

OPTIMIZED SUBSTRATE FORMULATION USING INDUSTRIAL INSTANT COFFEE RESIDUE FOR GROWING AMARANTH AND CHIA MICROGREENS

Erika Piña-Moreno¹, Otto Raúl Leyva-Ovalle², Mirna López-Espíndola¹,
Adriana Contreras-Oliva¹, Emmanuel de Jesús Ramírez-Rivera³,
José Andrés Herrera-Corredor^{1*}

¹Colegio de Postgraduados – Campus Córdoba, Programa de Innovación Agroalimentaria Sustentable, Km. 348 Carretera. Fed. Córdoba-Veracruz, Amatlán de los Reyes, Veracruz México, C.P. 94946.

²Facultad de Ciencias Biológicas y Agropecuarias – Universidad Veracruzana, Josefa Ortiz de Domínguez s/n. Colonia Centro. Amatlán de los Reyes, Veracruz. México C.P. 94950.

³Tecnológico Nacional de México/ Instituto Tecnológico Superior de Zongolica, Km. 4 Carretera S/N Tepetitlanapa, 95005 Zongolica, Veracruz, México.

* Author for correspondence: jandreshc@colpos.mx

ABSTRACT

The objective of the study was to determine the potential of instant coffee residue as material for substrates in the production of amaranth and chia microgreens using the mixture experimental design methodology. The residue was mixed with sand and sawdust following a simplex centroid design. Sawdust was favorable for substrate aeration. Sand, sawdust, and coffee residue mixture improved the water-holding capacity of the mixture (74 %), whereas a mixture of coffee residue and sand resulted in an adequate pH substrate (6.7). Both, substrate bulk density and particle size were observed to influence plant development. The formulation composed entirely of sand (100%) demonstrated remarkable improvements in mean stem diameter, root length, and hypocotyl height compared to other mixtures, establishing it as the best substrate for amaranth and chia growth. This finding underscores the potential of this substrate to develop an efficient and environmentally friendly growing medium for microgreens, thus contributing to urban agriculture and sustainable organic waste management.

Keywords: Substrate, industrial coffee residue, mixture design, regression, microgreens.

INTRODUCTION

According to the United Nations, the world population is projected to reach approximately 9.7 billion by 2050, with a significant concentration of this growth occurring in urban areas. By 2050, it's estimated that 68% of the global population will reside in urban centers, compared to 55% today, adding an estimated 2.5 billion people to urban populations (Porras and González, 2016; Burgos, 2018). Population growth implies a higher demand for food and living space leading to the loss of agricultural land. The cultivation of microgreens is an alternative for fresh vegetable production

Citation: Piña-Moreno E, Leyva-Ovalle OR, López-Espíndola M, Contreras-Oliva A, Ramírez-Rivera EJ, Herrera-Corredor JA. 2025. Optimized substrate formulation using industrial instant coffee residue for growing amaranth and chia microgreens. *Agrociencia*. <https://doi.org/10.47163/agrociencia.v59i5.3439>

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: April 03, 2025.

Approved: July 21, 2025.

Published in Agrociencia:
July 28, 2025.

This work is licensed under a Creative Commons Attribution-Non-Commercial 4.0 International license.



that has recently gained interest given its minimal space and time requirements for production in addition to its nutritional and sensory advantages (Di Gioia *et al.*, 2015). Microgreens are versatile in terms of cultivation, being able to be grown both at home and at complex agricultural facilities. Ebert (2022) indicated that cultivation of microgreens at home is completely viable. Nolan *et al.* (2018), pointed out that this variety of methods allows for different types of substrates to be used for expanding the options beyond conventional soil. Currently, peat moss, perlite, and coconut fiber have been used as substrates for growing microgreens. However, these can be expensive or difficult to access. The food industry produces organic waste when processing food. For example, in the production of instant coffee, a large amount of roasted coffee bean residues (spent coffee grounds) is generated after extraction. These coffee residues (CR) have no clear use and are usually deposited on large plots until they decompose. The use of mixture designs for the formulation of substrates can help identify a viable combination of these residues blended with other materials for growing microgreens. Coffee is one of the most popular beverages on the planet. Population growth suggests that coffee production and consumption will continue to increase in the future, including its by-products and residues. Its demand has grown significantly in recent years due to several factors such as urbanization, and economic and social development. Coffee is also one of the most important commodities in the international trade (Murthy and Naidu, 2012). The International Coffee Organization (ICO) in the 2023/2024 season projected a 5.8 % increase in world production, reaching 178 million 60 kg bags of parchment coffee (Johnson *et al.*, 2021). In 2021, an increase in coffee demand was observed, with an annual growth of 2.4 %. Regarding coffee in Mexico, in 2016 there were consumed 87,300 Mg of roasted coffee beans, 35,339 Mg of ground coffee and 47,344 Mg of instant coffee. Out of instant coffee production, it is estimated that 45 % ends up as waste, which is equivalent to approximately 21,304.80 Mg (Euromonitor, 2017). Other coffee residues, such as husk and pulp (among others), have traditionally been used for agricultural or industrial purposes, such as fertilizers, animal feed, composting, or elimination of harmful substances to produce gibberellic acid. However, these uses only take advantage of a minimal part of the available residues (Antonio *et al.*, 2021). Residues from the industrial production of instant coffee (CR) have a high content of neutral detergent fiber (45.2 %), which includes hemicellulose, cellulose and lignin-bound substances, and acid detergent fiber (29.8 %), consisting of cellulose and lignin (Vardon *et al.*, 2013). These characteristics suggest that it may be a viable substrate for microgreens production.

The production of microgreens has aroused interest in recent years. Their consumption has increased due to their high nutritional and functional value, as they are rich in phytochemicals, vitamins, and minerals (Kyriacou *et al.*, 2016). Microgreens stand out for their superior nutritional profile and represent a significant innovation in the field of fresh vegetables. A study by Di Gioia *et al.* (2015) examined twenty-five varieties of these plants revealed that, compared to traditional vegetables harvested at their optimum ripeness, microgreens contain significant antioxidant levels and vitamins

such as C, E, and K, as well as carotenoids such as β -carotene, lutein, and zeaxanthin, that can be up to twelve times higher (Xiao *et al.*, 2012). In addition, microgreens can be sustainably produced and can be adapted to different cultivation systems. They can be grown in greenhouses with hydroponic or soilless systems, or on a small scale at home for self-consumption (Renna *et al.*, 2018). These advantages, together with their attractive sensory properties, stimulate interest in their research.

