Agricultural Lignocellulosic Waste and Volcanic Rock Combinations Differentially Affect Seed Germination and Growth of Pepper (*Capsicum annuum* L.)

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The effect of three substrates derived from combining agricultural lignocellulosic residues and a volcanic rock called tezontle (40/60; v/v) was tested on the germination and biomass production of five varieties of pepper (Capsicum annuum L.) grown under greenhouse conditions. The three substrates consisted of sugarcane bagasse and tezontle (SBTZ), coffee husk and tezontle (CHTZ), and filter cake from the clarification of sugarcane juice and tezontle (FCTZ), whereas the pepper varieties tested were Sven F1, Sympathy F1, Zidenka F1, Yolo Wonder, and California. Physical analyses of the substrates indicated that they had suitable properties, except for the percentage of readily available water, which was low in all the substrates. With regard to the chemical analyses, the best substrate was FCTZ. The highest germination percentage and the shortest time in which maximum germination was reached were also both found with the FCTZ substrate. Additionally, the greatest plant height and the highest shoot and biomass production were also recorded with the FCTZ substrate. In terms of varieties, those that responded best to the substrates were Sven F1, Sympathy F1, and Zidenka F1.

Keywords: Sugarcane bagasse; Filter cake; Coffee husk; Tezontle; Soil; Mineral substrates

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INTRODUCTION

Substrate quality is one of the factors that most influences germination and seedling development in the nursery. A good substrate has physical and chemical properties that promote rapid and healthy growth of plants and act jointly, although the physical properties related to water-air availability for plant roots are the most important in the study of materials used as growth media in containers or pots (Cabrera 1999; Vence 2008).

The mixture of materials is crucial for obtaining a suitable substrate for seedling production in containers, especially if they are local or regional materials, or alternatives to those normally used in precision farming, such as peat, perlite, and agrolita (a mineral substrate obtained from expanded perlite). One of the advantages of using alternative regional substrates is their availability and lower cost, and their use helps reduce the environmental pollution caused by the disposal of agricultural residues and strengthens sustainable agriculture (Humpert 2000; Cruz-Crespo *et al.* 2012a).

Currently, some of the most commonly used alternative substrates are coffee pulp, rice husks, sawdust, sugarcane bagasse, grape pomace, and olive pomace, among others (De Medeiros *et al.* 2008; De Grazia *et al.* 2007; Gomes *et al.* 2008; Kaciu *et al.* 2009). In Mexico, it is common to use combinations of organic and inorganic substrates in different ratios to produce seedlings in the greenhouse (San Martín-Hernández *et al.* 2012; López-Baltazar *et al.* 2013).

In the present study, the physical and chemical characterization of substrates based on three agricultural lignocellulosic residues, namely coffee husk, filter cake from sugarcane processing, and sugarcane bagasse, mixed with the volcanic rock known in Mexico as tezontle, was carried out. Additionally, their use in the germination and seedling production of five varieties of pepper (*Capsicum annuum* L.) under greenhouse conditions was assessed.

Sugarcane bagasse is the residue that remains after the sugarcane stalk or body is milled (Basanta *et al.* 2007). It is a lignocellulosic material composed mainly of cellulose, hemicellulose, and lignin, which is obtained as a byproduct or waste after the juice is extracted from sugarcane, and which represents between 25 and 40% of the total material processed (Pernalete *et al.* 2008). Structurally, the parts that make up the sugarcane bagasse have an average length of 2.5 mm; particle size mainly depends on the milling process and the sugarcane variety. Freshly ground material contains about 45 to 52% water (retained by the material), 6 to 8% soluble solids, and about 45% fibers (water-insoluble organic fraction) (Alarcón *et al.* 2006).

Filter cake results from the filtering and washing of the settled mud during the sugarcane juice clarification process. This material contains low levels of lignin, but high levels of cellulose, hemicellulose, and colloids from the organic matter originally dispersed in the juice, together with organic and inorganic anions that precipitate during clarification (Berrospe-Ochoa *et al.* 2012; Salgado *et al.* 2001). For every ton of processed raw material, around 30 to 50 kg of filter cake, which has high water holding capacity (Hernández-Melchor *et al.* 2008) and contains approximately 25% dry matter, is obtained (Basanta *et al.* 2007).

