El Colegio de la Frontera Sur

Efecto de las propiedades fisicoquímicas y microbiológicas de extractos de composta y lombricomposta en la emergencia de plántulas de tomate

Tesis

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Por

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Las personas abajo firmantes, miembros del jurado examinador de: **Samia Berenice Flores Solórzano** hacemos constar que hemos revisado y aprobado la tesis titulada "Efecto de las propiedades fisicoquímicas y microbiológicas de extractos de composta y lombricomposta en la emergencia de plántulas de tomate", para obtener el grado de **Maestra en Ciencias en Recursos Naturales y Desarrollo Rural**.

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Dedicatoria

A mi madre, por su complicidad y escucha activa.

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Resumen

Los extractos de composta y lombricomposta representan una alternativa al uso de fertilizantes y plaguicidas sintéticos, debido a sus efectos benéficos en plantas. El objetivo de este trabajo fue identificar la relación entre el efecto en la germinación de semillas de tomate y las características microbiológicas y fisicoquímicas de extractos obtenidos por distintos métodos. Los factores evaluados fueron: el método de extracción (aireado / no aireado), proporción (composta:agua) y proceso de transformación de los residuos (composta / lombricomposta). La materia prima empleada fue una mezcla de fibra de palma africana, pulpa de café y residuos orgánicos de mercado. Se encontró relación entre la diversidad microbiana y el índice de germinación, así como una dependencia del contenido de nutrientes con el método de extracción empleado. Mediante el análisis discriminante canónico identificamos que los factores que optimizaron el efecto de los extractos en el índice de germinación de semillas, y su relación entre ellos, fueron: los obtenidos de composta, la proporción 1:3 (composta:agua) y el método no aireado.

Palabras clave

Compostaje; Fibra de palma; Aireación; Diversidad microbiana; Índice de germinación.

Introducción

Las prácticas actuales de agricultura como la siembra intensiva, uso de agroquímicos y monocultivo ocasionan el deterioro de la calidad del suelo y la generación de residuos. La inadecuada disposición de esos residuos conduce a problemas ambientales como la contaminación del suelo y del agua debido a lixiviados, la contaminación del aire por la generación de gas en la descomposición, y contaminación visual por la degradación del paisaje (FAO 1990).

Entre las opciones para tratar los residuos agrícolas están el compostaje y el lombricompostaje. Moreno y Moral (2008) definen el compostaje como la descomposición biológica aerobia de la materia orgánica en condiciones controladas, seguida por la estabilización y maduración del producto. El lombricompostaje es el proceso de la transformación acelerada de materia orgánica realizado por lombrices de tierra. Comparado con la composta, en la lombricomposta se obtienen concentraciones más altas de nutrientes y mayor diversidad microbiana (Tognetti et al. 2005). Ambos productos se emplean en el suelo para mejorar el rendimiento de los cultivos y la calidad del mismo (Pane y Zaccardelli 2014; Kiyasudeen et al. 2016).

Otra forma de aplicarlos es en preparados con agua; estos preparados se denominan extractos de composta y tienen efectos benéficos para las plantas; su aplicación a manera de riego o rocío lo convierte en algo fácil de emplear por los agricultores, de esta manera se busca reducir el uso de fertilizantes y plaguicidas (Ingham 2005; Scheuerell y Mahaffee 2002; Bess 2000).

Diversas investigaciones (Pane et al. 2012; Dionne et al. 2012; Koné et al. 2009) han demostrado el efecto positivo de los extractos de composta en aplicación a cultivos para reducir la incidencia de patógenos. Los resultados sugieren que microorganismos específicos (agentes potenciales de control biológico) juegan un papel importante en el efecto supresor contra los patógenos. No obstante, los autores recomiendan realizar más estudios para identificar microorganismos antagonistas contenidos en los extractos.

Aún no existen trabajos que evalúen si la diversidad microbiana de los extractos depende del proceso de tratamiento de los residuos orgánicos (composta contra lombricomposta). Así mismo, son necesarios más estudios para considerar si las propiedades de los extractos dependen del método de extracción. Nuestra hipótesis planteada fue que el método empleado para obtener los extractos influye en la diversidad general de los microorganismos y las características fisicoquímicas de los productos, lo que repercutirá en el efecto de germinación de semillas al ser aplicado.

El objetivo de este trabajo fue identificar la relación entre los parámetros microbiológicos y fisicoquímicos de los extractos obtenidos bajo distintos métodos y conocer el efecto de los mismos en la germinación de semillas. A su vez, se evaluaron los factores relacionados a estos parámetros: método de extracción, proporción (composta:agua) y proceso de transformación adecuado para la mezcla, en este caso compuesta por residuos orgánicos de mercado, pulpa de café y fibra de palma de aceite. Estos residuos provienen de las principales actividades económicas del estado de Chiapas.

El documento presente está organizado en capítulos, donde primero hacemos una introducción al tema, luego exponemos el artículo derivado de este trabajo y finalmente, las conclusiones y recomendaciones basadas en los resultados obtenidos. La literatura citada al final es la utilizada en la introducción o capítulo introductorio, la literatura indicada como referencias pertenece a lo citado en el artículo.

Artículo científico

Compost and vermicompost extraction methods affect the microbial composition, physicochemical properties and effectiveness of seed germination

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Compost and vermicompost extraction methods affect the microbial composition, physicochemical properties and effectiveness of seed germination

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Abstract

Compost and vermicompost extracts represent an alternative to synthetic fertilizers and pesticides because of their beneficial effects on plants. This study identified the relationship between the effect on tomato seed germination and the microbiological and physicochemical characteristics of extracts obtained using different extraction methods. The factors evaluated were the extraction method (aerated/nonaerated), ratio (compost:water) and waste transformation process (compost/vermicompost). The raw material used was a mixture of oil palm fiber, coffee pulp and organic market waste. Relationships were found between microbial diversity and the germination index and between the nutrient content dependence and the extraction method used. Using canonical discriminant analysis, we identified the factors that optimized the extracts' effects on the germination index of the seeds. These factors were extracts obtained from compost, a compost:water ratio of 1:3, and use of the nonaerated method. We also identified the relationship between them.

Keywords

Composting; Palm fiber; Aeration; Microbial diversity; Germination index.

1. **Introduction**

Compost extracts are the products of water preparations using material resulting from the biological transformation of organic waste (compost), which must have known physicochemical properties and maturity (Brinton et al., 1996). The compost:water ratio used most often ranges from 1:3 to 1:10 (weight:volume), which is achieved by agitation and incubated under specific aeration and temperature conditions (Scheuerell and Mahaffee, 2004). The extracts can be derived from compost or vermicompost products, both of which are aerobic, bio-oxidative processes that stabilize organic matter. Using vermicompost products can lead to greater nutrient availability and microbial activity during the extraction process (Dominguez et al., 1997, Subler et al., 1998); applying vermicompost products to soil has been shown to increase plant growth (Kaur et al., 2015, Kashem et al., 2015, Namayandeh and Shirdareh, 2015).