The design of experiments with mixtures methodology is a variant of response surface methodologies. It is a tool that allows for optimizing substrate formulations based on specific objectives such as yield and plant development. Ceglie *et al.* (2015) successfully used this methodology to identify a replacement for peat in tomato, melon, and lettuce seedlings for transplanting. This methodology is based on varying the proportions of the components that make up a mixture to determine the synergistic effect of a combination of two or more components on a desired characteristic of the mixture. The study by Salamanca *et al.* (2015) highlights the use of blend design to optimize the manufacture of mango purees and contribute to the development of dairy products, such as yogurt. This process was supported by the application of sensory testing and experimental techniques for ingredient mixing. The objective of the study was to evaluate the potential of instant coffee residue as material for substrates in the production of amaranth and chia microgreens using the mixture experimental design methodology.

MATERIALS AND METHODS

Materials

Pine wood sawdust used in the study was purchased from a lumberyard in Córdoba, Veracruz, Mexico, with a particle size of approximately 2 mm. Sand was purchased from the local construction materials store in Córdoba, Veracruz, México. The residue from the industrial production of instant coffee (CR) was donated by Café Tostado de Exportación (CATOEX) Cordoba, Veracruz, Mexico.

Amaranth (*Amaranthus hypochondriacus* L.) seeds, Laura variety, grown in 2022, were purchased from a producer in Tlaxcala, Mexico. Chia seeds (*Salvia hispanica* L.) were purchased from a local natural products store (Naturista, located in Cordoba, Veracruz, Mexico).

Germination tests

According to the International Seed Testing Association (ISTA) the germination process was performed using an inter-folded towel (Sanitas, Kimberly-Clark S. A. B. de C. V. Mexico City, Mexico) which provided the necessary moisture for chia and amaranth seeds. The seeds (100) were placed on the distilled water-saturated paper before being rolled. The rolls were put in a polyethylene bag and stored at room temperature (26 °C) over a plastic tray. The first count was made on the third day visually identifying

the germinated seeds. The germination percentage was calculated with the formula (germinated seeds/total seeds) × 100. The test was performed in duplicate.

Chemical composition of mixture materials.

Chemical composition of coffee residue, sand, and sawdust (Table 1) used for the substrate formulation was determined at the FYPA Soil Analysis Laboratory in Fortín, Veracruz, Mexico. Moisture content, ashes, organic matter, total carbon, total nitrogen, and carbon/nitrogen ratio were determined according to the NMX-FF-109-SCFI-2008 (DOF, 2008) standard. Minerals were determined using an Atomic Absorption Spectrometer PERKIN ELMER AAnalyst-400 (Shelton, Connecticut, USA). Phosphorus was determined by UV-VIS using a PERKIN ELMER LAMBDA-25 UV-VIS Spectrometer (Shelton, Connecticut, USA).

Table 1. Chemical composition of mixture materials.

Variable	CR	SN	SD
Moisture (%)	2.33	0.0	15.5
Ashes (%)	0.78	98.0	0.25
Organic matter (%)	99.22	0.0	NA
Total carbon (%)	57.55	0.0	53.87
Total nitrogen (%)	0.90	0.0	0.35
Carbon/nitrogen ratio	63.94	0.0	153.9
Calcium (CaO) (%)	0.148	2.17	41.2
Magnesium (MgO) (%)	0.147	1.17	13.8
Sodium (Na ₂ O) (%)	0.044	1.51	1.1
Potassium (K ₂ O) (%)	0.159	0.0	26.5
Phosphorus (P ₂ O ₅) (%)	0.017	0.18	3.9
Iron (Fe) (%)	0.0209	0.30	NA
Copper (Cu) (%)	0.0050	0.0	NA
Zinc (Zn) (%)	0.0015	0.0	NA
Manganese (Mn) (%)	0.0036	0.0	NA

NA: Not available. SN=Sand, SD=Sawdust, CR=Coffee residue from industrial production of instant coffee.

Conditioning of industrial production of instant coffee residue (CR)

Before using the residue from the industrial production of instant coffee in the formulation of substrates for microgreens, a composting process was carried out to stabilize it. The composting process was carried out for 3 months according to the manual for aerobic composting (Navarro and De la Tierra, 2003).

Formulation of substrates for microgreens

Formulation of substrates using sand, sawdust, and CR followed a simplex mixture design with centroid. The proportions were based on weight. The codes assigned to the formulations and the percentages of each ingredient are presented in Table 2.

Table 2. Substrate formulations for microgreens production.

Formulation	Code	Sand (%)	Sawdust (%)	Coffee residue (%)
1	SN	100	0	0
2	SN/SD	50	50	0
3	SN/CR	50	0	50
4	SN/SD/CR	33.33	33.33	33.33
5	SD	0	100	0
6	SD/CR	0	50	50
7	CR	0	0	100

SN=Sand, SD=Sawdust, CR=Coffee residue from industrial production of instant coffee

Physical characterization of substrates

The characterization of substrates was carried out according to the Mexican Official Standard NOM-021-RECNAT-2000 (DOF, 2002), which establishes the standards for fertility, salinity, and soil classification. The analysis included the measurement of porosity, aeration capacity, water retention capacity, bulk density, and particle density.

2.7. pH and electrical conductivity (EC) of substrates

A pH meter was used to determine the pH of a suspension formed by the substrate and distilled water in a 1:2 ratio. To prepare the suspension, 30 g of substrate were weighed and mixed with 60 mL of distilled water in plastic cups. The suspension was then allowed to rest for at least 30 min and shaken again for 20 s before measuring pH with a Thermo Scientific™ Orion™ 3-star pH meter (©2010 Thermo Fisher Scientific Inc.) The electrical conductivity determination was carried out using a Hanna determinator model HI 9811-5, following the same procedure for pH.

Substrate sterilization

Before placing the seeds on the substrates, they were sterilized. A steamer with a 70 L capacity loaded with 2 L of water was used. Bags containing sand, sawdust, and CR were heat-treated at 70-80 °C for one hour to reduce microbial contamination. Finally, the ingredients were mixed according to Table 1 to formulate the substrates based on the simplex design with centroid.

Microgreens growing

Microgreen cultivation took place in a greenhouse with a transparent plastic cover at the Facultad de Ciencias Biológicas y Agropecuarias of the Universidad Veracruzana

(18° 51' 38" N, 96° 54' 10" W). In this setup, 55 cm x 28 cm x 6 cm trays were filled with 1.30 Kg of the substrate mixtures (formulations), reaching a height of 2.5 cm.

A disinfection solution was prepared with 1 L of water and 100 mL of chlorine (5 % initial concentration) in a 2 L container. The seeds, weighing 121.5 g, were immersed in this solution for 15 min, shaking them with a glass rod for an additional 5 min. They were then sieved and washed with distilled water to remove chlorine residues and dried at room temperature on absorbent paper. Nine grams of chia and amaranth seeds were sown. Seeds were distributed over the substrates and covered with black plastic layer for three days to facilitate germination (Di Gioia *et al.*, 2015). After germination, seedlings were nourished with a hydroponic SOLUPONICS® STANDARD A+B UNIVERSAL hydroponic solution (INVERFARMS Hidroponía Querétaro, Mexico,) applied daily by subirrigation (Di Gioia *et al.*, 2017). The pH was adjusted to 6.0 with nitric acid (65% purity) to neutralize the bicarbonates present. 0.054 mL L⁻¹ of nutrient solution was used for this condition. The electrical conductivity was adjusted to 2300 mS cm⁻¹. The resulting osmotic potential was -0.083 MPa. (Table 3).