Coffee husk results from removing the exocarp, mesocarp, and endocarp from the coffee cherry to obtain coffee beans (Mazzafera 2002), making it an excellent source of cellulose, hemicellulose, and lignin. Mora (1999) states that coffee husk is a material that has low moisture holding capacity and that it is good for oxygenating substrate when making mixtures, but that it has a very short lifespan, as it decays in a short time. It is a good source of humus and organic matter, so it represents an environmentally and economically viable alternative as processed material in agricultural production (Leifa *et al.* 2001).

Tezontle is a volcanic rock that is very abundant in Mexico. It is an inert material with pH values near neutral, low CEC, good aeration, and moisture-holding capacity that is dependent on particle diameter. Additionally, tezontle does not contain toxic chemicals and has physical stability. Bulk and true densities increase with decreasing particle size, while total porous space increases with increased particle size. Concerning pH, electrical conductivity (EC), cation exchange capacity (CEC), and organic matter content, tezontle shows values of 7.3, 0.15 dS m⁻¹, 2.7 cmol_c kg⁻¹, and 0%, respectively (Cruz-Crespo *et al.* 2012b; Trejo-Téllez *et al.* 2013). In soilless culture, the recommended particle size is 3 mm, while in substrate mixtures, better plant performance has been demonstrated with 5 mm particle size (San Martín-Hernández *et al.* 2012; Trejo-Téllez *et al.* 2013).

EXPERIMENTAL

Plant Material

For this study, seeds of five varieties of blocky pepper (*Capsicum annuum* L.) were used. The genotypes were as follows: Sven F1, Sympathy F1, Zidenka F1, Yolo Wonder, and California, produced and marketed by different seed companies.

Substrates

The substrates evaluated were composed of different agricultural residues mixed with volcanic rock known locally as tezontle, with particle size of 5 to 5.6 mm in diameter. The substrate mixtures had 40% agricultural residue and 60% tezontle and generated the following treatments:

- 1. SBTZ: Sugarcane bagasse (SB) + Tezontle (TZ).
- 2. CHTZ: Coffee husk (CH) + Tezontle (TZ).
- 3. FCTZ: Filter cake (FC) + Tezontle (TZ).

The substrates were placed in polystyrene trays with 200 cavities, in which the seeds of the five varieties of pepper were sown at a depth of 1 cm. Irrigation was applied by saturation before and after planting, using distilled water during the experimental phase.

Germination process

Prior to germination, the pepper seeds were placed in germination trays (containing the substrates under study) and were kept in a Shel Lab L115 automatic germination chamber for 15 days, with lighting for 12 h and a temperature of 25 °C, with daily watering. Then, the standards set by the International Seed Testing Association (ISTA, 2013) were followed. Once germination began, the trays were moved to a greenhouse to prevent seedling etiolation.

Treatments and experimental design

A factorial treatment design (3 X 5) was used in which the study factors were the substrate and the pepper variety. The substrate factor levels were as follows: sugarcane bagasse and tezontle, coffee husk and tezontle, and filter cake and tezontle in 40/60 proportions (v/v); the variety factor levels were as follows: Sven F1, Sympathy F1, Zidenka F1, Yolo Wonder, and California.

Combining the factor levels resulted in 15 treatments, which were distributed in a greenhouse in a split-plot design, where the big plot was the substrate and the small plot was the variety. The experimental units were comprised of seedlings, with 32 experimental units per treatment.

Variables Evaluated

Bulk density (BD) and true density (TD)

Bulk (BD) and true density (TD) were determined according to the protocols described by Martínez and Roca (2011).