Using compost extracts in agriculture increases crop yield and quality and suppresses pathogenic microorganisms in plants (Islam et al., 2016). The observed benefits depend on factors such as the stability and quality of the substrate used, the compost:water ratio, the incubation time and the extraction method (Hegazy et al., 2015). Extraction methods are classified mainly as either aerated or nonaerated. In the aerated method, the extract receives a constant air flow during the extraction, and the air flow must provide more than 5 mg/l of oxygen to promote aerobic organismal growth. In the nonaerated method, once the compost:water mixture is made, it requires no oxygen input, thus saving energy (Ingham, 2005).

Controversy exists between the advantages and disadvantages of the two methods. Pant et al. (2009) concluded that the extraction method used does not affect the nutrient content or plant growth when applied; however, Marín et al. (2013) showed higher nutrient concentrations in the extracts obtained with aeration. Regarding the extracts' effects on plant growth, Xu et al. (2012b) compared the two extraction methods and observed greater growth in plants stimulated with aerated compost extract. However, no information is available on how the products of these methods stimulate plant growth or seed germination or whether

this effect is related to the extracts' microbial diversity. Xu et al. (2012a) obtained results that differed from others in the literature; these authors found that each residue has characteristics specific to its origin and that these physical and biological characteristics impact the extracts' effectiveness. Using the same waste mixture to compare extraction methods would allow determining each method's contribution to the microbial diversity and physicochemical characteristics of the extracts as well as whether this influences plant development.

Among the microorganisms present in the extracts are beneficial bacteria that compete with disease-causing microorganisms (Ingham, 2005). Also present are actinomycetes that promote plant growth and degrade recalcitrant compounds (Bhatti et al., 2017). Additionally, some fungi have protective mechanisms for plants and soil; fungal biomass retains macro and micronutrients, degrades toxic materials and plant residues, helps form soil structure and improves water retention capacity (Ingham, 2005; Bagyaraj and Ashwin, 2017). The diversity and microbial abundance of the extracts depend on environmental and nutritional factors that are difficult to replicate in the laboratory. Therefore, we hypothesized that the method used to obtain the compost extracts influences the microorganismal abundance and diversity in the products, which could alter the extracts' effectiveness when applied to plants.

Plate counting using specific culture media is a useful method for estimating the number of microbial species culturable from the extracts (Diánez et al., 2018); however, this method represents the minimal microbial community and depends on substrate selectivity, growth conditions and species abundance. Molecular methods provide more information on microbial diversity by including nonculturable species equally important in microenvironmental functions. Molecular methods may help determine the relationships between microbial diversity and the effects of compost and vermicompost extracts.

To date, no studies have evaluated whether the extracts' microbial diversity depends on the organic waste treatment process (compost compared with vermicompost). Thus, more studies are needed to consider whether the extracts' properties depend on the extraction method. Furthermore, the relationship between the extracts' physicochemical and

microbiological characteristics and their effect on seed germination has not been evaluated, and this could optimize the extraction process and expand the extracts' uses.

This work identified the relationship between the microbial and physicochemical parameters of extracts obtained using different methods and determined their effects on seed germination. The factors related to these parameters were also evaluated, including the extraction method, ratio (compost:water) and the adequate transformation process for the mixture, which was composed of organic market waste, coffee pulp and oil palm fiber obtained via the main economic activities of the state of Chiapas.

2. **Materials and methods**

2.1. Biological material

The experiment was conducted at the El Colegio de la Frontera Sur, in Tapachula, Chiapas, Mexico.

The residues used were coffee pulp (*Coffea canephora* L.) from Finca Alianza, oil palm fiber (*Elaeis guineensis* Jacq.) from the Iztaccíhuatl oil processor, and organic waste from the San Juan Market in the city of Tapachula, Chiapas, Mexico. Moisture content, pH, total nitrogen and total carbon were determined from samples of these residues.

2.2. Composting and vermicomposting

A homogenous mixture of the coffee pulp, oil palm fiber and market waste (CP:PF:MW) was developed at a 1:1:1 ratio. The ratios were based on the waste volume to achieve a carbon/nitrogen ratio (C/N) near 30 (Jhorar et al., 1991). A pile 95 cm high and 210 cm long was formed, after which it was manually turned over once weekly. The temperature, moisture, pH and electrical conductivity (EC) of the mixture were monitored during the 24-day precomposting period. The temperature was measured daily at the center and sides of the pile at a depth of 20 centimeters, and samples were taken every five days from the center and sides of the pile to measure pH and EC.

After 24 days, the C/N ratio and moisture of the mixture were determined, and the pile was divided for the simultaneous 80-day composting and vermicomposting processes.

Temperature and moisture content were monitored every five days, and the weekly composting turnovers were continued. The vermicomposting was conducted in two plastic tares of 69 × 40 cm containing 52 kg of the mixture and 2,000 earthworms (*Eisenia fetida* Sav.)/m^2. Lastly, the percentages of organic matter, organic carbon, total nitrogen, pH, electrical conductivity, humic substances and total coliforms were determined for both products. Total coliforms were determined using the most-probable-number method (ISO 4831, 1991).

2.3. Preparation of compost and vermicompost extracts

The extracts were prepared in triplicate using 12 treatments (six for the compost and six for the vermicompost) with half of each group being extracted via the aerated method (Shrestha et al., 2011) and the other half without aeration. In both cases, three mixing ratios of the compost or vermicompost and water (weight:volume) were used: 1:3, 1:5 and 1:10. The 1:3 ratio contained 3 kg of mixture and 900 mL of tap water; the 1:5 ratio contained 2 kg of mixture and 10 L of water; and the 1:10 ratio contained 1 kg of mixture and 10 L of water. These mixtures were placed in 20-L plastic pails with lids and incubated for 72 hours at room temperature (28–32 °C). The aeration treatments were continuously aerated with a 2.5 W air pump (SAP-300) with a maximum flow rate of 1.8 L/min. Two controls were used with only water, one aerated and one nonaerated. After the incubation, the extracts were filtered with galvanized steel-woven mesh, and 400 mL of the samples were stored in 500-mL amber glass flasks.

Immediately after filtering, the extracts were diluted to plate and count the viable microorganisms; the extracts were then stored at 4 °C for further analysis.

2.4. Physicochemical parameters

Nitrogen in the compost and vermicompost was determined using a micro-Kjeldahl method (NOM-021-SEMARNAT-2000) (DOF, 2002); the percentages of moisture and organic matter were measured using the NMX-FF-109-SCFI-2008 standard (SEGOB, 2008). Humic substances were extracted using the Kononova method (1966), with $Na_2P_2O_7 \bullet 10H_2O$ as the extractant solution, using 5 g of compost or vermicompost. The pH and EC of compost and

vermicompost mixtures and extracts was measured with a multielectrode CONDUCTRONIC PC18 portable.

For the compost and vermicompost extracts, the total nitrogen content was determined by the persulfate digestion method, and the total phosphorus content was determined by the molybdovanadate method with acid persulfate digestion, both by colorimetry using HACH® DR/890 equipment (2013) for wastewater because the samples were similar in organic matter content.

2.5. Microbial diversity of the extracts

The microbial diversity was studied by determining the bacterial, actinomycetes and fungal colony-forming units (CFUs) and analyzing the diversity by denaturing gradient gel electrophoresis (DGGE) (bacteria and actinomycetes).