Table 3. Composition of the nutrient solution.

Nutrient	Soluponics formula (mmol L ⁻¹)	Water analysis (mmol L ⁻¹)	Nutritional contribution (mmol L ⁻¹)
NO ₃ ⁻	15	0	15
NH ₄ ⁺	1	0	1
PO ₄ ⁻	2	0	2
K ⁺	7	0	7
Ca ²⁺	5	1.23	6.23
Mg ²⁺	1.5	0.75	2.25
SO ₄ ²⁻	2	0	2
HCO ₄ ⁻	0	1.28	0.5
BO ₃ ⁻	13.9		
Fe-EDTA	28		
Zn-EDTA	3.3		
Cu-EDTA	1.7		
Mn-EDTA	15.2		
MoO ₄ ²⁻	0.7		

Microgreen growth assessment

The hypocotyl length of ten randomly selected seedlings per treatment was measured every 24 h after germination for 15 days. A ruler in centimeters was used to measure from the beginning of the hypocotyl to the cotyledonary leaves. Similarly, stem diameter and root length were measured in centimeters using a vernier and a ruler respectively every 24 hours for 15 days.

Microbiological analysis

The microbiological quality of the culture media and the microgreens produced in them were carried out according to NOM-113-SSA1-1994 (DOF, 1995) and NOM-110-SSA1-1994 (DOF, 1994) standards. Random samples of 10 g of microgreens were taken from each tray and from different substrates. Microgreens were cut with sterile scissors and serially diluted in sterile water. Then, they were seeded in duplicate on bile red violet agar for total coliform counts and on potato dextrose agar for fungal and yeast counts. The plates were incubated at 35 °C for 24 ± 2 h and 48 ± 2 h, respectively.

Statistical analysis

To analyze the effect of the formulation (as a whole) on the substrate characteristics (porosity, aeration, water retention, pH, EC, bulk, and particle densities), a completely randomized experimental design was used. Mean comparisons were performed using Tukey's test. Further analysis to identify the impact of formulation ingredients on physicochemical characteristics of substrates according to the mixture design were analyzed using a second-order mathematical model as follows.

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \epsilon.$$

Where: Y= the response variable (porosity, aeration, water retention, pH, EC, bulk, and particle densities); X1= proportion of sand in the mixture; X2 = proportion of sawdust in the mixture; X3 = proportion of coffee residue in the mixture, and ϵ = the error term. A factorial design with three factors: species (2 levels: amaranth and chia), formulation (7 levels = 7 formulations), days (15 levels) was used to analyze the evolution in the growth of microgreens of both species (chia and amaranth) through time.

All statistical analyses were conducted using a significance level $\alpha = 0.05$ in R software ver. 4.3.2 with the integrated development environment R Studio ver. 2023.12.1.

RESULTS AND DISCUSSION

Germination tests

The amaranth germination percentage was 90 % after 72 h. According to Wang *et al.* (2023), amaranth has a high germination capacity at moderate temperatures. According to his results, 80-90 % of seeds germinated between 19 and 28 °C after 72 h. As for the germination percentage in chia, 93 % germinated after 72 h at room temperature. Differences in germination percentage between species could likely be due to genetic differences.

Physical characteristics of substrates

Table 4 shows the physical characteristics of the different formulations. The highest values of porosity were found in the formulations SN/SD/CR, SD, and CR. As expected,

Table 4. Results of physical analysis of substrates.

Formulation	Mixture	Porosity (%)	Aeration capacity (%)	Water retention capacity (%)	Bulk density (Mg m ⁻³)	Particle density (Mg m ⁻³)
1	SN	40.05±0.07 ^e	10.10±0.14 ^b	29.95±0.07 ^d	0.79±0.90 ^a	2.63±0.04 ^a
2	SN/SD	59.15±1.48 ^c	11.00±1.41 ^b	49.45±1.06 ^c	0.52±0.04 ^a	2.15±0.07 ^b
3	SN/CR	50.20±0.14 ^d	15.15±7.28 ^b	35.05±7.42 ^d	0.64±0.13 ^a	2.30±0.28 ^{ab}
4	SN/SD/CR	85.05±0.07 ^a	10.20±0.14 ^b	74.85±0.07 ^a	0.38±0.19 ^a	1.48±0.06 ^c
5	SD	80.90±0.57 ^a	30.45±0.64 ^a	50.45±1.20 ^b ^c	0.16±0.03 ^a	2.17±0.04 ^{ab}
6	SD/CR	70.05±0.07 ^b	20.15±0.07 ^{ab}	49.90±0.14 ^c	0.26±0.05 ^a	1.34±0.06 ^c
7	CR	82.75±3.46 ^a	20.20±0.14 ^{ab}	62.55±3.61 ^{ab}	0.45±0.06 ^a	0.44±0.05 ^d
Pr(>F)		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Mean of three replicates ± standard deviation. *Significant $\alpha = 0.05$. Means within the same column followed by different letters are significantly different (Tukey, $P < 0.05$). SN=100 % sand; SD/SN=50 % sawdust+50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100 % sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR= residue from industrial production of instant coffee 100 %.

the formulation composed of 100 % sand had the lowest porosity value. Martínez and Roca (2011) reported that organic substrates typically exhibit porosity values above 85 %. This parameter is critical to ensure adequate aeration and water retention in the substrate which are essential factors for optimal plant development. Regarding aeration capacity, the highest value was observed for the SD formulation, whereas the lowest values corresponded to the SN, SD/SN, CR/SN and SN/SD/CR formulations. According to Martínez and Roca (2011), the optimum range of aeration capacity is between 20 and 30 %; presented by CR, SD/CR and SD formulations. The formulations for water retention in substrates revealed significant data. The SN/SD/CR formulation showed the highest value, highlighting its efficiency in water retention. On the other hand, the SN formulation registered the lowest water retention and, the general range of water retention oscillates between 29.95 and 74.85 %. The SD formulation presented the lowest bulk density value, whereas the SN formulation showed the highest value. The results obtained in the measurement of the particle density of the substrates indicate that the SN formulation had the highest real density. The results are consistent with the parameters published by Martínez and Roca (2011), except in the case of the CR formulation.

Chemical properties of substrates

Chemical properties of substrates are presented in Table 5. The formulations were found located in an acidic pH range. According to Di Gioia *et al.* (2015), a suitable substrate for the cultivation of microgreens should have a pH ranging between 5.5 and 6.5. In addition, it is crucial that the electrical conductivity is less than 500 mS cm⁻¹, thus indicating that the amount of nutrients present in the substrate is not excessive

Table 5. Chemical properties of substrates.

