Sustainable valorization of *Moringa oleifera* Lam. co-products and zoo waste

Valorização sustentável dos coprodutos de *Moringa oleifera* Lam. e resíduo de zoológico

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ABSTRACT

Moringa oleifera (moringa) stands out as a promising plant in several segments, being produced worldwide. However, its co-products, particularly valves and seed husks, which represent more than 70% of its fruit, remain underutilized. Therefore, this work aimed to assess the use of parts of the moringa fruit in conjunction with sediment from an artificial pond in a zoo enclosure inhabited by *Tapirus terrestris* (tapir), exploring the potential treatment of these wastes, using *Eisenia foetida* earthworms. Five experimental conditions were analyzed, whose waste proportions were varied. The vermicomposts were not phytotoxic and differed regarding the C/N ratio; those that received parts of the moringa fruit had a higher C/N ratio. As commonly observed in stabilization processes, the contents of P, K, Ca, and electrical conductivity increased, while carbon and pH decreased during stabilization. Plant development of *Catharanthus roseus* was evaluated using, in addition to the vermicomposts, two commercial composts. The vermicomposts provided better development of *C. roseus* than the commercial composts, with T2 (65% sediment+35% fruit valves) and T3 (50% sediment+35% valves+15% seed husks) standing out as the best treatments. Vermicomposting associated with moringa co-products and zoo waste is a viable alternative via aerobic treatment, favoring waste management and the search for sustainability.

Keywords: tapir; vermicomposting; waste mitigation.

RESUMO

A *Moringa oleifera (moringa)* tem se destacado como uma planta promissora em diversos segmentos, sendo produzida mundialmente. No entanto, seus coprodutos, entre eles valvas e cascas das sementes, que representam 70% dos seus frutos, não são valorizados. Diante disso, este trabalho teve por objetivo avaliar o uso de partes do fruto de moringa em conjunto com sedimento de lagoa artificial de recinto de zoológico utilizada por *Tapirus terrestris* (anta), a fim de verificar essa alternativa de tratamento desses resíduos utilizando minhocas *Eisenia foetida*. Foram analisadas cinco condições experimentais, em que se variaram as proporções dos resíduos. Os vermicompostos não se apresentaram fitotóxicos e diferiam quanto à relação C/N, já que, nos que receberam partes dos frutos de moringa, essa razão foi maior. Comumente a processos de estabilização, os teores de P, K, Ca e condutividade elétrica aumentaram, enquanto carbono e pH diminuíram ao longo da estabilização. Foi avaliado o desenvolvimento vegetal de *Catharanthus roseus* utilizando-se, além dos vermicompostos, mais dois compostos comerciais. Os vermicompostos propiciaram melhor desenvolvimento de *C. roseus* do que os comerciais, sendo T2 (65% de sedimento+35% valvas dos frutos) e T3 (50% de sedimento+35% valvas+15% cascas das sementes) os melhores tratamentos. A vermicompostagem associando coprodutos de moringa e resíduos de zoológico é uma alternativa viável via tratamento aeróbio, favorecendo o seu gerenciamento e a busca pela sustentabilidade.

Palavras-chave: anta; vermicompostagem; gerenciamento de resíduos.

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Introduction

Moringa oleifera Lamarck, originally from northwest India, stands out worldwide due to its various properties (Kavithambika et al., 2020), being used in different industrial sectors, pharmaceuticals, cosmetics production, biodiesel generation, and water treatment. Additionally, due to its nutrient-rich profile including proteins, vitamins, fatty acids, and minerals, it serves as a valuable component in both human and animal nutrition (Masih et al., 2019; Trigo et al., 2020). Studies indicate that moringa has biologically active molecules, with antibacterial, antifungal (Morgan et al., 2020; Mohanty et al., 2021), anti-inflammatory (Brilhante et al., 2017), and antitumor properties (Barhoi et al., 2021). The global market for moringa constituents was worth US$ 6.9 billion in 2020 and is projected to grow by 9.5% by 2028 (Factors FA, 2020; Patil et al., 2022).

Moringa oleifera is a perennial plant, widely cultivated in tropical regions, resilient even under adverse conditions. The fruit of the moringa tree is a simple loculicidal capsule with three valves, measures 45 to 90 cm, containing globose seeds, where the almond is covered with a three-winged shell (Jattan et al., 2021). Only the seed of the moringa fruit holds commercial interest, and despite the increasing usage of this plant, research on its by-products remains limited. It was found that citrus fertilized with moringa seed cake resulted in better fruit development while reducing the need for chemical fertilizer and costs. Enhanced results were observed when the cake was incorporated with organic compounds (El-Hadidy et al., 2022).

Studies also indicate that, despite its numerous benefits, certain parts of the moringa fruit may be toxic (Tahir et al., 2018, 2020; Attah et al., 2022). Among aerobic stabilization practices, vermicomposting is preferable, as earthworms are used as bioindicators due to their sensitivity (Saint-Denis et al., 2001) and as standard organisms for toxicological experiments (Caspers, 1984). Vermicomposting stands out as an economically and environmentally attractive alternative for organic waste management (Jjagwe et al., 2019), converting it into fertilizers and nutrient-rich humic substrates (Yuvaraj et al., 2021), requiring less management for large-scale operations (Enebe and Erasmus, 2023).

However, to ensure effective organic waste stabilization, parameters such as moisture control, pH, carbon/nitrogen (C/N) ratio, and complete compost maturation must be maintained. The C/N ratio influences the energetic and structural action of decomposing organisms (Voběrková et al., 2017; Gusain and Suthar, 2020). Achieving the ideal C/N ratio involves supplementing high-carbon plant by-products with nitrogen-rich animal waste (Cotta et al., 2015).

Traditional solid waste management techniques such as incineration, pyrolysis, and gasification have high investment costs (Alshehrei and Ameen, 2021) and often release toxic and harmful substances into the environment. Thus, for a zoo to achieve sustainability goals, all its activities must neutralize their environmental impact (Pérez-Godínez et al., 2017; WAZA, 2020). Therefore, the use of organic waste from zoos to produce fertilizers, composts, biofertilizers, and plant extracts to improve plant development emerges as an economically viable and sustainable waste management methodology, capable of replenishing soil nutrients (Dores-Silva et al., 2013; Alshehrei and Ameen, 2021; Cappelini et al., 2021; Kachangoon et al., 2022).