2.5.1. Determining colony-forming units

Viable microorganisms were counted using serial dilutions (10^{-1} to 10^{-5}) of the extracts, with 1% peptone water, sterilized in an autoclave. In duplicate, 100 µl of each dilution was inoculated into Petri dishes with culture medium according to microorganismal group. Nutrient agar was used for bacterial growth, and oat agar was used for actinomycetes (Franco-Correa, 2008), both with nystatin (100,000 U/L). The bacteria were incubated at 37°C for 24 hours, and the actinomycetes at 25°C for five days. Colonies were counted at the 10^{-4} and 10^{-5} dilutions. Fungal CFUs were counted 48 and 96 hours after sowing in potato-dextrose agar medium (PDA) with chloramphenicol (0.50 mg/mL); 10^{-1} and 10^{-2} dilutions were counted and incubated at 25 °C. The results are reported in log-base 10 of the CFU/mL for each treatment.

2.5.2. Diversity analysis by DGGE

DNA was extracted from the compost and vermicompost extracts following the methodology of Cheng et al. (2016) with some modifications. First, 6 mL of each sample was centrifuged at 8,000 RCF for five minutes to obtain a pellet of 0.10 g. The pellet was washed with 1 mL of prewash buffer (pH 10.0) consisting of 100 mM Tris, 100 mM Na_2HPO_4, 1% polyvinylpyrrolidone (PVP), 100 mM $NaCl_2$, 0.05% Triton X-100 and 4% skim milk (Xi et al.,

2006). The pellets were then vortexed for one minute, incubated at 55 °C for five minutes and centrifuged at 12,000 RCF for 5 min. The supernatant was then discarded, and the procedure was repeated three times.

Next, 0.6 mL of $CaCl_2$ (0.5 M) and 1 mL of sterile water were added to the prewashed samples and centrifuged at 12,000 RCF for 10 min. The supernatant was discarded, and the cells were lysed with 1 mL of buffer at pH 8.0 (100 mM Tris-HCl, 100 mM Na_2HPO_4, 1.5 M NaCl, 1% cetyl trimethylammonium bromide [CTAB]), 200 µL of s*odium* dodecyl sulfate (SDS) and glass beads. The mixture was vortexed and centrifuged at 8,000 RCF for 15 min. Next, 500 µL of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the supernatant, stirred and centrifuged at 10,000 RCF for 10 min. The supernatant was transferred, and 500 µL chloroform-isoamyl alcohol (24:1) was added, vortexed again and centrifuged at 10,000 RCF for 10 min. Next, 500 µL of isopropanol was added to the supernatant, and 5 µL of sodium acetate (3 M pH 5) was left to precipitate at -20 °C and centrifuged at 13,000 RCF for 20 min. The precipitate was washed with 70% ethanol and resuspended in 30 µL of ultrapure water.

The bacterial 16S rDNA fragments were amplified by polymerase chain reaction (PCR) using the oligonucleotides, F984GC (CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGGGCGCAACGC GAAGAACCTTAC) and R1378 (CGGTGTGTACAAGGCCCGGGAACG) (Nübel et al., 1996). Semi-nested PCR was used for the actinomycetes; in the first reaction, the oligonucleotides F243 (GGATGAGCCCGCGGCCTA) and R1378, in the second reaction, F984GC and R1378 were used (Heuer et al., 1997). The 18S fungal region was amplified using GCFUNG (GCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCATTCCCCGTTAC CG) and NS1 (GTAGTCATATGCTTGTCTC) (Guillén-Navarro et al., 2015).

The PCR mixture for the bacterial amplification and the first PCR for actinomycetes were performed using 1 µL of DNA, 1x buffer, 5% dimethyl sulfoxide (DMSO), 0.2 pM mL^{-1} of

each primer and dNTP, 1.5 nM $MgCl_2$ and 1 U of *Taq* DNA polymerase for a final volume of 50 µL. The amplification conditions were as follows: initial denaturation at 96 °C for 5 min, followed by 35 cycles of denaturation at 96 °C for 1 min, alignment at 62 °C for 1 min and extension at 72 °C for 1 min, ending with a final extension at 72 °C for 10 min. The second actinomycetes amplification reaction was performed using 1 µL of annealed PCR product without DMSO, using the following protocol: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 45 s and extension at 72 °C for 2 min, with a final extension at 72 °C for 5 min. The 18S fungal region could not be amplified after several attempts under different PCR conditions.

DGGE was performed using a DCode™ Universal Mutation Detection System (Bio-Rad, USA). Equal amounts of the PCR products (500 ng) were placed in polyacrylamide gels with a denaturing gradient of 20–60% of urea and formamide, and electrophoresed in 1% TAE buffer (40 mM TAE, 2 mM Tris-acetate, and 1 mM Na_2EDTA, pH 8.5). The bacteria were analyzed using 8% acrylamide, and the actinomycetes were analyzed using 6% acrylamide. Both gels were electrophoresed at 90 Volts and 60 °C for eight hours. Sybr Gold at 1x was used to stain the gels.

2.6. Germination test

The seeds were soaked for 24 h in both the controls and the compost and vermicompost extracts diluted to 7.5% in distilled water (previous testing revealed this to be the adequate dilution percentage). The seeds were then placed in 241-cell seedling trays with substrate previously prepared with 35% palm fiber, 25% soil and 40% vermicompost. Ten tomato seeds (organic *Solanum lycopersicum* L.) were seeded in triplicate for all extracts, and 10 seeds for each control (aerated and nonaerated) were seeded in a randomized design. Seedling emergence was verified over 15 days. For the analysis, the germination index, also known as Kotowski's coefficient of velocity (1926), was calculated as $IG = \Sigma n_i/\Sigma n_i t_i$, where IG is the germination index, n_i is the number of seeds germinated on day i, and t_i is the number of days between sowing and seedling emergence.

2.7. Experimental design and statistical analysis

The compost and vermicompost extract treatments were prepared in a 2 × 2 × 3 factorial design in blocks of three (one block per repetition). The factors to be considered were the transformation process of the organic matter (composting or vermicomposting), the extraction method (aerated or nonaerated), and the ratio used (1:3, 1:5, or 1:10 [w:v]). In total, 12 treatments (C10, compost nonaerated extract with ratio 1:10; Ca10, compost aerated extract with ratio 1:10; V10, vermicompost nonaerated extract with ratio 1:10; Va10, vermicompost aerated extract with ratio 1:10; C5, compost nonaerated extract with ratio 1:5; Ca5, compost aerated extract with ratio 1:5; V5, vermicompost nonaerated extract with ratio 1:5; Va5, vermicompost aerated extract with ratio 1:5; C3, compost nonaerated extract with ratio 1:3; Ca3, compost aerated extract with ratio 1:3; V3, vermicompost nonaerated extract with ratio 1:3; Va3, vermicompost aerated extract with ratio 1:3) were analyzed with three repetitions, an aerated control with water and air flow for 72 hours and a nonaerated control with only water.

The DGGE banding pattern was analyzed using 2010 Gel Analyzer software. The Shannon-Weaver index (1963) was used to evaluate the general bacterial and actinomycetes diversities in each sample. The richness index (S) was used to represent the total number of bands per treatment, and the Jaccard index (Real and Vargas, 1996) was used to determine the similarities between microorganismal groups from the extracts aerated vs nonaerated and compost vs vermicompost.