Formulation	Mixture	pH	Electrical conductivity (mS cm ⁻¹)
1	SN	5.53±0.09c	0.62±0.02e
2	SN/SD	5.80±0.11bc	92.10±0.14a
3	SN/CR	6.70±0.18a	11.00±0.00de
4	SN/SD/CR	5.61±0.10bc	103.00±1.41a
5	SD	5.72±0.04bc	27.50±2.12cd
6	SD/CR	5.81±0.02 ^{bc}	38.00±16.97bc
7	CR	5.96±0.06 ^b	59.00±0.00b
Pr(>F)		<0.0001*	<0.0001*

Mean of three replicates ± standard deviation. *Significant $\alpha = 0.05$. Means within the same column followed by different letters are significantly different (Tukey, $P < 0.05$). SN=100 % sand; SD/SN=50 % sawdust+50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100 % sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR= residue from industrial production of instant coffee 100 %.

and that it has the right conditions for healthy microgreens growth (Di Gioia *et al.*, 2015). All the formulations used in this study were found to have an adequate EC. The simplex coordinate system in mixture design allows for visual identification of the change in the property of interest as a function of the ingredient proportions in the mixture (Figure 1). In the case of porosity (Figure 1a), the evenly spaced lines in the triangle suggest that soil porosity was homogeneously distributed but decreased when increasing the sand content in the mix. This is reasonable given that sand is a compact and dense material whereas sawdust and CR are more porous. According to Moreno-Reséndez and Valdés-Perezgasga (2005), the use of sand in combination with vermicompost is effective in replacing other substrates. They found that vermicompost mixed with different levels of sand resulted in a rich and complete nutritional composition capable of integrally satisfying the nutritional needs of plants during their development. This alternative also promotes healthy plant growth due to the diversity of essential nutrients it offers. In case of aeration (Figure 1b), a clustering of curved lines towards sawdust indicated a rapid increase in substrate aeration. Regarding water retention (Figure 1c), a reduced variability was found increasing gradually towards sawdust and CR content. According to Morales-Maldonado and Casanova-Lugo (2015) sawdust particle size has a significant impact on air porosity and water holding capacity. Depending on their size, they can increase porosity while reducing water holding capacity, or vice versa. Formulation SN showed an increase in bulk density with a value of 0.8. On the other hand, formulation 5 with SD indicates a lower particle density, as shown by the clustering of lines near the lower vertex, reflecting lower values in this measure, specifically a value of 0.2. It is common to observe that as particle size decreases, both bulk and particle densities tend to increase.

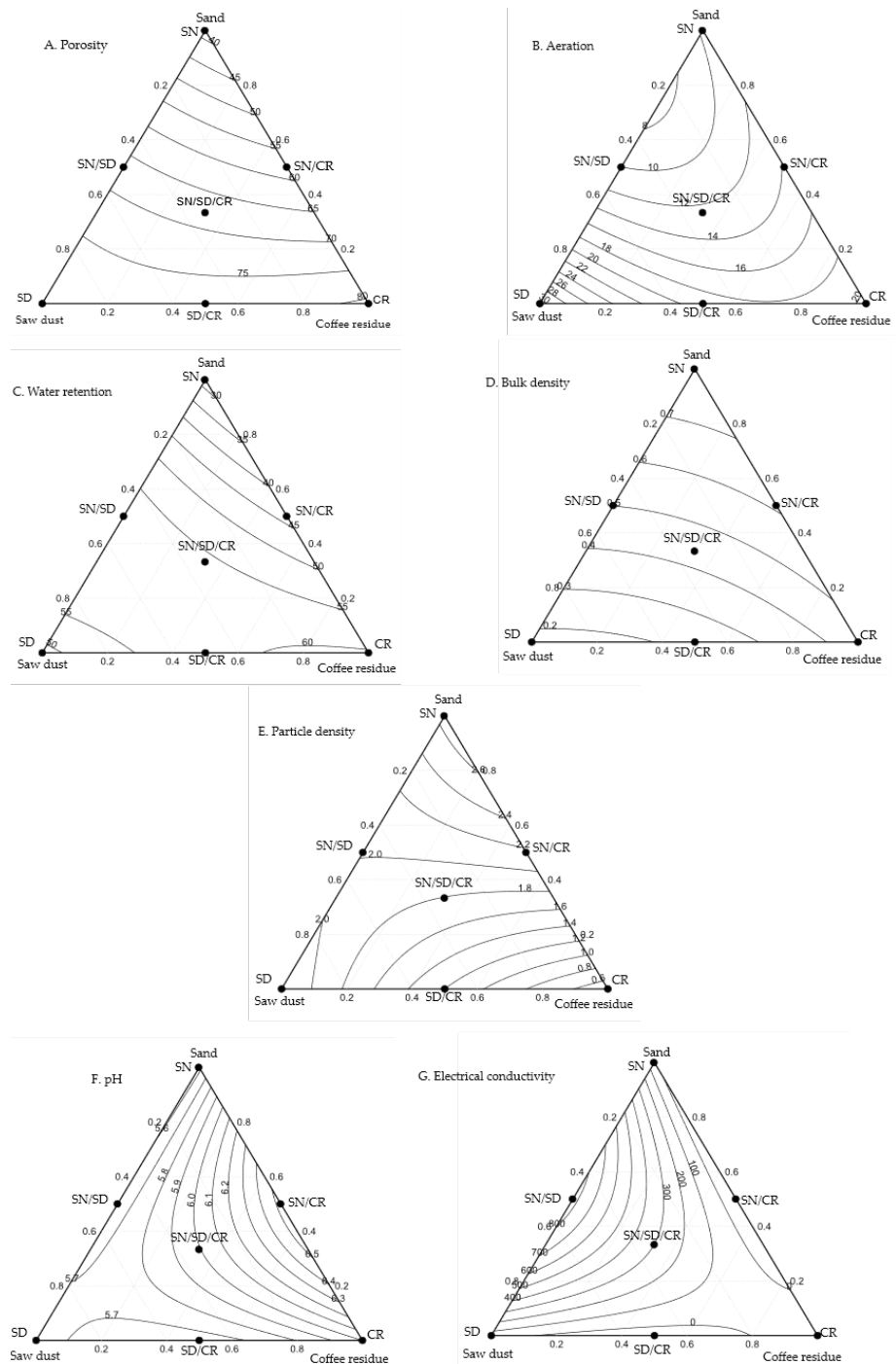


Figure 1. Use of the Simplex Coordinate System for Visualization of the impact of ingredients on the physicochemical characteristics of substrates. SN=100 % sand; SD/SN=50 % sawdust+ 50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100 % sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR= residue from industrial production of instant coffee 100 %.