Thus, this work was carried out at Refúgio Biológico Bela Vista, home to the Roberto Ribas Lange Zoo, which hosts, among other animals, the largest mammal in southern America, the tapir (*Tapirus terrestris*), weighing between 180 and 300 kg (Thoisy et al., 2014). Tapirs are an herbivorous species and a great disperser of seeds, serving as a crucial bioindicator of ecosystem health and biodiversity conservation (Dario, 2014; Ticiani et al., 2021). However, tapirs defecate in water (Bodmer, 1991), leading to the accumulation of organic material in captive environments, posing a risk to the health of animals and causing environmental problems such as eutrophication, greenhouse gases emissions, bad smells, and contamination of soil and water bodies. However, currently, in addition to sheltering animals, zoos also contribute significantly both environmentally and economically, providing scientific advancements by enabling the development of effective preservation techniques (WAZA, 2020).

In view of the above, the aim of this work was to evaluate the use of parts of the *Moringa oleifera* fruit associated with sediment from a pond in a zoo enclosure used by *Tapirus terrestris*, for stabilization by vermicomposting, in order to provide valorization and sustainable management for these two waste streams produced on a large scale.

Materials and methods

Characterization of the parts of the *Moringa oleifera* fruit

Moringa oleifera fruits were collected from six specimens located in the city of Foz do Iguaçu, Brazil, in the summer of 2022. Then, the fruit valves (capsules) were manually removed, and the seeds were peeled. The moringa fruit parts were dried in a forced-air oven at 40°C for 48 h, weighed, and their volumes were measured.

The valves, seed husks, and almonds were milled in a knife mill, sieved through a 28-mm mesh granulometric sieve, and used to determine parameters of hydrogen-ion potential (pH), electrical conductivity (EC) (Voběrková et al., 2017), total solids (TS), ash (FS), organic matter (VS), and moisture (M) (AOAC, 2005). The seed oil was chemically extracted using hexane solvent via Soxhlet extraction method (IUPAC, 1979).

The milled samples (valves, seed husks, almond, and cake) along with the seed oil underwent chemical characterization of functional groups using Fourier transform infrared spectroscopy (FT-IR), with KBr pellets (Stevenson, 1994) in the range from 4,000 to 400 cm^{-1} with spectral resolution of 4 cm^{-1}, using a Frontier by Perkin Elmer FT-IR spectrometer. Valves and seed husks were evaluated by scanning electron microscopy (SEM), metallized with gold-palladium to a thickness of 6-9 nm, using an SC7620 Quorum Mini Sputter Coater/Glow Discharge System metallizer. The scanning microscope used was a Zeiss (EVO-MA10), operating at a voltage of 20 kV.

To determine the total organic carbon (TOC) content, the samples, previously dried in an oven at 105°C, were calcined in a muffle furnace at 550°C for 6 h (Cunha-Queda et al., 2003). Extracts obtained from sulfuric acid digestion were used to determine the total Kjeldahl nitrogen (TKN) content using the Kjeldahl distiller (Malavolta et al., 1997). The C/N ratio was determined by the TOC/TKN ratio, and for determination of crude protein, a conversion factor of 6.25 was applied (van Soest, 1994).

In the phytotoxicity study, parts of the moringa fruit (valves, husks, whole seeds), in addition to the cake and almond oil, were evaluated for the determination of the germination index (GI). An extract was obtained in the proportion of 1:10 (m/v) in distilled water from moringa fruit parts kept under agitation for 30 min, at 60°C and 160 rpm. A volume of 5 mL of the extract was added to a Petri dish containing sterilized cellulose filter paper and 20 seeds of *Lactuca sativa* (lettuce) (Zucconi et al., 1981). Petri dishes containing the seeds were incubated at 22°C with 8 h of light for 48 h using a germination chamber (biochemical oxygen demand — BOD). Four control petri dishes were prepared with distilled water. Four replications were performed for each sample. GI was calculated as the product of the percentage of viable seeds, the number of germinated seeds, and root growth after 48 h (Equation 1):

$$IG = \frac{NGext\ .LRext}{NGcont\ .LRcont}.100 \tag{1}$$

Where:

NG_{ext}: number of germinated seeds in the extract;
NG_{cont}: number of germinated seeds in the control;
LR_{ext}: average length of radicles in the extract; and
LR_{cont}: average length of radicles in the control.

Characterization of the pond sediment

The pond sediment was collected at Refúgio Biológico Bela Vista – Roberto Ribas Lange Zoo, located in the city of Foz do Iguaçu, Brazil. Within this setting, an artificial pond with a cemented bottom, designated for use by *Tapirus terrestris* (tapirs), was selected. The pond is characterized by its dimensions: an area of 75 m^2, a flow rate of 2.870 $m^3\ h^{-1}$, a volume of 36.209 m^3, and ana average depth of 0.482 m.

Fresh tapir manure and sediment collected from the pond used by these animals were characterized in terms of pH, EC (Voběrková et al., 2017), and C/N ratio. The C/N ratio was determined through calcination and sulfuric acid digestion, respectively (Malavolta et al., 1997; Cunha-Queda et al., 2003).

Vermicomposting

The practice used to stabilize the parts of the moringa fruit and the pond sediment used by the tapirs was vermicomposting. For that, moringa valves, seed husks, whole seeds and seed cake were dried, weighed, and milled.

Approximately 5,000 L of pond sediment was collected on the designated cleaning day, with the aid of a suction pump coupled to a specialized truck for transport. The sediment was distributed among 13 fiberglass boxes of 1,000 and 500 L. Each box was equipped with a drainage system consisting of a layer of crushed stone and fabric for water drainage. After 1 week of drainage, the solid waste (sediment) was relocated to a covered area and was turned over every 3 days, for pre-stabilization. After 15 days, the pre-composted sediment underwent vermicomposting.

The pre-composted sediment was distributed among 20 vermireactors of 20 L each, covered with shade cloth and with holes in the base for liquid flow and compost oxygenation. The vermireactors were arranged in a completely randomized design (CRD), with five treatments and four replications (T1: 70% Sediment+30% fruit and vegetable scraps; T2: 65% Sediment+35% moringa valves; T3: 50% Sediment+35% moringa valves+15% moringa husks; T4: 50% Sediment+40% moringa valves+5% moringa husks+5% whole seed; T5: 50% Sediment+40% moringa valves+5% moringa husk+5% cake). Valves were chipped in a woodchipper, into pieces measuring between 2 and 4 cm. All treatments showed an initial C/N ratio of 22 (Melo et al., 2020), determined by the TOC/TKN ratio (Malavolta et al., 1997; Cunha-Queda et al., 2003). Each vermireactor contained the equivalent of 1 kg of TS related to waste and 15 earthworms of the Eisenia foetida species.