Statistical analyses were performed using R Core Team statistical software (2018). Analysis of variance (ANOVA) with multiple factors (type II) and Tukey's test (HSD) were used to compare means by independent variables. We also performed a multivariate analysis of variance (MANOVA) II with Pillai's test and the canonical discriminant analysis to evaluate differences between treatments and the variables related to each group.

3. **Results**

3.1. Composting and vermicomposting

The initial total nitrogen and organic carbon contents (Table 1) of the waste allowed us to reach a C/N ratio of 28 in the mixture of the three residues (CP:PF:MW) in equal parts.

During the precomposting process, the C/N ratio decreased to 20, and the nitrogen and pH increased, with the latter stabilizing when the composting (7.9) and vermicomposting (7.5) ended. The final nitrogen contents were 3.11% in the compost and 3.54% in the vermicompost, with C/N ratios of 15 and 13, respectively.

Table 1. Physicochemical characteristics of the sources used for the production of compost and vermicompost extracts.

Source	Total nitrogen (%)	Organic matter (%)	C/N	pH	EC (dS/m)
Coffee pulp	2.30	93.06	23	4.7	2.83
Oil palm fiber	1.45	95.99	38	5.9	1.76
Organic market waste	2.51	94.04	22	5.5	1.08
Mix (1:1:1)	1.92	92.07	28	5.2	1.40
Precomposting (24 days)	2.58	93.41	20	9.2	1.84
Final composting	3.11	79.42	15	7.9	0.72
Final vermicomposting	3.54	81.50	13	7.5	0.77

C/N, carbon to nitrogen ratio; EC, electrical conductivity.

During precomposting, the temperature was maintained at 60 °C for ten days, and then, it began decreasing towards day 18. For the vermicomposting, prior to introducing the worms, the temperature of the mixture was adjusted to 34 °C, and the pile height was lowered. After adding the worms, the pile was maintained between 28 and 30 °C. Conversely, the compost pile temperature continued to decrease until day 70 and then stabilized to between 33 and 36 °C until the process ended (supplementary material, Fig. S1).

The percentage of humic substances (total extractable carbon) of the compost (6.80%) was greater than that of the vermicompost (5.65%). The compost also had a higher total coliform content (184 NMP/g) than did the vermicompost (110 NMP/g).

3.2. Determining physicochemical parameters of the compost and vermicompost extracts

The nitrogen and total phosphorus of the extracts differed significantly ($p < 0.001$) between the ratios, with the 1:3 ratio retaining the highest elemental content (Fig. 1). The aeration factor also differed significantly (phosphorus: $p < 0.001$, nitrogen: $p < 0.05$) for both variables: treatments with constant airflow had higher nitrogen and phosphorus contents than did the nonaerated treatments. No differences were observed between the compost and vermicompost. The aerated control (water with air flow) presented 7 mg/L of total nitrogen and 1.7 mg/L of total phosphorus. The nonaerated control (water only) presented 5.7 mg/L of nitrogen and 0 mg/L of phosphorus.

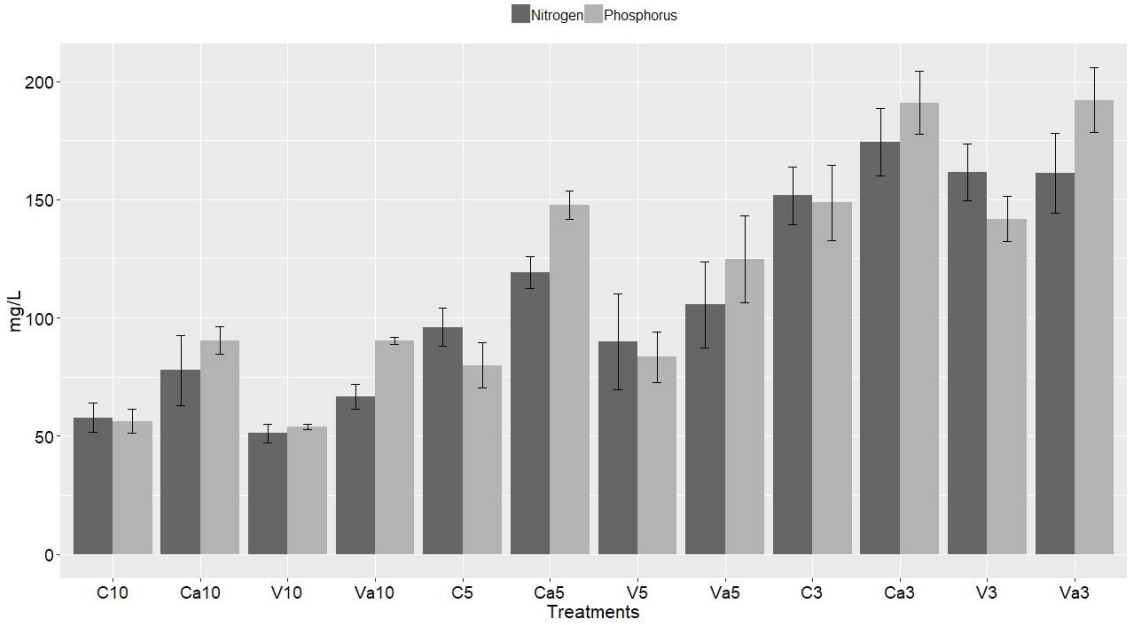


Fig. 1. Total nitrogen and phosphorus from the extracts. Means ± standard error. C, compost; a, aerated; V, vermicompost; 10, ratio 1:10; 5, ratio 1:5; 3, ratio 1:3.

The pH and EC of the extracts showed similar tendencies; both increased as the compost or vermicompost ratio increased (Fig. 2). The pH was higher by 0.2 in the aerated extracts than

in the nonaerated ones, and the electrical conductivity was also higher for the aerated extracts with a difference of 0.2 to 0.5 dS/m. The pH of the aerated control was 7.25, and the EC was 0.23 dS/m. The values for the nonaerated extract were 7.1 and 0.22 dS/m, respectively.