This is because smaller particles can settle more densely and reduce the proportion of pores between them, resulting in a higher bulk density value.

In relation to pH, an increase (6.5) in this value was found to be oriented towards a combination of 50 % sand and 50 % CR (Formulation 3) and a lower value (5.7) towards the sawdust content in the mixture. This effect is attributed to the presence of acidic compounds in the mixture that have an adverse impact on complete plant growth. Regarding electrical conductivity, the lowest values were found in formulation SN (100 % sand) whereas the highest values were found in formulation SN/SD/SR composed of 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee.

Microgreen growth

In general, it was found that the substrate formulation significantly influenced microgreen growth variables such as stem diameter, root length and hypocotyl height (Table 6) with p values less than 0.0001.

In formulation SN showed a stem diameter of 0.21 mm, which was significantly different compared to formulations SN/SD, SN/CR and CR. In addition, the root length of formulation SN was 2.34 cm, marking a difference with formulations SN/CR, SN/SD/CR and CR. These differences may be indicative of how each formulation affects plant development in different aspects of growth. The hypocotyl height of formulation SN was 3.01 cm, also distinguishing it from formulations SN/SD, SN/CR and CR. These results suggest that formulation SN has a unique effect on plant growth, which could be crucial to better understand the influence of formulation variables on plant morphology.

Table 6. Average growth variables by formulation.

Formulation	Mixture	Stem diameter (mm)	Root length (cm)	Hypocotyl height (cm)
1	SN	0.21±0.11a	2.34±1.09a	3.01±1.24a
2	SN/SD	0.08±0.04bc	1.83±1.16b	1.29±0.78b
3	SN/CR	0.07±0.05cd	0.98±0.94c	0.77±0.66c
4	SN/SD/CR	0.10±0.02b	2.35±1.11a	1.44±0.64b
5	SD	0.09±0.05b	2.05±1.27ab	1.22±0.61b
6	SD/CR	0.09±0.03b	1.80±1.12b	1.27±0.69b
7	CR	0.05±0.05d	0.91±1.04c	0.75±0.78c
Pr(>F)		<0.0001*	<0.0001*	<0.0001*

Mean of three replicates, standard deviation. *Significant $\alpha=0.05$. Means within the same column followed by different letters are significantly different (Tukey, $P < 0.05$). SN = 100 % sand; SD/SN= 50 % sawdust+50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100 % sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR = residue from industrial production of instant coffee 100 %.

This pattern was confirmed from a multivariate perspective (Figure 2) where it was found that, according to the map of individuals (Figure 2a), formulation SN was located far away from the other formulations in the highest position of dimension 1, which represents the highest variability (84.23 %). Formulations SN/SD, SN/SD/CR,

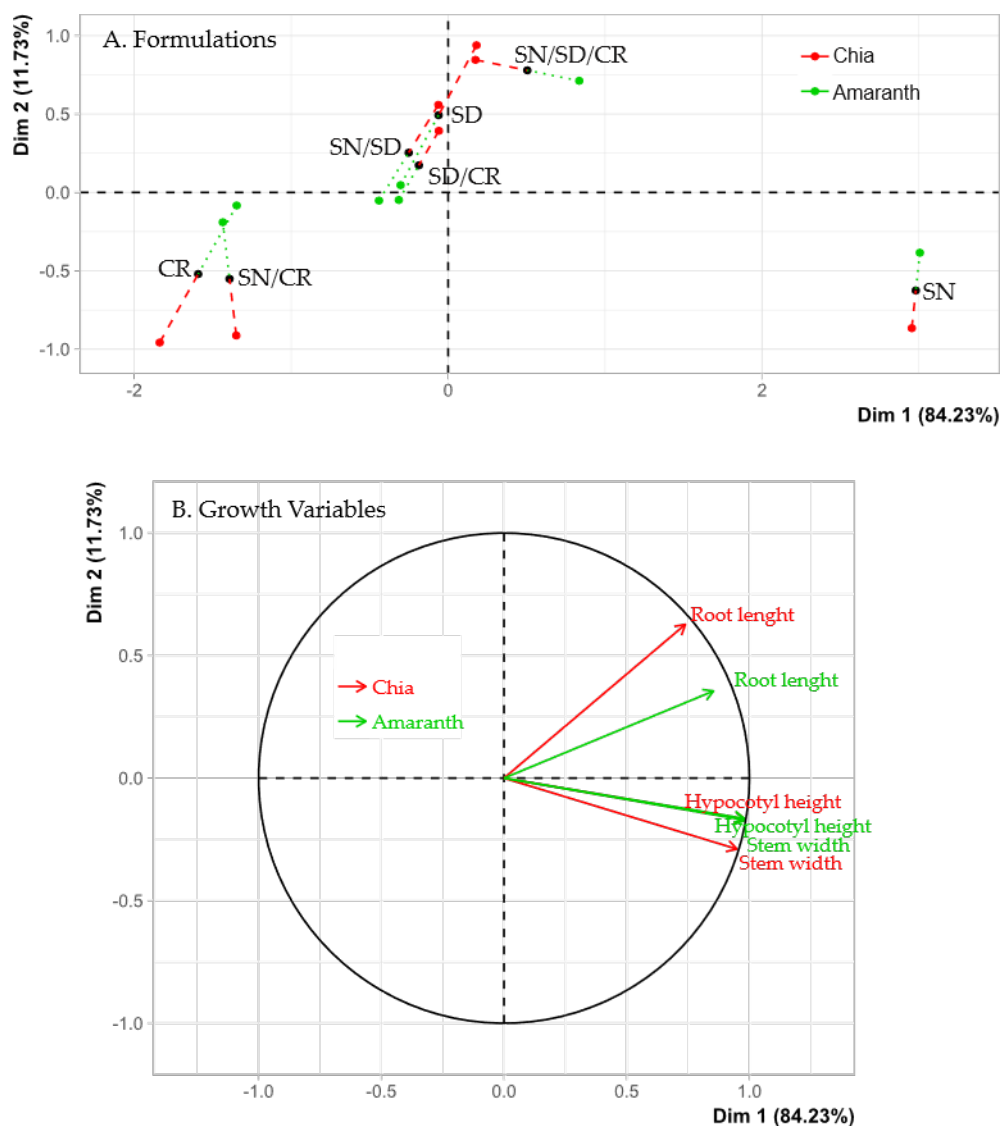


Figure 2. Comparison between species in terms of formulations and growth variables. SN=100 % sand; SD/SN=50 % sawdust+50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100 % sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR= residue from industrial production of instant coffee 100 %.