After 7, 30, 60, and 90 days of stabilization of moringa co-products and pond sediment, the resulting vermicomposts were evaluated for pH, EC (Voběrková et al., 2017), TS, FS, VS, M (AOAC 2005), and C/N ratio (Malavolta et al., 1997; Cunha-Queda et al., 2003).

For the determination of total phosphorus (TP) in the vermicomposts, the molybdenum blue spectrophotometry method was used (Malavolta et al., 1997) while the quantification of potassium (TK) and calcium (TCa) was conducted using a flame photometer after nitric-perchloric acid digestion (EMBRAPA, 2009). These elements were determined at the beginning of the stabilization process and at the end, after 90 days.

In the vermicompost phytotoxicity assays, the methodology by Zucconi et al. (1981) was followed. For this purpose, the vermicomposts were dried at room temperature and an extract was obtained in a ratio of 1:10 (m/v) in distilled water, from vermicompost subjected to agitation for 30 min at 60°C and 160 rpm. Subsequently, 5 mL of the extract was added to a Petri dish containing sterilized cellulose filter paper and 20 seeds of *Lactuca sativa* (lettuce). Petri dishes containing the seeds were incubated in a germination chamber (BOD) set at 22°C with 8 h of light for 48 h. Four control Petri dishes were prepared with distilled water. Four replications were performed for each sample. GI was calculated as the product of the percentage of viable seeds, the number of germinated seeds, and root growth after 48 h (Equation 1).

To evaluate plant development, vermicomposts produced by the five treatments developed in vermicomposting (T1, T2, T3, T4, and T5), along with two other commercial composts — one produced by vermicomposting (T6) and the other by composting (T7) — were employed. The substrates were arranged in polypropylene trays, where three *Catharanthus roseus* seeds were placed per cell. The setup was organized in CRD with eight replications. After 10 days, thinning was performed, keeping one seedling per cell. The same treatments were prepared with different doses of organic compounds: I. 100% organic compound; II. 75% organic compound+ 25% sterile sand; III. 50% organic compound+50% sterile sand.

After 40 days of sowing, seedling growth efficiency was analyzed using the Dickson quality index (DQI) (Equation 2). For this, six plants were randomly selected, and measurements were taken.

$$DQI = \frac{TDM}{\left(\frac{H}{SD}\right)+\left(\frac{SDM}{RDM}\right)} \quad (2)$$

Where:
DQI: Dickson Quality Index;
H: plant height (cm);
SD: stem diameter (cm);
SDM: shoot dry mass (g);
RDM: root dry mass (g); and
TDM: total dry mass (g).

In addition to DQI, the plants were also evaluated for the number and diameter of leaves (mm), root length (cm), root/shoot dry matter ratio (R/S), and shoot height/stem diameter ratio (H/D).

Statistical analysis

The dependent variables (pH, EC, TS, fixed solids and volatile solids, in addition to TOC, total nitrogen, C/N ratio, GI, calcium, phosphorus, potassium, number of leaves, leaf diameter, root and stem size) were evaluated across treatments (T1, T2, T3, T4, T5) via one-way analysis of variance (ANOVA) when the data were in normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test). Upon statistical significance, multiple comparisons of means were performed using the Tukey-honestly significant difference (Tukey-HSD) post-hoc test. For variables whose normality and homoscedasticity assumptions were not confirmed, the non-parametric Kruskal-Wallis test was applied, followed by the Dunn's follow-up test.

Considering that plant development variables are interconnected with the plant's growth pattern, an integrative multivariate analysis was performed using a multivariate principal component analysis (PCA), considering the use of 100, 75, and 50% organic compounds. Data quality was assessed using the Kaplan-Meyer-Olkin (KMO) method, using the matrix of variables only with KMO values greater than 0.5. Factor loadings were extracted, being considered as latent variables and compared using the two-way ANOVA, with fixed factors encompassing the treatments (T1 to T7) and the use of the dose of the compounds (50, 75, and 100%). In case of statistical significance, the Tukey-HSD test was used.

All analyses were carried out in the R program (R Core Team, 2022), employing a significance level of 0.05 in the statistical tests.

Results and discussion

Characterization of the parts of the *Moringa oleifera* fruit

A large-scale production of *Moringa oleifera* seeds yields significant volumes of materials with no added value. This was confirmed by the quantification of the parts of the moringa fruit, revealing that 77% (±3.19) of the mass and 99% (±0.13) of the volume correspond to its co-products: valves and seed husks. This volume of moringa co-products is justifiable due to the presence of numerous pores in these materials, as evidenced through scanning electron micrographs (Figure 1), which portrays the spongy characteristic of the fruit, providing low density and consequently large volume.

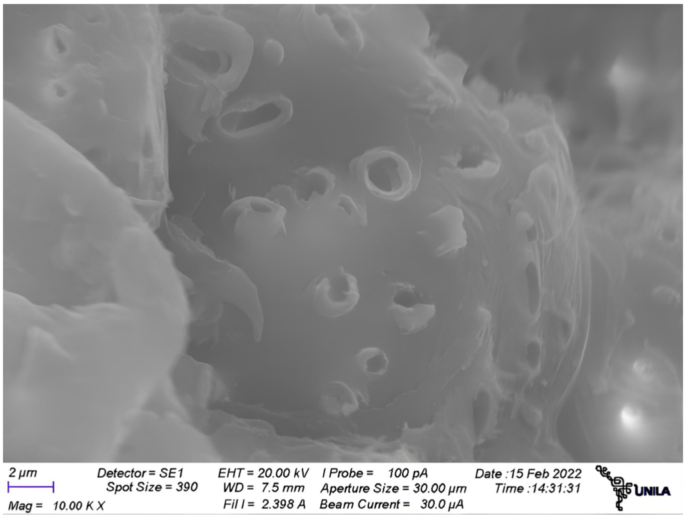
The characterization of the parts of the moringa fruit showed that the almond (38.28%±0.17) and the cake (47.03%±0.10) had a higher percentage of proteins than seed husks (18.59%±0.00) and fruit valves (9.84%±0.00).

This finding aligns with previous studies that indicated the abundance of proteins in moringa seeds (Masih et al., 2019; Trigo et al., 2020; El-Hadidy et al., 2022), ranging between 19 and 47% (Bhutada et al., 2016; Gharsallah et al., 2021). El-Hadidy et al. (2022) observed results consistent with the characterization of moringa cake as reported in this study. However, these percentages may vary depending on the location and physiology of the plant (Rébufa et al., 2021).