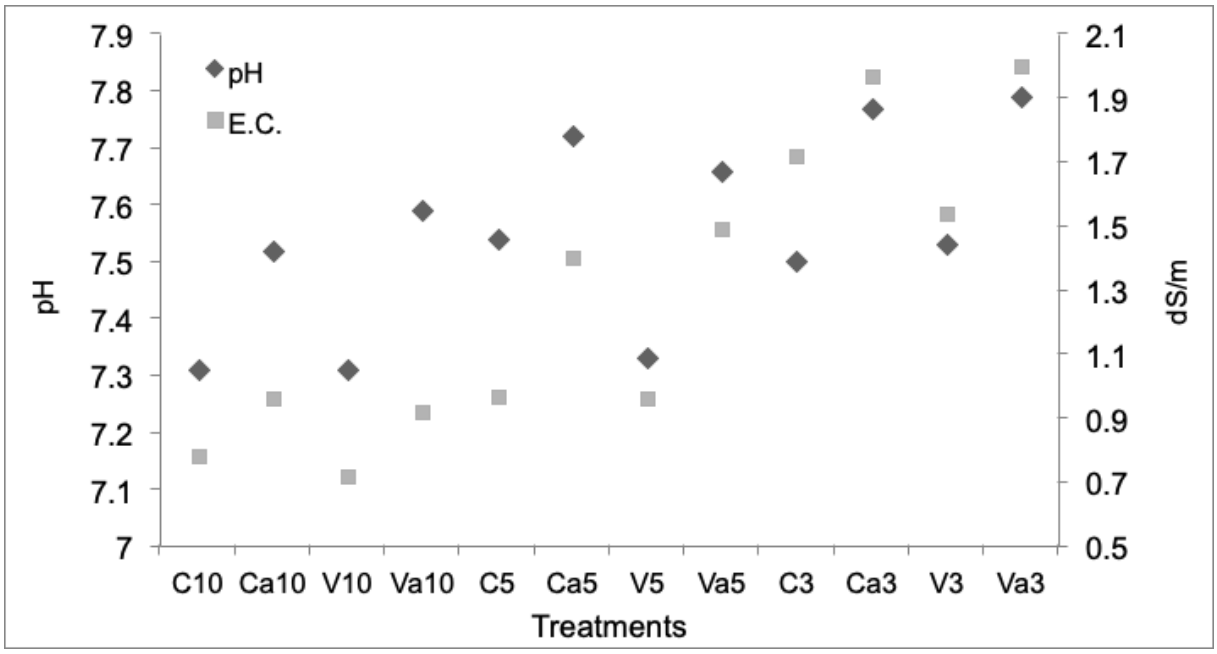


Fig. 2. pH and electrical conductivity (E.C.) from the extracts. C, compost; a, aerated; V, vermicompost; 10, ratio 1:10; 5, ratio 1:5; 3, ratio 1:3.

3.3. Microbial diversity in the extracts

3.3.1. Colony-forming units

Microbial diversity as determined by the CFUs showed that the nonaerated compost extracts at the 1:3 ratio contained the most microorganismal colonies (bacteria, actinomycetes and fungi) (Fig. 3). Bacterial and actinomycetes CFUs differed significantly between treatments with different ratios (w:v) ($p < 0.01$). Cultured fungi and yeasts presented differences statistically significantly between aerated and nonaerated extracts ($p < 0.05$), at between compost and vermicompost extracts ($p < 0.001$) and between ratios ($p < 0.001$). The controls yielded no CFUs for any of the microorganismal group dilutions.

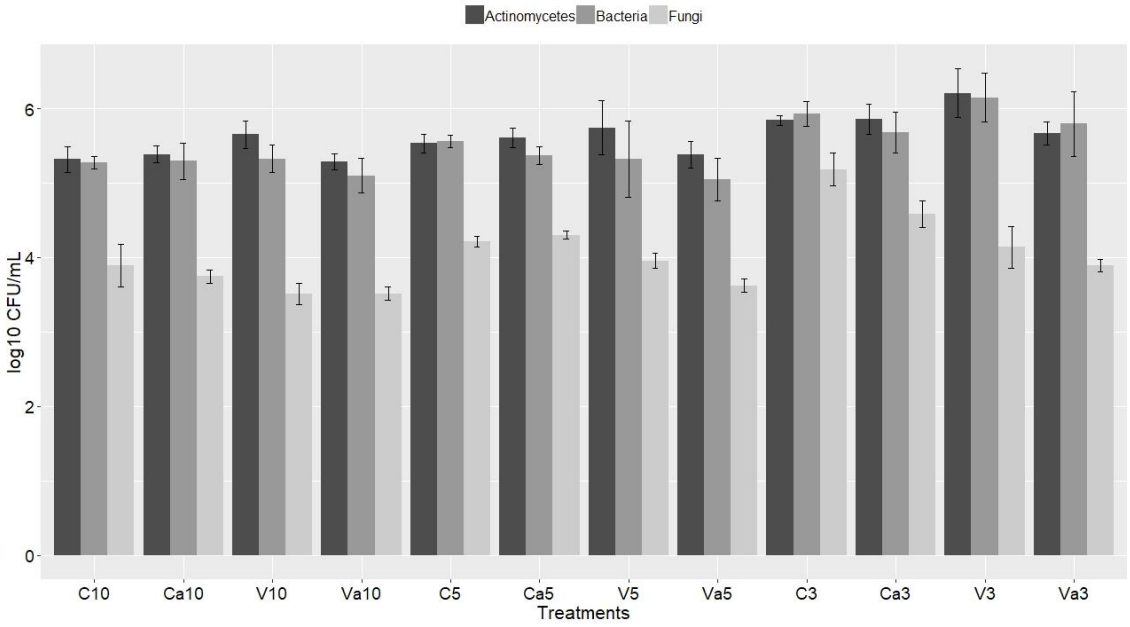


Fig. 3. Colony-forming units (CFU) of bacteria, actinomycetes, and fungi in extracts. Means ± standard error. C, compost; a, aerated; V, vermicompost; 10, ratio 1:10; 5, ratio 1:5; 3, ratio 1:3.

3.3.2. Diversity analysis by DGGE

The Shannon-Weaver indices derived from the DGGE analyses showed that all treatments had high bacterial diversities (greater than 1), and this was maintained between the treatments (Table 2). The differences in banding patterns between the compost and vermicompost extracts are reflected in Jaccard's index, which showed a similarity between 56% and 64% between the aerated and nonaerated extracts. A smaller similarity (44% to 61%) was observed between the compost and vermicompost extracts (Table 2).

Table 2. Shannon-Weaver Index (H) and Jaccard Index from compost and vermicompost extracts.

Shannon-Weaver Index (H)			Jaccard Index(J)					
			Aerated / Non-aerated			Compost / Vermicompost		
Treatments	Bacteria	Actinomycetes	Treatments	Bacteria	Actinomycetes	Treatments	Bacteria	Actinomycetes
C10	1.06	1.10	Ca / C10	0.56	0.64	C / V10	0.50	0.61
Ca10	1.10	0.98						
V10	1.07	0.88	Va / V10	0.61	0.36	Ca / Va10	0.58	0.31
Va10	1.22	0.83						
C5	1.10	0.69	Ca / C5	0.58	0.43	C / V5	0.44	0.11
Ca5	1.13	0.69						
V5	1.10	0.69	Va / V5	0.58	0.33	Ca / Va5	0.55	0.33
Va5	1.14	0.84						
C3	1.24	1.07	Ca / C3	0.62	0.20	C / V3	0.61	0.23
Ca3	1.20	0.74						
V3	1.04	0.60	Va / V3	0.64	0.28	Ca / Va3	0.55	0.22
Va3	1.07	0.70						

C, compost; a, aerated; V, vermicompost; 10, ratio 1:10; 5, ratio 1:5; 3, ratio 1:3

All treatments shared seven bands in the bacterial DGGE profile, and two bands were more intense than the others, indicating that these populations were more abundant in the extracts. The aerated extracts generally presented greater species richness (S) since more bands were observed (Fig. 4a).

For actinomycetes, the diversity was lower than in the bacteria; the extracts with the highest Shannon index were compost extracts C10 and C3. Compost extracts generally responded better without aeration, while vermicompost extracts were more diverse with aeration. As the compost or vermicompost ratio increased in the extracts, the community similarity (Jaccard's index) decreased; the nonaerated extracts at ratios of 1:10 conserved the most species among them. In the DGGE profile for actinomycetes, the extracts shared only one band, which was more intense than the others (Fig. 4b).