SD, and SD/CR also clustered in a central position of dimension 1. These formulations were characterized by being located towards the sawdust content in the mixture in the simplex coordinate system. Finally, formulations SN/CR and CR were in the lowest position in dimension 1 and are those oriented towards CR content. Regarding the correlation between variables (determined by the angle formed by these variables in Figure 2b), it was similar for both species. Hypocotyl height and stem diameter were found to be closely correlated, whereas the correlation of these variables with root length was lower. These results suggest that both species had higher growth of hypocotyl and stem diameter in the substrate composed only with sand whereas formulations SN/SD, SN/SD/CR, SD, and SD/CR had higher root growth in the formulations composed with sand and sawdust. Mineral nutrition from chemical sources, such as Steiner solution, becomes truly relevant and is utilized by seedlings once the first true leaves appear and metabolism shifts to autotrophic. Before this point, application of the nutrient solution has little effect and can be counterproductive if used at normal concentrations. In the case of chia (*Salvia hispanica* L.), the appearance of true leaves occurred between 7 and 14 days after sowing. In amaranth (*Amaranthus hypochondriacus* L.), this occurred between 15 and 17 days. Once the true leaves have appeared, the nutrient solution may have the following influence: promoting a more robust and less etiolated stem; the roots will develop and branch more; and the hypocotyl will be shorter and more robust. According to Table 2, if substrates containing coffee grounds (CR) and pine sawdust (SD), as well as their formulations, were watered only with water after the first true leaves appeared, the seedlings would grow more than those using sand as a substrate, due to the elements contained in each.

The magnitude of the differences in the growth variables is presented in Table 7. The values for chia indicate that this species had a significantly higher stem diameter than amaranth. For root length, chia significantly exceeded the amaranth in this variable. For hypocotyl height, as with the other variables, chia has a significantly higher hypocotyl height than amaranth. These data suggest that chia tends to have more robust growth compared to amaranth under the study conditions.

Figure 3 shows the effect of significant interactions among factors (time, formulation, and species) for growth variables. Figure 3a shows that formulation SN favored an increase in stem diameter, reaching 0.3 mm, in contrast to the other formulations that

Table 7. Comparison of growth variables by species.

Species	Stem diameter (mm)	Root length (cm)	Hypocotyl height (cm)
Amaranth	0.09±0.07 ^b	1.29±0.97 ^b	1.22±0.96 ^b
Chia	0.11±0.07 ^a	2.21±1.29 ^a	1.56±1.13 ^a
Pr(>F)*	<0.0001	<0.0001	<0.0001

*Mean of three replicates ± standard deviation. Means within the same column followed by different letters are significantly different (Tukey, $P < 0.05$).

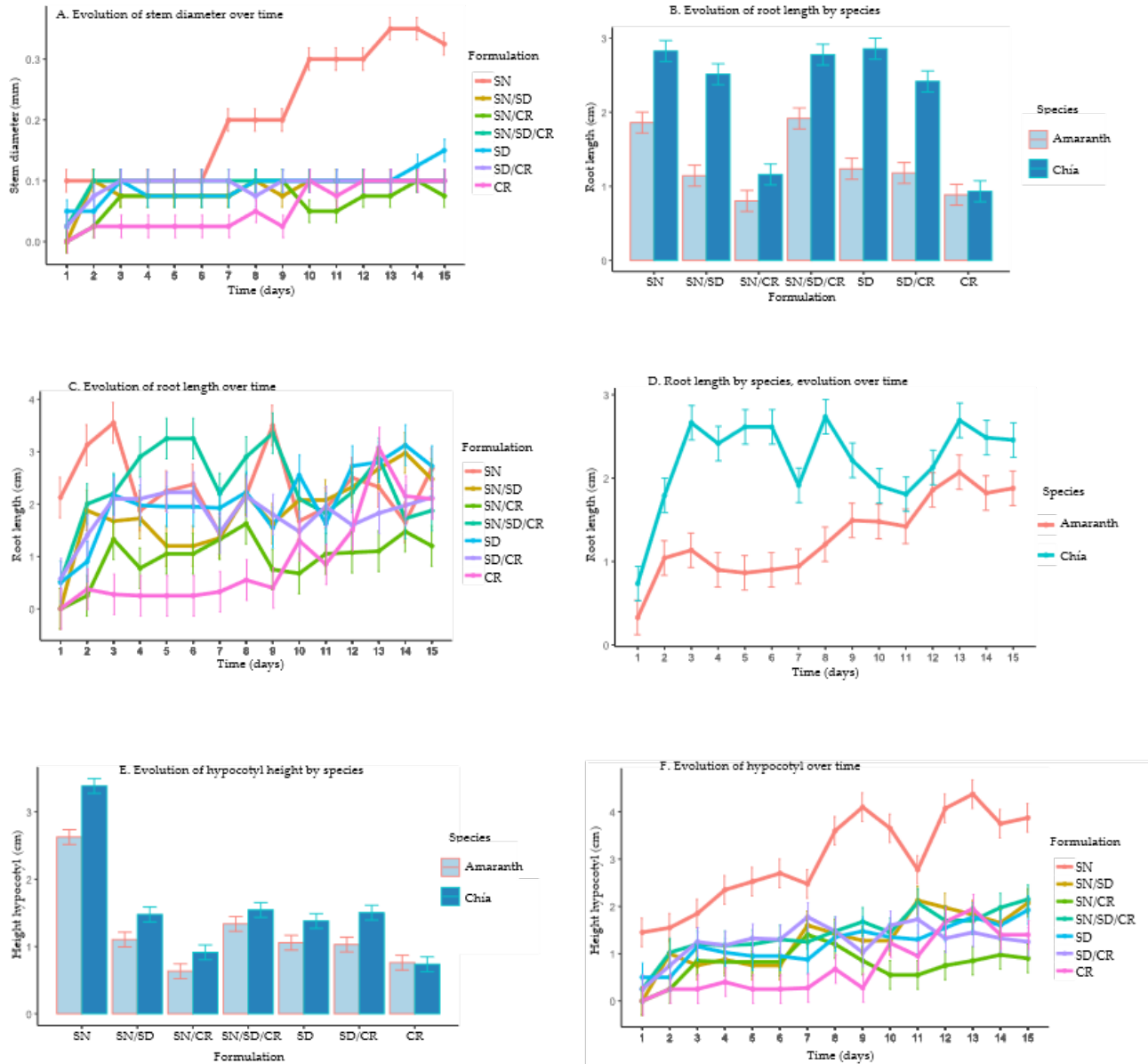


Figure 3. Growth variables of microgreens in amaranth and chia as a function of significant interactions (Tukey, $P < 0.05$) between time, species, and formulation factors. SN=100 % sand; SD/SN=50 % sawdust+50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100 % sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR= residue from industrial production of instant coffee 100 %.