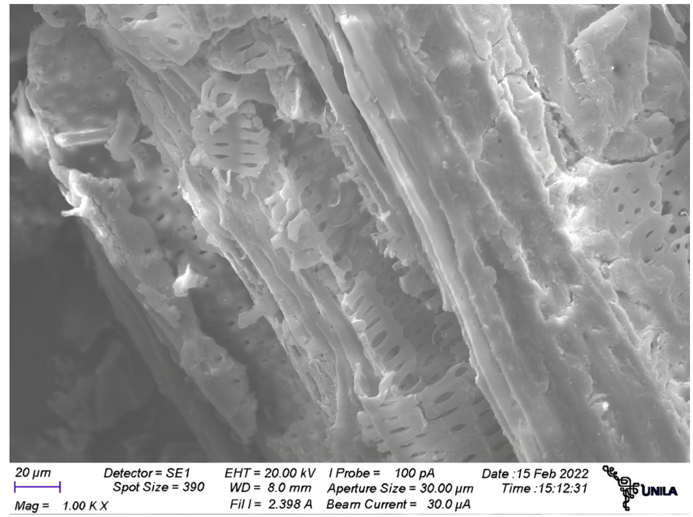
The seeds used in the stabilization process had an oil percentage of 38.70% (±1.79), in line with previous findings indicating substantial oil content in moringa seeds, reaching up to 40% (Bhutada et al., 2016; Gharsallah et al., 2021).

FT-IR analysis of the samples (Figure 2) allowed the identification of functional groups within the compounds of the moringa fruit. Intense bands in the region of 3,300 cm^{-1} are present in all samples, except oil, and can be attributed to the vibrational stretching of the OH bond of carbohydrates, proteins, polysaccharides, and lignin units. The presence of high protein content in these samples is further indicated by the stretching of the NH bond within this band. Additionally, subtle bands around 2,925 and 2,852 cm^{-1} suggest the symmetric and asymmetric stretching of CH bonds of the alkane groups, present in lipids and lignin, which also corroborates with peaks close to 1,748, attributed to acetyl and carboxyl groups found in lipids and proteins (Rébufa et al., 2021; Kachangoon et al., 2022).

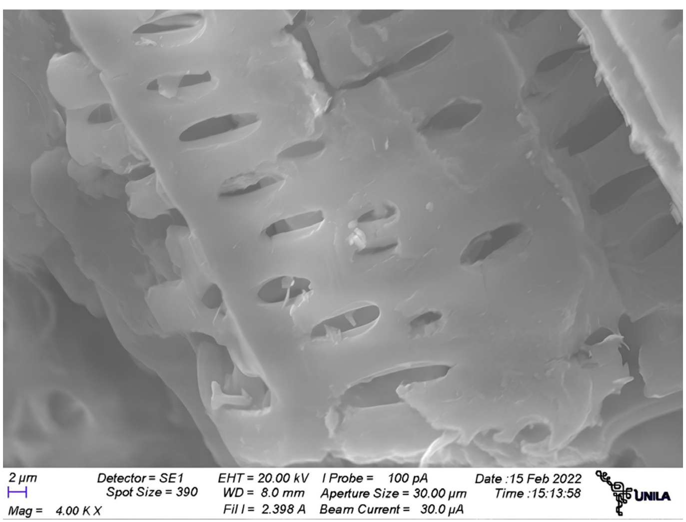
The bands observed at 1,660 and 1,547 cm^{-1} are attributed to C=O stretching and N-H bending of amides in the protein portion (Ezeamaku et al., 2018). Weak bands at 1,460 cm^{-1} are attributed to vibration of the C=C bond present in lignin (Meneghel et al., 2013).



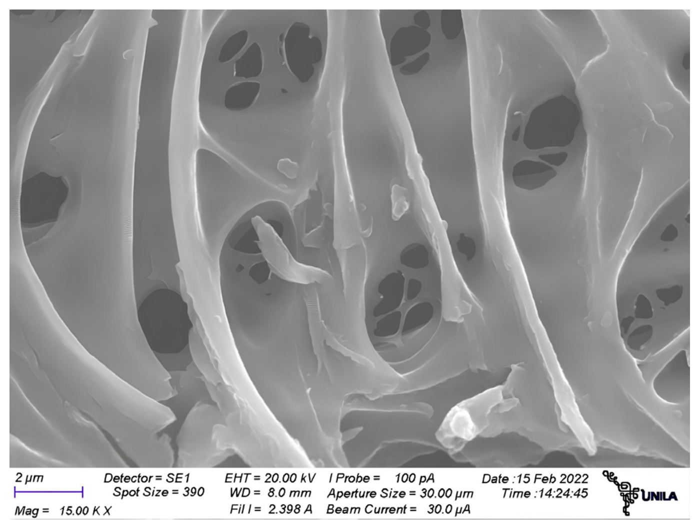
(A)



(B)



(C)



(D)

Figure 1 – Scanning electron micrographs of (A and B) moringa seed husks; C and D) moringa valves.

The spectral region from 1,100 to 900 cm^{-1} is characteristic of polysaccharides, with bands below 950 cm^{-1} attributed to C–O stretching and C–H aromatic deformation, indicative of lignin presence. In addition, within this range, ring vibration and CH_2, characteristics for polysaccharides, are verified (Rébufa et al., 2021).

FT-IR analysis of moringa fruit parts revealed a multitude of functional groups, proving their complex composition. It also confirmed that oil extraction reduced the fatty acid content in the seeds but did not affect their protein content.

Bioassays indicating GIs above 80% suggest the absence of phytotoxic substances (Bernal et al., 2009). Parts of the moringa fruit were found to be phytotoxic in 100% solutions and 50% dilutions. Starting from 25% dilutions, seed pod and husk solutions acted as phytostimulants, presenting GIs of 103%. However, cake and whole seeds remained phytotoxic even at 5% dilution.

In light of the evidence of the toxicity of moringa cake and whole seed, new phytotoxicity tests were carried out with lower dilutions. Samples containing moringa whole seed, which contained oil, were phytotoxic even at 1% dilutions, with toxicity levels higher than those of the cake (%GI moringa whole seed: 67, 79, 82, 88; %GI cake: 94, 96, 118, 96; at dilutions: 1, 0.5, 0.1, and 0.01, respectively:). There was no significant difference between the dilutions of moringa whole seeds (coefficient of variation [CV]=25.41; p=0.602) and cake (CV=13.56; p=0.171).