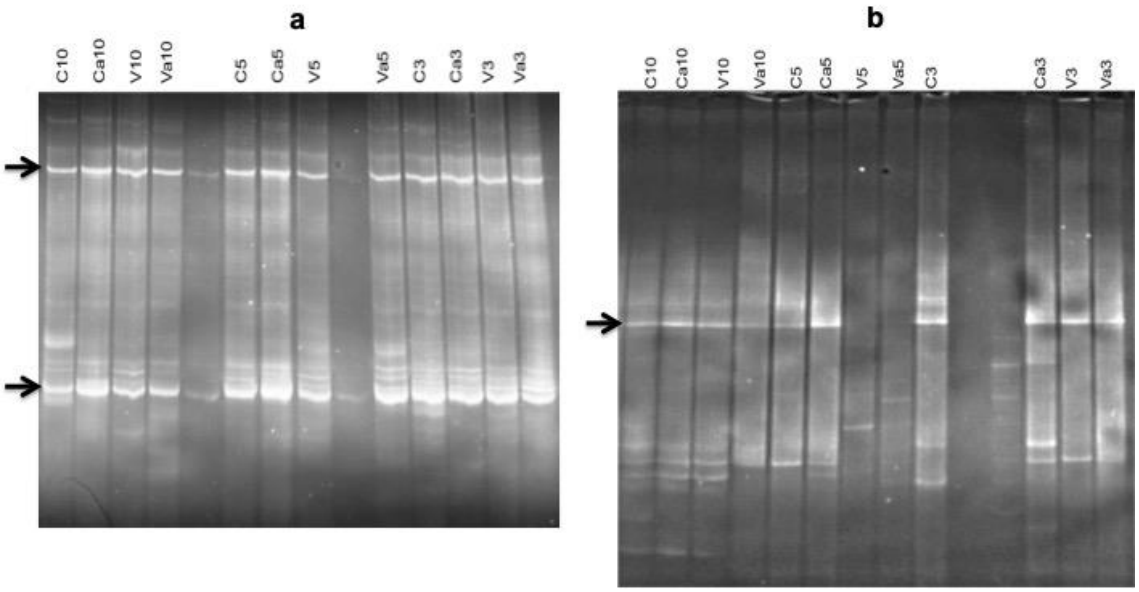


Fig. 4. Bacterial (**a**) and actinomycetes (**b**) DGGE profiles of amplified 16S rDNA fragments obtained from compost and vermicompost extracts. C, compost; a, aerated; V, vermicompost; 10, ratio 1:10; 5, ratio 1:5; 3, ratio 1:3.

3.4. Germination test

Eighty percent germination was achieved within ten days of sowing for all extracts. The C3 treatment had the highest germination rate since it reached 80% germination five days after sowing and 93% germination seven days after sowing (Fig. 5). No trend was observed between ratios; however, the nonaerated extracts showed higher germination rates than the aerated extracts. The germination indices for the controls were 0.23 for the aerated control and 0.16 for the nonaerated control.

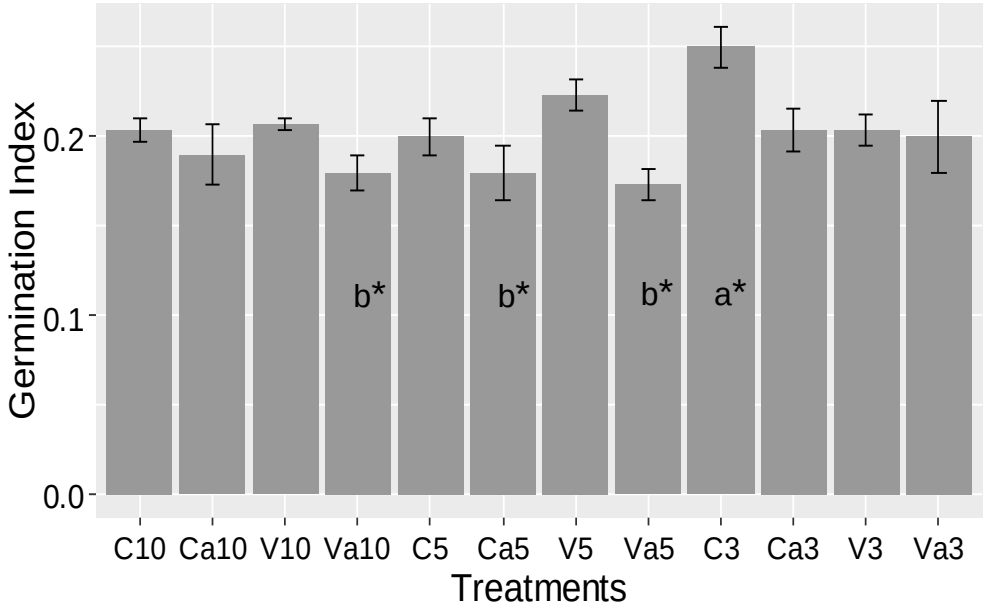
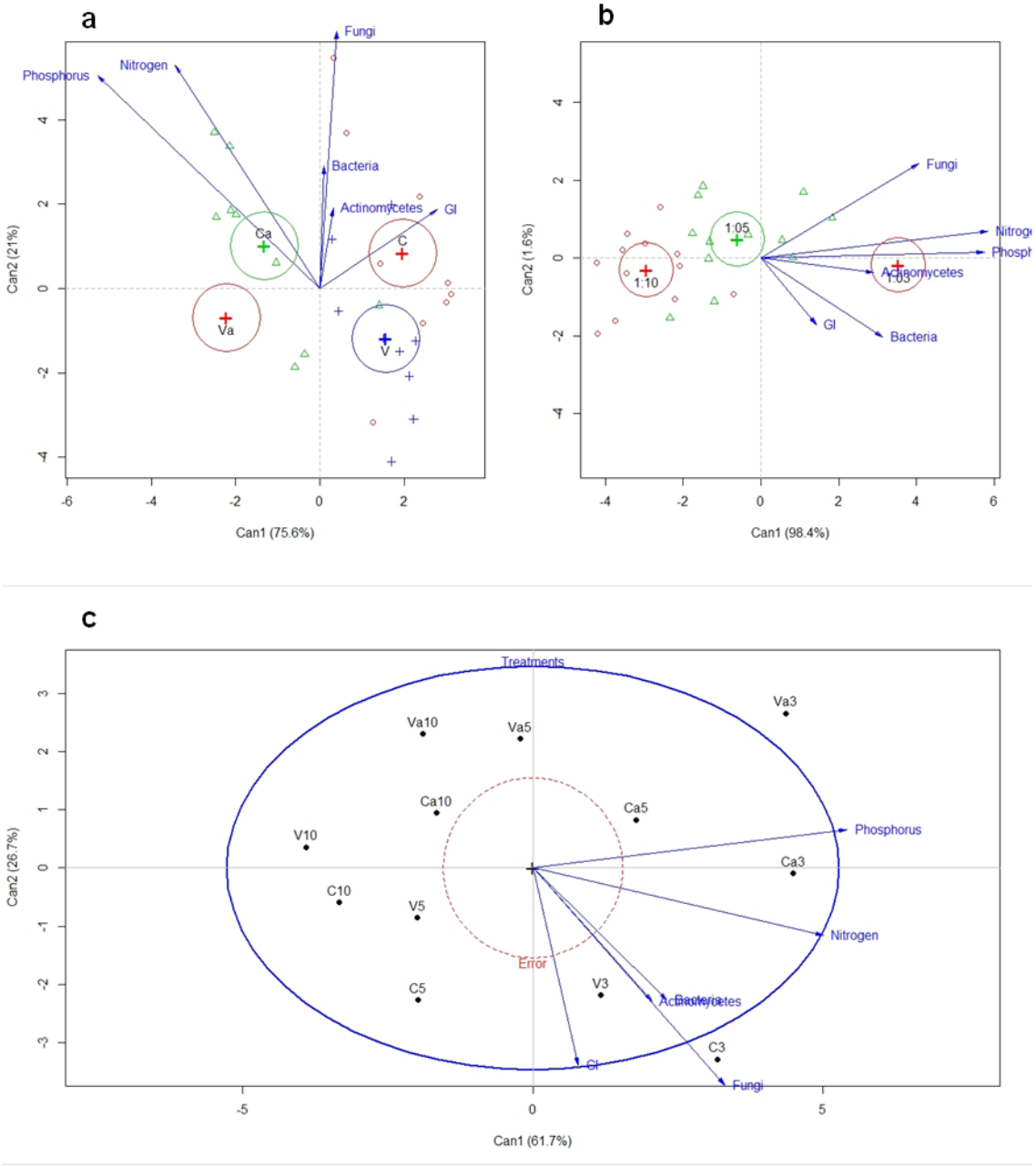