remained at 0.1 mm. In Figure 3b, formulations SN, SN/SD/CR and SD, promoted root growth in the chia species, reaching 2 to 3 cm, whereas formulations SN/CR and CR were not as effective. In the case of amaranth, formulations SN and SN/SD/CR promoted root growth of 2 cm. Figure 3c revealed that, overall, formulations SN and SN/SD/CR promoted more pronounced root growth over time, with roots reaching 3 cm, comparatively superior to the other formulations that ranged between 1 and 2 cm in length. The decrease in root length concurs with the appearance of true leaves, which could encourage their branching. This can be attributed to the physical and chemical characteristics of each of the substrates evaluated, which plays an essential role in plant development. Figure 3d shows that chia had higher root length with values between 2 and 3 cm, surpassing amaranth, which root length varied in a range of 1 to 2 cm. Figure 3e formulation SN proved to be the most effective achieving hypocotyl growth between 2.5 and 3 cm in the species tested (amaranth and chia), in contrast to formulations SN/CR and CR, where the growth was less than 1 cm. According to Figure 3f, the same formulation SN excels in terms of hypocotyl height of the microgreens, achieving 4 cm, whereas formulations SN/SD, SN/SD/CR and SD present a height of 2 cm. These results agreed with the study of Flores-Pacheco *et al.* (2016) who found the increase in plant height is due to the capacity of the sand substrate to retain nutrients. Sand allows for a better accumulation of nutrients that are essential for microgreen growth. This is due to high nutrient retention of sand in hydroponic systems is due to a combination of surface nutrient uptake by the sand particles; precise control of irrigation and nutrient solution delivery; and rapid root uptake that prevents excessive leaching. Under overwatering conditions, its retention capacity will be limited.

Microbiological analyses

Microbiological results (Table 8) showed that formulations SN/SD/CR and SD had high levels of bacterial CFU. On the other hand, formulation SN registered the lowest number of bacteria. A notable presence of fungi was observed in the formulation SN/SD/CR, with a count of 40.45×10^2 CFU g^{-1} . This increase could be related to the high-water retention shown in Table 1. So, this could create optimal conditions for fungi growth. Regarding microgreens, chia microgreens showed a microbial density of 127.09×10^{13} CFU g^{-1} , whereas amaranth microgreens showed a lower density with 11.02×10^{13} CFU g^{-1} . These findings are consistent with Chandra *et al.* (2012) who indicate that bacteria can easily grow in young and delicate tissues such as those of microgreens. It has also been noted that bacterial growth can be stimulated by sugars and other organic substances released during endosperm decomposition at germination. According to the regulatory framework proposed by the U. S. Environmental Protection Agency (EPA) and the European Union (EU), the tolerance for total coliforms is 100 UFC g^{-1} (Alimentarius, 2003). In a study Castagnino *et al.* (2020) found that 'rapini' (*Brassica rapa* L.) sprouts may contain high levels of bacteria due to the organic media used in their cultivation. These bacteria can cause a disease

Table 8. Microbiological analysis.

Formulation	Mixture	Moisture (%)	Bacteria (UFC g ⁻¹)	Fungi (UFC g ⁻¹)
1	SN	13.52	14.45 X 10	Negative
2	SN / SD	53.00	51.22 X 10 ²	Negative
3	SN / CR	45.59	31.43 X 10 ³	Negative
4	SN/ SD /CR	45.62	98.38X 10 ³	40.45 X10 ²
5	SD	78.53	81.27 X 10 ³	Negative
6	SD/CR	56.88	27.48 X 10 ³	Negative
7	CR	46.84	39.31 X 10 ³	Negative
	Chia microgreens	93.75	127.09 X 10 ¹³	Negative
	Amaranth microgreens	89	11.02 X 10 ¹³	Negative

SN=100 % sand; SD/SN=50 % sawdust+50 % sand; SN/CR=50 % sand + 50 % residue from industrial production of instant coffee; SN/SD/CR= 33.3 % sand + 33.3 % sawdust + 33.3 % residue from industrial production of instant coffee; SD= 100% sawdust; CR/SD= 50 % residue from industrial production of instant coffee + 50 % sawdust, CR= residue from industrial production of instant coffee 100 %.

known as soft rot which significantly reduces the shelf life of these fresh vegetables and may favor the development of pathogens.

CONCLUSIONS

The use of experimental mix design methodologies to optimize substrate composition is a strategy that has proven to be effective. The mixture with a higher percentage of sand shows a decreased in porosity. Sawdust improved the aeration of the substrate, although this also led to an increase in the variability of water retention capacity, particularly when the proportion of sawdust and CR increased in the mixture. With respect to pH, a balanced mixture of 50 % sand and 50 % CR resulted in an adequate pH of 6.7 for microgreens production. This study revealed that the best substrate for chia and amaranth microgreen growth was sand when irrigated with a nutrient solution. Coffee residue seemed not to be adequate for growing chia or amaranth microgreens. However, the possibility of combining it with other materials to improve its properties is a promising avenue that deserves further research. This sustainable approach not only facilitates waste management, but also promotes the production of local, fresh, and nutritious food, thus supporting a more sustainable and efficient agriculture.

ACKNOWLEDGMENTS

The authors would like to extend their gratitude to the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) for their invaluable support and the scholarship awarded

to the first author. Their contribution has been instrumental in the project's success. We also express our sincere thanks to the Colegio de Postgraduados, Campus Córdoba, for the support and training provided throughout these years of study, which have been crucial to our academic and professional development.

REFERENCIAS

- Alimentarius C. 2003. Codex alimentarius commission. Code of practice for food quality. Rome: FAO/WHO.
- Antonio CG, Romero LAR, Trejo JFG, Pérez AAF. 2021. Revaluation of coffee crop wastes: Towards a circular economy. *Digital Ciencia@ UAQRO* 4 (6): 71–79.
- Burgos ME. 2018. Los desafíos del futuro: crecimiento poblacional y desarrollo. *Journal of Social Sciences* 6 (11): 179–185. <https://doi.org/10.18682/jcs.v0i11.900>.
- Castagnino A, Marina J, Benvenuti S, Marin CMA. 2020. Microgreens and sprouts, two innovative functional foods for a healthy diet in Km 0. *Horticultura Argentina* 39 (100): 55–95.
- Ceglie FG, Bustamante MA, Ben Amara, M Tittarelli F. 2015. The challenge of peat substitution in organic seedling production: optimization of growing media formulation through mixture design and response surface analysis. *PLoS One* 10 (6): e0128600. <https://doi.org/10.1371/journal.pone.0128600>
- Chandra D, Kim JG, & Kim YP. 2012. Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. *Horticulture, Environment, and Biotechnology*. 53: 32–40. <https://doi.org/10.1007/s13580-012-0075-6>
- Diario Oficial de la Federación. 1994. Preparación y dilución de muestras de alimentos para su análisis microbiológico. Norma Oficial Mexicana. https://dof.gob.mx/nota_detalle.php?codigo=4883170&fecha=16/10/1995#gsc.tab=0. (Retrieved: May 2024)
- Diario Oficial de la Federación. 1995. Norma Oficial Mexicana que establece el método para la cuenta de microorganismos coliformes totales en placa.. https://www.dof.gob.mx/nota_detalle.php?codigo=4880115&fecha=25/08/1995#gsc.tab=0. (Retrieved: June 2024)
- Diario Oficial de la Federación. 2000. Norma Oficial Mexicana que establece las especificaciones de fertilidad, salinidad y clasificación de suelos.. https://dof.gob.mx/nota_detalle.php?codigo=717582&fecha=31/12/2002#gsc.tab=0(Retrieved: January 2024)
- Diario Oficial de la Federación. 2008. Norma Oficial Mexicana que establece las especificaciones para humus de lombriz (lombricomposta)-especificaciones y métodos de prueba. https://www.dof.gob.mx/nota_detalle.php?codigo=5044562&fecha=10/06/2008#gsc.tab=0. (Retrieved: March, 2024)
- Di Gioia F, Mininni C, Santamaria P. 2015. Come coltivare micro-ortaggi. In F. Di Gioia & P. Santamaria (Eds.), *Microgreens: Novel fresh and functional food to explore all the value of biodiversity* (pp. 51–80). ECO-logica. Bari, Italy.
- Di Gioia F, De Bellis P, Mininni C, Santamaria P, Serio F. 2017. Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (*Brassica rapa* L.) microgreens. *Journal of the Science of Food and Agriculture* 97 (4): 1212–1219. <https://doi.org/10.1002/jsfa.7852>
- Ebert AW. 2022. Sprouts and microgreens-Novel food sources for healthy diets. *Plants*. 11(4), 571. <https://doi.org/10.3390/plants11040571>