Moringa oil proved to be phytotoxic in all dilutions analyzed (%GI: 64, 78, 76, 76, 76, 73; at dilutions: 10, 5, 1, 0.5, 0.1, 0.01, respectively), with no significant difference between dilutions (p>0.05), corroborating previous studies, indicating that after oil extraction, the moringa cake does not present toxicity (Tahir et al., 2018, 2020; Attah et al., 2022).

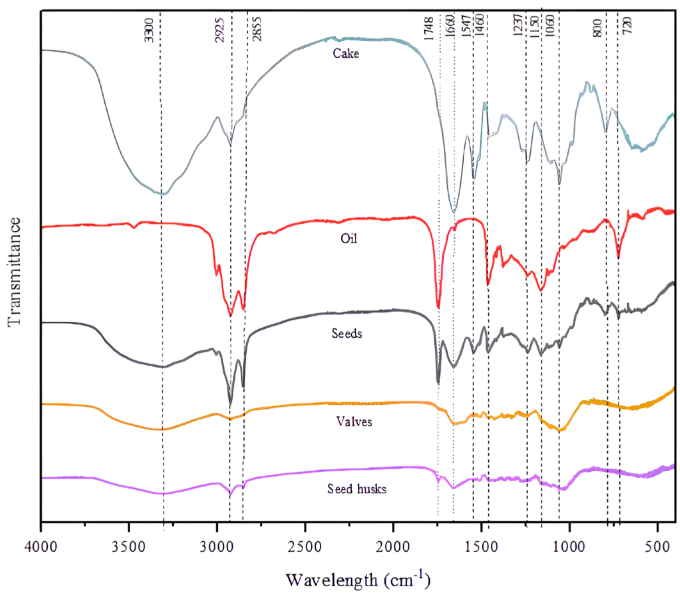


Figure 2 – FT-IR spectra of Moringa oleifera fruit parts.

Vermicomposting stabilization process

The pond sediment had a more acidic pH (6.34±0.02), in addition to greater EC (EC: 2.13 mS cm^{-1}±0.15), lower C/N ratio (17.30±0.36), and percentage of carbon (TOC: 34.32%±0.91) than fresh *Tapirus terrestris* manure (pH: 6.95±0.07, EC: 1.09±0.08; C/N: 34.28±0.13; TOC: 48.00%±0.42), which are characteristic of materials in the stabilization process (Cotta et al., 2015; Li et al., 2016; Melo et al., 2020).

pH is a critical parameter that directly affects microbial activation (Voběrková et al., 2017) and although the final vermicomposts were acidified (Table 1), which can be attributed to the mineralization of phosphorus and nitrogen compounds and the production of fulvic and humic acids (Ndegwa and Thompson, 2000), the pH range for the vermicomposting process typically falls between 5 to 9 (Melo et al., 2020).

In this study, fluctuations in pH and EC values were observed throughout the incubation period (Figure 3). EC increased in the last week of analysis, indicating higher salinity in the substrates and signaling compost maturity (Voběrková et al., 2017; Gusain and Suthar, 2020). This increase in EC can be attributed to the release of different ions resulting from the decomposition of organic substances and grinding of materials carried out by earthworms (Garg et al., 2006; Pérez-Godínez et al., 2017; Vico et al., 2018; Ripp et al., 2020). Despite the increase in this parameter in the final vermicomposts, EC remains within the recommended range, which should be less than 4 mS cm^{-1} (Pérez-Godínez et al., 2017; Meng et al., 2019).

The C/N ratio serves as a valuable indicator of compost maturity (Meng et al., 2019). Across all treatments, there was a reduction in the C/N ratio, since the loss of mass via microbial respiration decreases the carbon content while concurrently concentrates nitrogen, thus reducing the C/N ratio (Li et al., 2016; Melo et al., 2020).

Table 1 – Characterization of vermicomposts produced with pond sediment used by Tapirus terrestris and parts of Moringa oleifera fruit.

Parameters	Treatments						
	T1	T2	T3	T4	T5	CV	p-value
pH	5.89±0.25	6.35±0.40	6.58±0.56	5.67±0.43	5.58 ±0.08	7.82	0.0354
EC (DM cm^{-1})	1.74^{ab}±0.19	1.12^{b}±0.18	1.60^{ab}±0.48	2.12^{a}±0.34	2.13^{a}±0.19	17.47	0.0015
TS (%)	27.21^{a}±1.24	20.02^{b}±0.75	19.66^{b}±0.77	21.47^{b}±1.54	21.19^{b}±1.70	5.35	0.0000
Ash (DM%)	52.10^{a}±0.49	45.77^{b}±0.92	41.77^{c}±0.75	41.29^{c}±1.39	39.29^{d}±0.91	1.84	0.0000
VS (DM%)	47.88^{d}±0.80	54.21^{c}±0.92	58.21^{b}±0.75	58.70^{b}±1.39	60.70^{a}±0.91	1.45	0.0000
TOC (DM%)	26.65^{d} ±0.44	30.12^{c}±0.51	32.34^{b}±0.42	32.61^{b}±0.77	33.72^{a}±0.50	1.41	0.0000
TKN (DM%)	1.92±0.14	2.01±0.10	2.01±0.00	2.01±0.10	2.04±0.20	6.85	0.7362
C/N	13.87^{c}±0.87	15.65^{b}±0.13	15.74^{ab}±0.62	16.78^{ab}±0.37	16.94^{a}±0.66	3.74	0.0000
GI (%)	164.34±23.27	138.21±39.95	121.29±38.91	161.12±35.56	150.13±18.29	22.04	0.0705
TP (DM%)	1.03^{ab}±0.03	0.87^{c}±0.08	1.01^{abc}±0.02	0.96^{bc}±0.04	1.13^{a}±0.10	6.65	0.0011
TK (DM%)	0.88^{c}±0.09	1.39^{b}±0.01	1.83^{a}±0.18	1.84^{a}±0.13	1.76^{a}±0.07	5.04	0.0000
TCa (DM%)	1.07±0.11	0.92±0.02	1.08±0.05	0.89±0.24	0.94±0.09	13.06	0.2957

Means with different letters within a row are significantly different (p<0.05); T1: 70% *T. terrestris* pond sediment+30% fruit and vegetable scraps; T2: 65% *T. terrestris* pond sediment+35% *M. oleifera* valves; T3: 50% *T. terrestris* pond sediment+35% *M. oleifera valves*+15% *M. oleifera* husks; T4: 50% *T. terrestris* pond sediment+40% *M. oleifera* valves+5% *M. oleifera* husks+5% *M. oleifera* whole seed; T5: 50% *T. terrestris* pond sediment+40% *M. oleifera* valves+5% *M. oleifera* husks+5% *M. oleifera* seed cake; CV: coefficient of variation (%); p-value of one-way ANOVA; EC: electrical conductivity; DM: dry mass basis: TS: total solids; VS: volatile solids; TOC: total organic carbon; TKN: total Kjeldahl nitrogen; C/N: carbon/nitrogen ratio; GI: germination index; TP: total phosphorus; TK: total potassium; TCa: total calcium.