Fig. 5. Effect of compost and vermicompost extracts on seed germination index of tomato. Means ± standard error, dissimilar letters indicate significant differences according to Tukey's HSD test ($P < 0.05$). C, compost; a, aerated; V, vermicompost; 10, ratio 1:10; 5, ratio 1:5; 3, ratio 1:3.

3.5. Relationship between physicochemical and microbiological parameters

The multivariate analysis shows highly significant differences ($p < 0.001$) between the treatments and between the ratios. Canonical discriminant analysis showed that the differences between the aerated and nonaerated extraction methods were explained by 75.6% in axis 1,

and the differences between the compost and vermicompost were explained by 21% in axis 2 (Fig. 6a). Nitrogen and phosphorus were strongly correlated with the aerated compost extracts, which is in contrast to the microbiological variables and germination index, which were mostly explained by the nonaerated compost extracts. Fig. 6b shows that the differences are represented by the macroelements and the actinomycetes CFUs because these variables correspond to what is explained by axis 1 (98.4%). These variables, and to a lesser extent the germination index and the other microbiological variables, suggest that the best treatments are extracts with a 1:3 ratio.



373

374 **Fig. 6.** Canonical discriminant analysis between extracts and characteristics (nitrogen,
375 phosphorus, germination index and microorganisms). **a**) Correlation between compost and
376 vermicompost, aerated and non-aerated extracts. **b**) Correlation between ratios 1:10, 1:5 and
377 1:3. **c**) Correlation between all the treatments. Treatments are significantly different according to
378 Type II MANOVA Pillai's test ($P < 0.001$). C, compost; a, aerated; V, vermicompost; 10, ratio
379 1:10; 5, ratio 1:5; 3, ratio 1:3, GI, germination index.

380

Fig. 6c shows that all the variables are strongly related to the extracts with 1:3 ratio. The macroelements were not correlated with the microbiological variables. Germination index and microbiological variables tends to increase along axis 2, which indicates its relationship with the nonaerated extracts (axis 2 explains the differences between the aerated and nonaerated methods).

4. **Discussion**

The mixture of the three residues (CP:PF:MW) adequately achieved the optimal C/N ratio at the beginning of composting; the ratio should be between 25 and 35 (Jhorar et al., 1991) to avoid nutrient loss without delaying decomposition. After monitoring the compost and vermicompost for 100 days, the maturity parameters showed a stable end product. The final C/N ratio was less than 20, the pH was between 5.5 and 8.5, the nitrogen percentage was between 1 and 4%, and the coliform content was less than 1000 NMP/g (SEGOB, 2008); these values are adequate levels for using in soil and extract production.

Aerated extraction further improved the nitrogen and total phosphorus values, pH and EC likely because aeration promotes organic matter oxidation (which can trigger nitrification processes). Conversely, in the nonaerated treatments, the lack of oxygen in the environment can lead to denitrification processes (the passage of inorganic forms of nitrogen to nitrogen gas, Van Loosdrecht, 1998) and phosphorus assimilation by microorganisms. Increasing the nitrogen and phosphorus values available in the aerated extracts increased the concentration of dissolved salts as measured by the electrical conductivity (Sanchéz-Monedero, 2001). However, all EC levels were suitable for growing plants such as tomatoes (FAO, 2013).

The physicochemical parameters were also affected by the ratio, with 1:3 being the ratio that conserved the highest elemental content, producing the most organic matter (3 kg of compost/vermicompost per 9 liters of water). Hegazy et al. (2015) compared compost extraction

406 ratios and also observed that as the compost ratio increased, the nutrient concentration,
407 conductivity, pH and total bacterial, fungal and actinomycetes CFUs increased.
408 In our study, the number of colony-forming units also increased as the ratio increased, although
409 the microbial diversity (H) determined by the DGGE showed that the actinomycetes had the
410 greatest diversity at the lowest ratio (1:10). In addition, fewer fungal colonies were observed
411 compared with other groups, while the bacterial group had the most CFUs. This could explain
412 the difficulty in amplifying the 18S region in the fungal diversity analysis in the compost and
413 vermicompost extract samples. Therefore, other strategies will be required to determine the
414 fungal structure since the effect of nonculturable fungi could be relevant in developing
415 greenhouse seedlings.
416 Aeration and vermicompost negatively impacted the bacterial and fungal groups; however, these
417 factors could be positive depending on which microorganisms are eliminated. The selective
418 feeding of bacteria and fungi by *Eisenia fetida* could favor selecting beneficial microorganisms
419 and reducing microbial biomass (Schonholzer et al., 1999). In this case, the raw material
420 (compost and vermicompost) presented fewer total coliforms in the vermicompost, as has been
421 described in other works (Monroy, 2008).
422 In the DGGE bacterial profiles, most aerated extracts were more diverse than those in the
423 nonaerated extracts, while the plate cultures contained more CFUs in the nonaerated extracts
424 and tended to decrease when the compost or vermicompost ratios decreased. Diánez et al.
425 (2018) indicated that aeration promotes greater bacterial diversity in compost and vermicompost
426 extracts. Our results suggest that conditions in the aerated treatments favored the growth of
427 some bacterial groups considered nonculturable per the molecular method used (DGGE), while
428 the nonaerated extracts favored culturable groups and presented reduced overall diversity.
429 Either the waste mixture or the transformation process promoted microorganismal species that
430 were unaffected by the extraction factors, as shown in the DGGE bands that were repeated in all
431 extracts. However, Jaccard's index showed that most species were not conserved between

extracts, aeration or product type, which, similar to the ratio, determined the selectivity or proliferation of microorganisms. Bacteria showed the greatest similarity between communities, mainly when comparing the aerated and nonaerated extracts, since approximately 60% of the bacterial community presented no differences between extraction methods.