Water holding

To determine the water holding curve of the evaluated substrates, the hanging column method proposed by De Boodt *et al.* (1974) was used. From the water holding curves, the water release curves in the substrates, which provides information on total porosity (TP), solid matter, hardly available water (HAW), reserve water (RW), easily available water (EAW), and no available water (NAW), were determined.

pH and electrical conductivity (EC)

The pH and EC values in the saturation paste extract were determined using distilled water as an extractant and stirring until a characteristic moisture point on the substrate surface was reached. The pH readings were determined with a potentiometer (OAKTON[®] pH/mV/°C meter, Series No: 43291). The EC was measured with a benchtop conductivity meter (Hanna, Mod. HI 4312).

Soluble ions

In the saturation paste extract, the ions in solution (P, K, Ca, Mg, Fe, Zn, Mn, B, and Mo) were determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-VARIAN equipment, model 725-ES). Nitrogen content was estimated by the micro-Kjieldahl method.

Cation exchange capacity (CEC)

The CEC in the substrate with pH below 7 was determined using the modified ammonium acetate (NH₄OAc 1 N, pH 7.0) method. In the substrates with pH values above 7 the CEC was determined with the sodium acetate (NaOAC 1N pH 8.2) method. In the resulting extracts, interchangeable Ca^{2+} , Mg^{2+} , K⁺, and Na⁺ were also determined.

Germination percentage

Germination in each experimental unit was evaluated by directly counting the number of emerged seedlings and estimating the germination percentage at the last evaluation date, 38 days after sowing (das).

Seedling height

At 62 das, seedling height was determined by measuring the length from the neck of the root to the leaf primordium of the seedlings with a tape measure.

Root and shoot dry weights and root/shoot ratio

Dry weight was determined by separating the shoot and root at harvest time and depositing them in separate paper bags for drying in a RIOSSA HCF-125D oven with forced air circulation at 70 °C for 72 h. Then, an OHAUS ADVENTURE TM PRO analytical balance was used to determine the shoot and root dry weights, expressed in mg, of each treatment. With the root and shoot dry weights, the root/shoot ratio was estimated.

Statistical Analysis

With the variables obtained, analysis of variance was performed according to the treatments and experimental designs used. For this purpose, SAS Ver 9.3 software was used (SAS 2011), and means were compared using the Tukey test ($P \le 0.05$).

RESULTS AND DISCUSSION

Physical Characterization of the Substrates

The physical characteristics of the substrates are shown in Table 1.

Table 1. Total Porosity, Air Space, Water Holding Capacity and True and BulkDensities of the Substrates Used for the Germination and Growth of Pepper(Capsicum annuum L.)

Substrate	Total porosity (TP; %)	Air space (AS; %)	Water holding capacity (WHC; %)	True Density (TD; g cm ⁻³)	Bulk density (BD; g cm ⁻³)
SBTZ	68	10	58	1.99	0.64
CHTZ	72	21	51	1.79	0.48
FCTZ	71	8	63	2.11	0.61

SBTZ: Sugarcane bagasse and tezontle; CHTZ: Coffee husk and tezontle; FCTZ: Filter cake and tezontle

According to Cabrera (1999), an ideal substrate must have a TP value of between 70 and 85%, WHC from 55 to 70%, and AS from 10 to 20%. The lowest TP (68%) was recorded in the substrate containing sugarcane bagasse, while the highest (72%) was observed in the substrate with coffee husk. Latshaw et al. (2009) reported 50% as the TP reference value; also, De Boodt and Verdondck (1972) indicated that the optimum TP for horticultural crops is higher than 85%. TP is the result of the sum of the liquid and gas phase volumes in the substrate and is related to the shape, size, and arrangement of the particles (Raviv et al. 2002). Mazarura (2013) indicates that to keep the oxygen level above 12% (value required for the root system to have adequate development and production of new roots), a substrate should have a TP value between 50 and 80%. Considering this reference, the evaluated substrates have optimum total porosity values; however, more conservative authors state that this value must be greater than 85% (Ansorena 1994; Zapata et al. 2005). Regardless of the TP reference value to be considered, it is more important to analyze the air space and water-holding capacity percentages, as if air space predominates, the substrate is considered light, but the plant may be subjected to water stress; on the contrary, if WHC predominates, the substrate becomes heavy and is susceptible to being waterlogged (Latshaw et al. 2009).