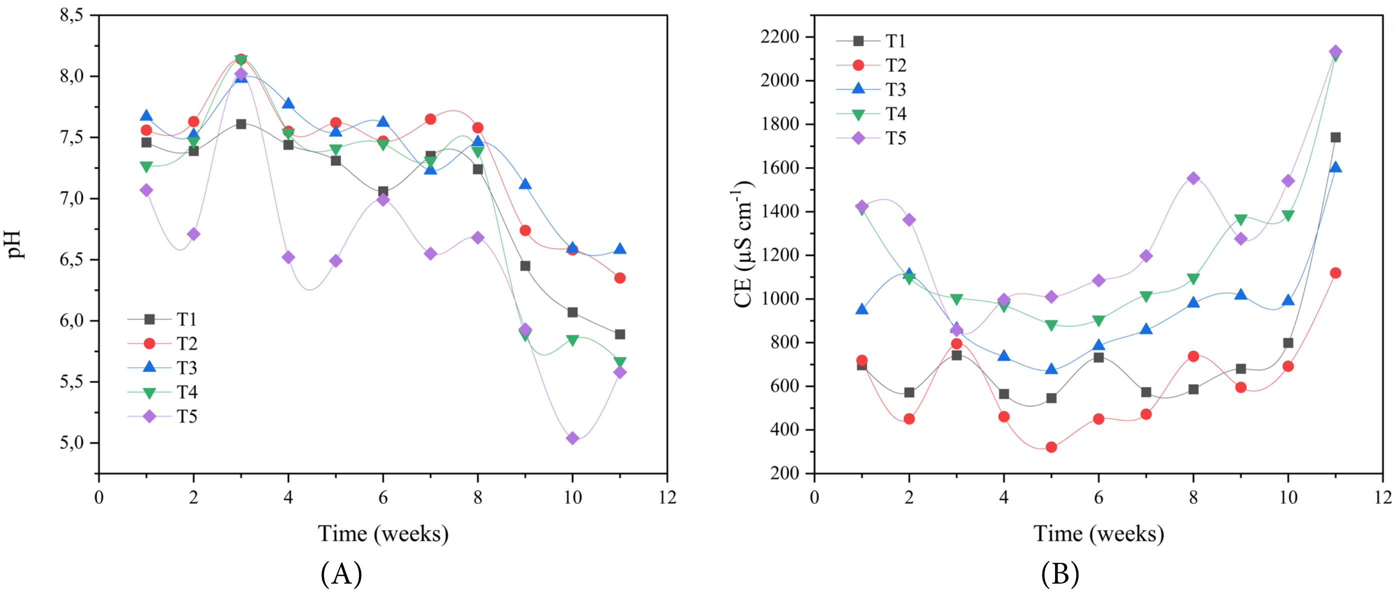


Figure 3 – (A) pH and (B) electrical conductivity of vermicomposts throughout the stabilization process.

C/N ratio (Li et al., 2016; Melo et al., 2020). Despite the decreasing trend in the C/N ratio, only T1 reached the limit recommended by IN No. 25, which institutes norms on specifications and guarantees, tolerances, and registration of organic fertilizers in Brazil (Brasil, 2009), set at a maximum of 14/1 (Table 1). This treatment did not receive moringa fruit products.

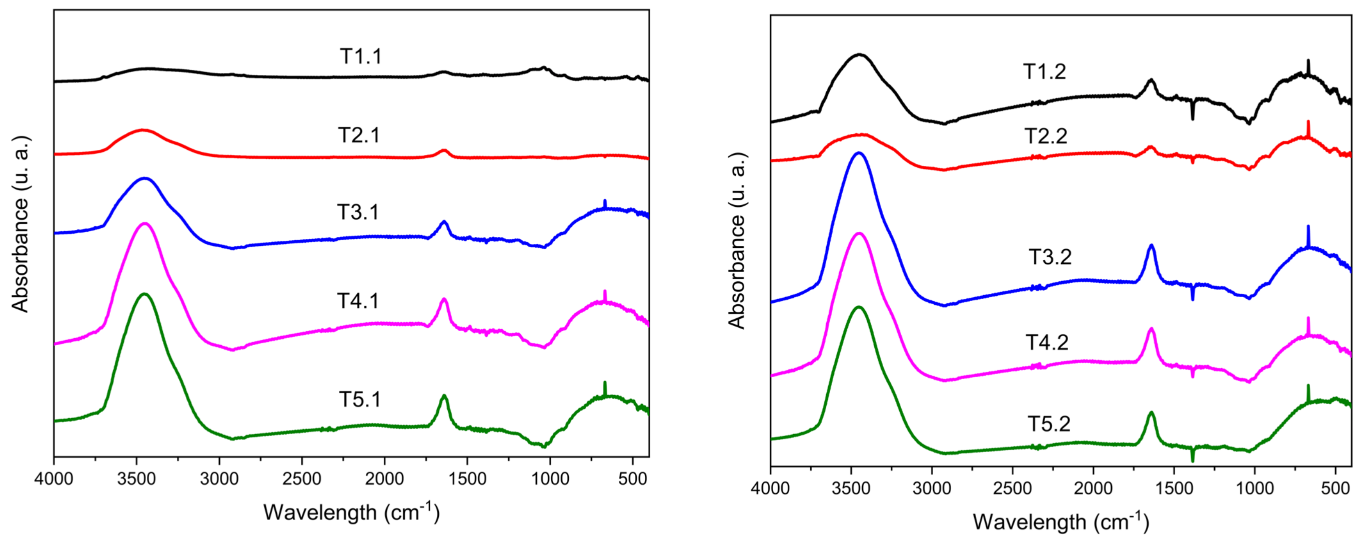
The reduction in biomass is directly linked to the fiber composition of plant residues, including lignin, a highly recalcitrant compound challenging to degrade, which delays the stabilization process (Nakhshiniev et al., 2012; Barragan-Fonseca et al., 2018; Orrico Junior et al., 2018). Fragments of the husks and valves of the moringa fruit, which is a lignite material (Oliveira Tavares et al., 2020), were still visible in the final vermicomposts. Figure 2 suggests the presence of lignin in the parts of moringa fruit, using FT-IR, which was also observed in other studies (Meneghel et al., 2013; Oliveira Tavares et al., 2020; Ishihara et al., 2021). However, the presence of lignin in organic compounds can be highly desirable, since these polymers are essential for the formation of organic matter in the soil, favoring increased soil organic matter content (Nkoa, 2014) and enhancing water absorption (Ishihara et al., 2021).