Actinomycetes diversity was positively influenced by the compost extracts. This microbial group decomposes the more degradation-resistant organic matter and contributes to producing humic substances in soils (Bhatti et al., 2017), which could explain the higher percentage of humic substances in the compost (determined before preparing the extracts). Actinomycetes produce spores and can therefore remain present at varying temperatures and in anaerobic environments for long periods, so their diversity index in nonaerated extracts does not decrease, but the population similarity varies between treatments, as indicated by Jaccard's index. Actinomycetes presence in the extracts is highly important, since actinomycetes promotes plant and root development and can contribute to pathogen control in plants (Jeffrey, 2007).

The extract with the highest bacterial diversity was the C3 treatment, which was also among those that showed the highest actinomycetes diversity. The C3 treatment extract also presented the most colonies in all cultured microbial groups and the best germination index. Our results suggest that a balance between microbial diversity and nutrient content in compost extracts can accelerate seed germination.

Nitrogen and phosphorus are necessary for crop development; however, excessive nitrogen and phosphorus in the seedling stage can trigger seedling loss and decrease the emergence rate (Ciampitti et al., 2006). The nonaerated extracts, in addition to having the lowest nutrient concentrations compared with the aerated extracts, also maintained similar nitrogen and phosphorus concentrations. In crops, a balance must be maintained between nutrients to promote their assimilation by the plant, and other nutrients can be affected by aeration, thus contributing to the germination effects. Arancon et al. (2012) concluded that low macronutrient concentrations are optimal for accelerating germination and attributed germination to

458 biomolecule production by microorganisms because they found concentrations of
459 phytohormones in their extracts.
460 Aerated extracts may contain more phosphorus-solubilizing microorganisms as well as
461 microorganisms involved in nitrification processes. Fritz et al. (2012) found *Nitrosospira* and
462 *Nitrosovibrio* bacteria in aerated extracts; these are aerobic bacteria that participate in
463 nitrification processes. These microorganisms could be related to the greater bacterial diversity
464 (H) in the aerated extracts and the higher content of nutrients in these extracts. Microorganisms
465 have also been found to be involved in producing plant growth hormones and antagonistic
466 metabolites of pathogens in plants (Pant et al., 2012; Marín et al., 2013), explaining how the
467 microbial diversity and community as a whole influence the extracts' effects.
468 Comparing the extracts (Fig. 6c) shows that all the variables were influenced by the compost
469 extracts and that nutrient content was strongly correlated with aeration. Thus, we conclude that
470 the aeration in the extracts favors the microbial metabolisms involved in oxidizing organic matter,
471 supported by the bacterial diversity (H), which was greater in the aerated extracts. Comparing
472 the ratios in the extracts revealed more microbial and macronutrient groups with higher compost
473 or vermicompost contents (ratio 1:3) since this increases the amount of organic matter, food and
474 surface available for microorganisms to develop. Analysis of all the extracts showed that
475 treatment Va3 had the highest macronutrient content, and C3 favored all the microbial groups.
476 Thus, the aeration method may be useful for other purposes, such as soil fertilization, since it
477 promotes increased macronutrients.
478 Diversity among culturable and nonculturable microorganisms was higher in the production of
479 nonaerated compost extracts at a 1:3 ratio. This treatment was unrelated to high macronutrient
480 contents, but it was related to seed germination, implying that microbial diversity promotes
481 germination rates in tomato seeds. Regarding the nutrient concentrations, most nonaerated
482 extracts showed a balance between nitrogen and phosphorus, a factor suggesting that it is a
483 determinant for germination.

Declaration of interest

None.

Acknowledgments

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Supplementary material

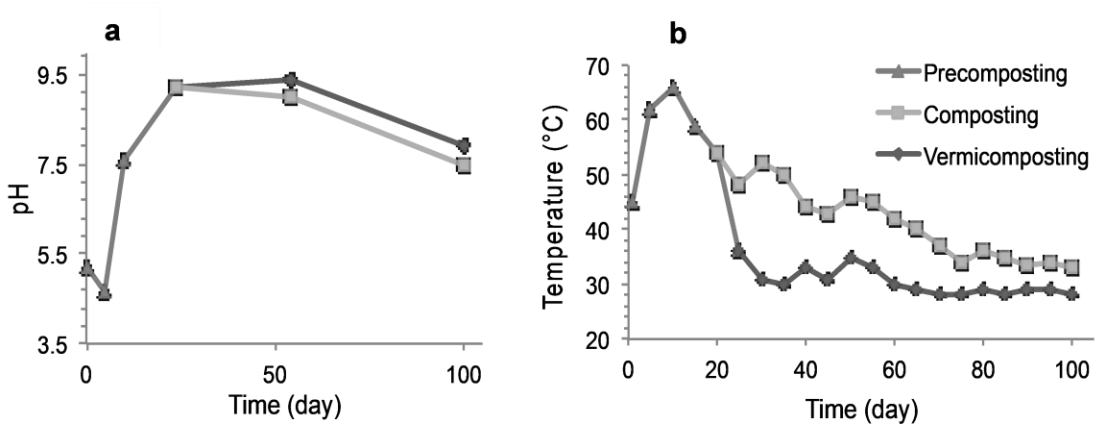

Fig. S1. Changes in (a) pH and (b) pile temperature at stages of precomposting, composting and vermicomposting of the waste mix of oil palm fiber, coffee pulp and organic market wastes.

Conclusiones

El método empleado para la producción de los extractos influye en sus características fisicoquímicas y microbiológicas. Las técnicas moleculares nos brindan un panorama más certero de la diversidad microbiana que existe en los extractos y las diferencias entre los métodos.

El método aireado fue útil para aumentar la cantidad de nutrientes y C.E. en los extractos, también promovió la diversidad microbiana. El compostaje demostró ser el proceso de transformación adecuado para la mezcla de residuos: fibra de palma, pulpa de café y residuos orgánicos de mercado (1:1:1). La proporción 1:3 favoreció a los parámetros evaluados, aumentando su concentración o diversidad.

La diversidad de microorganismos cultivables y no cultivables fue mayor en los extractos de composta no aireados con proporción 1:3, este tratamiento no mostró relación con altos contenidos de macronutrientes, pero si se relacionó con un mayor índice de germinación de semillas, implicando que la diversidad microbiana promueve la velocidad y el porcentaje en la germinación de semillas de tomate. En cuanto a la concentración de nutrientes, la mayoría de los extractos no aireados mostraron un equilibrio entre los contenidos de nitrógeno y fósforo, factor que sugiere ser determinante para la germinación.

Nuestros resultados nos permiten recomendar el uso de extractos de composta no aireados para favorecer la emergencia de plántulas de tomate. Es de suma importancia monitorear los procesos de compostaje o lombricompostaje y evaluar los productos obtenidos antes de elaborar el extracto y recordar que cada mezcla de residuos podría generar diferentes resultados.

Para posteriores trabajos sugerimos realizar identificación de microorganismos que podrían repercutir en los efectos de los extractos, ampliar las pruebas de emergencia de semillas a otro tipo de plantas e intentar otros métodos de PCR para conocer la diversidad de hongos en los extractos.

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