- Euromonitor C. 2017. Analysis of the coffee consumption market in Mexico 2016. Report of the study conducted by Euromonitor International. <https://amecafe.org.mx/>. (Retrieved: March, 2024)
- Flores-Pacheco, JA Pacheco, C F Murillo, Y Oporta, R Alemán Y. 2016. Hydroponic production of tomato (*Solanum lycopersicum*) and chiltoma (*Capsicum annuum*) with inert substrates. *Revista Científica de FAREM-Estelí*, 20: 73–81. <https://doi.org/10.5377/farem.v0i20.3069>
- Johnson SA, Prenni JE, Heuberger AL, Isweiri H, Chaparro JM, Newman SE, Uchanski ME, Omerigic HM, Michell KA, Bunning M, Foster MT, Thompson HJ, Weir TL. 2021. Comprehensive Evaluation of Metabolites and Minerals in 6 Microgreen Species and the Influence of Maturity. *Current Developments in Nutrition* 5 (2): <https://doi.org/10.1093/cdn/nzaa180>
- Kyriacou MC, Roupael Y, Di Gioia F, Kyratzis A, Serio F, Renna M, De Pascale S, Santamaria P. 2016. Micro-scale vegetable production and the rise of microgreens. *Trends in Food Science and Technology* 57: 103–115. <https://doi.org/10.1016/j.tifs.2016.09.005>
- Martínez PF, Roca D. 2011. Sustratos para el cultivo sin suelo. Materiales, propiedades y manejo. In: Flórez R., V.J. (Ed.). *Sustratos, manejo del clima, automatización y control en sistemas de cultivo sin suelo*. Bogotá: Editorial Universidad Nacional de Colombia. pp: 37–77.
- Morales-Maldonado, ER & Casanova-Lugo F. 2015. Organic and inorganic substrate mixtures, particle size and ratio. *Agronomía Mesoamericana*, 26(2):365–372. <https://doi.org/10.15517/am.v26i2.19331>
- Moreno Reséndez A, & Valdés Perezgasga MT. 2005. Tomato development on vermicompost/sand substrates under greenhouse conditions. *Agricultura Técnica*, 65 (1): 26–34. <https://doi.org/10.4067/S0365-28072005000100003>
- Murthy PS, Naidu MM. 2012. Sustainable management of coffee industry by-products and value addition-A review. *Resources, Conservation and Recycling* 66: 45–58. <https://doi.org/10.1016/j.resconrec.2012.06.005>. (Retrieved: April 2024)
- Navarro R, de la Tierra CA. 2003. Manual for aerobic composting. CESTA, Friends of the Earth El Salvador, pp: 2–5.
- Nolan C, Overpeck JT, Allen JR, Anderson PM, Betancourt JL, Binney HA, Brewer S, Bush M B, Chase BM, Cheddadi R, Djamali M, Dodson J, Edwards ME, Gosling WD, Haberle S, Hotchkiss SC, Hntley B, Ivory SJ, Kershaw AP, Kim S-H, Latorre C, Leydet M, Lézine A-M, Liu K-B, Liu Y, Mcglone AVL, Marchant RA, Momohara A, Moreno PI, Müller S, Otto-Bliesner, B Shen C, Stevenson J, Tahahara H, Tarasoy PE, Tipton J, Vincens A, Wend C, Xi O, Zheng Z, Jacksom ST. 2018. Past and future global transformation of terrestrial ecosystems under climate change. *Science* 361 (6405): 920–923. <https://doi.org/10.1126/science.aan5360>
- Porras AC, González AR. 2016. Aprovechamiento de residuos orgánicos agrícolas y forestales en Iberoamérica. *Academia y Virtualidad* 9 (2): 90–107. <http://dx.doi.org/10.18359/ravi.2004>.
- Renna M, Castellino M, Leoni B, Paradiso V M, Santamaria P. 2018. Microgreens production with low potassium content for patients with impaired kidney function. *Nutrients* 10 (6): 675. <https://doi.org/10.3390/nu10060675>
- Salamanca G, Méndez LMR, Osorio M, Arias NR. 2015. Experimental design of mixtures as a tool for the optimization of mango cremolacteos. *Colombian Journal of Agroindustrial Research* 2 (1): 16–24.

- Vardon DR, Moser BR, Zheng W, Witkin K, Evangelista RL, Strathmann TJ, Rajagopalan K, Sharma B K. 2013. Complete Utilization of spent coffee grounds to produce biodiesel, bio-oil, and biochar. *ACS Sustainable Chemistry & Engineering* 1 (10): 1286–1294. <https://doi.org/10.1021/sc400145w>.
- Wang, J., Li, J., Liu, W., Zeb, A., Wang, Q., Zheng, Z. and Liu, L. 2023. Three typical microplastics affect the germination and growth of amaranth (*Amaranthus mangostanus* L.) seedlings. *Plant Physiology and Biochemistry*, 194, 589–599. <https://doi.org/10.1016/j.plaphy.2023.01.012>
- Xiao Z, Lester GE, Luo Y, Wang Q. 2012. Assessment of Vitamin and Carotenoid Concentrations of Emerging Food Products: Edible Microgreens. *Journal of Agricultural and Food Chemistry* 60 (31): 7644–7651. <https://doi.org/10.1021/jf300459b>

Agrociencia