The percentages of TP, TK, and TCa had gradual increases during the stabilization period (Table 1), which is a common occurrence in such processes, supported by similar findings in other studies (Dores-Silva et al., 2013; Gusain and Suthar, 2020; Yuvaraj et al., 2021). This increase can be attributed to the presence of enzymes and microorganisms, present in the intestine of earthworms, that solubilize these macronutrients (Jjagwe et al., 2019).

The increase in TP was 33; 41; 59; 40, and 39%, respectively, according to the treatments; also, for TK the increase was 37; 69; 91; 82, and 76%, and for TCa: 60; 62; 47; 62, and 42%, following treatments T1, T2, T3, T4, and T5. Studies reveal the abundance of minerals such as calcium in moringa fruit (Ishihara et al., 2021), which represents an alternative for fertilization processes. However, nothing has been reported regarding the use of these materials in stabilization processes with future application in soils. Given their significance as essential nutrients for all forms of life and the depletion of natural reserves, the availability of these elements in a way that plants can assimilate, resulting from management practices, is crucial (Torri et al., 2017; Khan et al., 2022).

Assessment of compost maturity and toxicity includes the seed germination test, a valuable tool for such purposes (Bernal et al., 2009; Luo et al., 2018). Vermicomposts showed phytostimulant action, with a GI above 100% (Bernal et al., 2009). In addition, no statistical differences were observed between the treatments regarding the GIs ($p>0.05$). Among the parameters to assess compost maturity, FT-IR can aid in understanding structural changes in organic material (Asses et al., 2018). Vermicompost spectra showed characteristic bands of the functional groups at the beginning and end of the stabilization process, with different intensities of certain bands in the final composts (Figure 4), such as greater stretching in bridged -OH (~3,400 cm^{-1}) and C=C of aromatic groups (1,650–1,630 cm^{-1}), which confirms the mineralization of organic matter (Santos et al., 2021).

The FT-IR spectra of the final vermicomposts differ from those of the initial composts mainly by the presence of the band at 1,383 cm^{-1} due to C-O stretching of carbonate, Si-O absorption, and other silicate bonds in the region of 1,031 cm^{-1} (Castilhos et al., 2008; Asses et al., 2018). In addition, intense peaks were observed at 665 cm^{-1} (Figure 3B).



(A) (B)

Figure 4 – Fourier transform infrared spectroscopy spectra of vermicompost (A) pre- and (B) post-stabilization (90 days of stabilization).

According to Tavares et al. (2017), this spectral region is attributed to C-O bonds, which are common due to the presence of cellulosic structures.

Plant development

In the evaluation of plant development using 100% organic compound, the first principal component showed the most substantial contribution associated with shoot size (S), leaf diameter, TDM, SDM, DQI, RDM, number of leaves, SD, and root length, with the latent variable generated in Dimension 1 (Dim.1) (eigenvalue=8.85; variability=80.49%) (Figure 4).

The second principal component (Dimension 2 – Dim.2) showed a greater contribution of the variables R/S (root/shoot dry matter ratio) and H/D (shoot height/stem diameter ratio) (eigenvalue=0.99; variability=8.97%) (Figure 5). Upon comparing the factor loadings of Dim.1 (PC1), statistically significant differences were observed between the factor loadings of the treatments ($F_{6.41}$=53.11; $p<0.0001$), with the highest means observed between the groups T2 and T3 compared to the others ($p<0.05$). The positive scores of the factor loadings of Dim. 1 indicate higher values of S, leaf diameter, TDM, SDM, DQI, RDM, SD, and root length. When comparing the factor loadings of Dim. 2, there was statistical equality between treatments ($F_{6.41}$=0.898; p=0.507).

In the evaluation of plant development using 75% organic compounds plus 25% sterile sand, Dim.1 showed a greater contribution associated with DQI, RDM, SD, number of leaves, S, and leaf diameter (eigenvalue=6.78; variability=61.62%). Dim.2 showed a greater contribution of the variables TDM, SDM, root length, R/S, and H/D (eigenvalue=2.01; variability=18.25%). When comparing the factor loadings of Dim. 1, it was possible to verify statistically significant differences between the factor loadings of the treatments ($F_{6.41}$=26.24; $p<0.0001$), with the highest means observed in groups T2 and T3 compared to the others ($p<0.05$), followed by T1 and T4, and finally, T5, T7, and T6 with the lowest means. When comparing the factor loadings of Dim. 2 (root length), there was statistical equality between treatments ($F_{6.41}$=2.08; p=0.081).

In the evaluation of plant development, using 50% organic compounds plus 50% sterile sand, Dim.1 showed a greater contribution associated with S, TDM, SDM, leaf diameter, DQI, RDM, number of leaves, SD, R/S, and H/D (eigenvalue=8.99; variability=81.7%). Dim.2 showed a greater contribution only from the variable root length (eigenvalue=0.70; variability=6.37%). When comparing the factor loadings (Dim.1), statistically significant differences were observed between the factor loadings of the treatments ($F_{6.41}$=50.89; $p<0.0001$), with the highest mean observed in group T3 in comparison to the others ($p<0.05$), followed by T2, T1, and T4, and finally, T5, T7, and T6 with the lowest means. When comparing the factor loadings of Dim. 2 (root length), statistical equality was observed between treatments ($F_{6.41}$=1.98; p=0.096).

Since the first dimensions of the PCAs carried out for the use of 100, 75, and 50% compounds were the most explanatory of the variables related to plant development, the comparison was carried out considering the treatments and the doses of organic compounds. It was evident that there was a statistically significant effect of both factors ($F_{12.105}$=2.804; p=0.0023). Treatments T1, T2, and T3 showed statistically significant differences in the different percentages of organic compounds (p=0.0124; p=0.0267; p=0.0073, respectively).

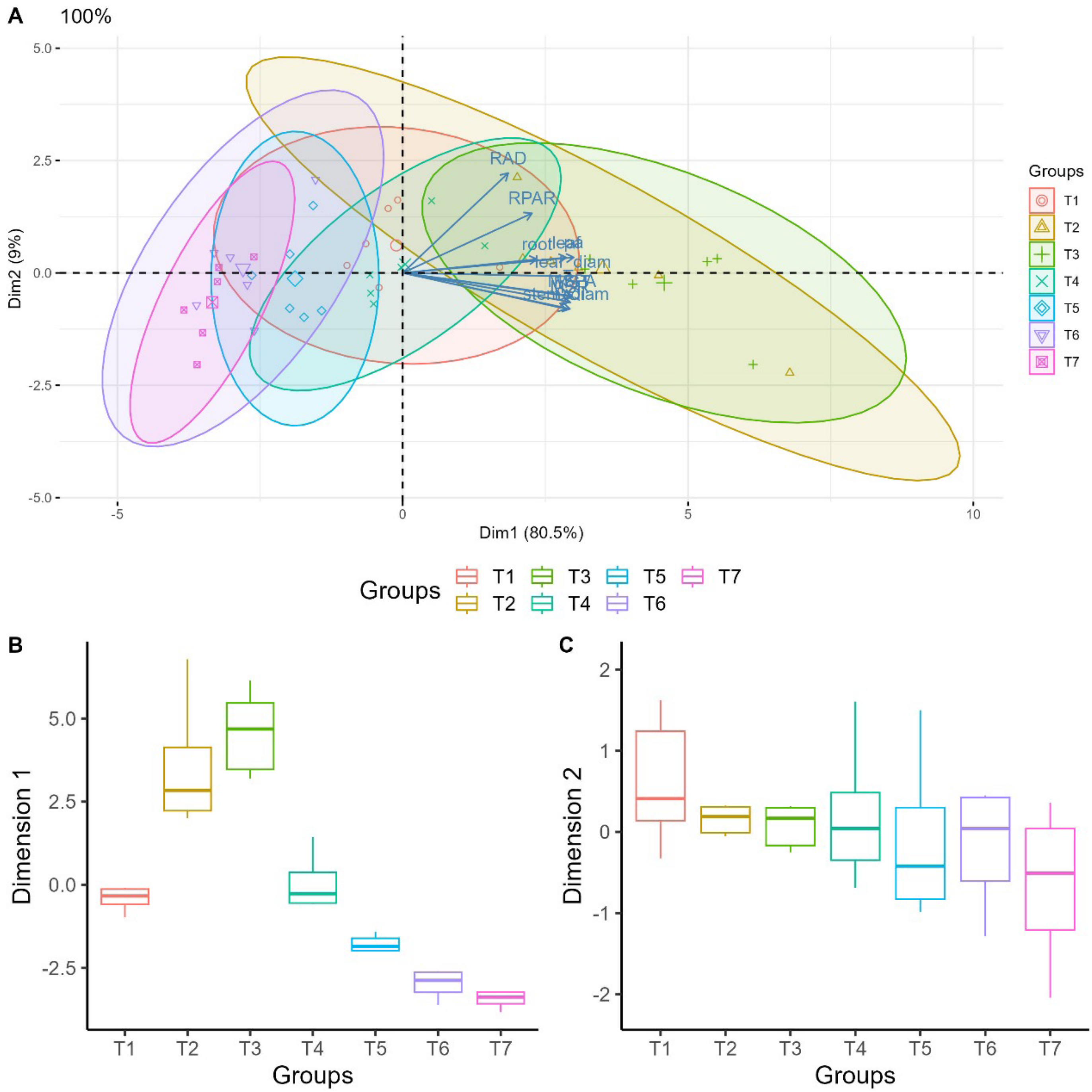


Figure 5 – (A) Principal component analysis ordination diagram, indicating Dimension 1 and Dimension 2. (B) Boxplot (median and interquartile range and interpercentile range) of Dimension 1 between treatments using 100% compound. (C) Boxplot (median and interquartile range and interpercentile range) of Dimension 2 between treatments using 100% compound.

Group T1 showed better plant development with the use of 50% compound+50% sand, group T2 showed the best development with the use of 75% compound+25% sand, and T3 with 100% compound. The other groups did not show statistically significant differences in relation to the use of different percentages of organic compound ($p>0.05$). This statement is confirmed by viewing Figure 6, which illustrates better plant development, such as shoot and root size, in addition to more robust stems (Ripp et al., 2020; Cappelini et al., 2021) with vermicompost produced with co-products of moringa and zoo waste (T1 to T5) compared to commercial composts (T6 and T7).

T1: 70% *T. terrestris* pond sediment+30% fruit and vegetable scraps; T2: 65% *T. terrestris* pond sediment+35% *M. oleifera* valves; T3: 50% *T. terrestris* pond sediment+35% *M. oleifera* valves+15% *M. oleifera* husks; T4: 50% *T. terrestris* pond sediment+40% *M. oleifera* valves+5% *M. oleifera* husks+5% *M. oleifera* whole seed; T5: 50% *T. terrestris* pond sediment+40% *M. oleifera* valves+5% *M. oleifera* husks+5% *M. oleifera* seed cake; T6: commercial compost originated by vermicomposting; T7: commercial compost originated by composting.

Figure 6 – Seedlings obtained from treatments with commercial composts (T6 and T7) and vermicompost produced from pond sediment used by Tapirus terrestris and Moringa oleifera.

Conclusions

Moringa co-products, such as valves and seed husks, represent about 77% of the mass and 99% of the volume of the fruit. These are carbon-rich, non-phytotoxic materials, unlike seeds, especially those containing a lipid portion. The vermicomposts produced were suitable for plant development, enriched with lignite material from the presence of this substance in the valves and seed husks. Throughout the maturation of the vermicomposts, there was a reduction in carbon and pH, alongside increases in EC and in the contents of K, P, and Ca, attributed to the release of ions throughout the stabilization process, without showing toxicity. The use of infrared spectroscopy revealed that the organic compounds present in vermicomposts are rich in aromatic groups, with a decrease in aliphatic groups at the end of the process. Vermicomposts produced with sediment from the pond used by *Tapirus terrestris* and parts of moringa fruit outperformed commercial composts in *Catharanthus roseus* plant development. The treatments that did not receive moringa seeds (T2 and T3) showed better seedling growth. Given the origin of wild animal waste, the use of vermicomposts for food production is not recommended, and further studies should be carried out regarding the use of moringa fruit parts together with other wastes, enabling the valorization of these co-products.

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Authors' contributions

RUBIO, F.: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft. COLDEBELLA, P.F.: supervision, writing – review & editing. BOROSKI, M.: supervision, writing – review & editing. GUIMARÃES, A.T.B.: formal analysis, validation. GONÇALVES, C.C.S.: investigation, project administration, supervision, visualization, writing – review & editing.

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