True and bulk density value intervals regarded as optimal are 0.5 to 0.75 and from 1.45 to 2.65 g cm⁻³, respectively (Ansorena 1994; Pastor-Sáez 1999; Zapata *et al.* 2005). In this regard, all the substrates can be considered as within the range identified as ideal.

The water release curves for the substrates were determined by applying suction intervals of 0, 10, 50, and 100 cm of water column pressure, and the main parameters of the physical properties for classifying the water contained in them were established (Fig. 1).

The EAW values in the analyzed substrates were negatively related with the percentages of nonavailable water (NAW); *i.e.*, as the readily available water decreased, nonavailable water increased, with this relationship being more evident in the SBTZ and CHTZ substrates (Table 2).



Fig. 1. Water release curves of the substrates used for the germination and growth of pepper (*Capsicum annuum* L.). A) SBTZ; B) CHTZ; C) FCTZ. Substrates in A, B, and C were added in a 40:60 proportion (v/v), respectively. TP = total porosity; HAW = hardly available water; RW = reserve water; EAW= easily available water; NAW = nonavailable water

Substrate	Nonavailable water (NAW)	Easily available water (EAW)	Reserve water (RW)	Hardly available water (HAW)
SBTZ	18.71	13.50	9.75	26.02
CHTZ	17.70	13.33	9.04	31.91
FCTZ	17.13	12.25	8.37	33.22
1012	11.10	12:20	0.07	00:22

Table 2. Percentage Indicators of the Water Release Curves of the SubstratesUsed for the Germination and Growth of Pepper (*Capsicum annuum* L.)

The value for easily available water (EAW) refers to the moisture held between container capacity and a nominated refill point for unrestricted growth; *i.e.*, in this moisture range the plants are neither waterlogged nor water-stressed (NSW 2004). De Boodt and Verdonck (1972) state that the optimum EAR range in substrates is between 20 and 30%. Therefore, none of the substrates evaluated in this research had appropriate values with regard to this indicator (Table 2).

The micropores in a substrate contain water that is not used by the plant in cases of normal hydration. It is therefore considered reserve water (RW) for use in a water stress situation (Gliński *et al.* 2011), and it is released at tensions between 5 and 10 kPa (Vence 2008). In this research, the substrates showed RW values ranging between 8.37 and 9.75% (Table 2).

Chemical Characterization of the Substrates

The pH and EC values of the substrates used are presented in Table 3. These variables are indicators of the form and concentration in which nutrients are available to plants. In particular, the pH plays a crucial role in the availability of micronutrients, with high pH values being associated with a reduction in their availability. A high pH is also associated with high concentrations of alkali ions. For its part, the EC value is indicative of the concentration of ionized salts in the substrate; high EC values can have negative effects on plant growth and metabolism (Brito *et al.* 2007).

Substrate	рН	EC (dS m ⁻¹)
SBTZ	7.81	0.404
CHTZ	6.01	3.163
FCTZ	7.89	1.900

Table 3. Values of pH and Electrical Conductivity (EC) in the Substrates Used for the Germination and Growth of Pepper (*Capsicum Annuum* L.)

Abad *et al.* (2001) stated that acceptable pH and EC values in substrates range from 5.2 to 6.3 and from 0.75 to 1.99 dS m⁻¹, respectively. Except for the CHTZ substrate, all of the substrates exceeded the aforementioned pH range. In the particular case of pepper, optimum pH values for good development range from 6.5 to 7; however, it can develop under acidic conditions with a pH value of 5.5 (Soler-Rovira *et al.* 2002; Abad *et al.* 2005). With regard to electrical conductivity (EC), only the FCTZ was within the reference interval. Of particular note is the high EC value in the substrate containing coffee husk, which can affect the development of saline-sensitive plants (Cabrera 1999).

It is also important to know the nutrient concentration in a substrate. Table 4 shows the nutrient concentrations determined in the saturation paste extract of the substrates. The

CHTZ substrate had the highest concentrations of the nutrients N, P, K, Fe, Zn, Mn, and B, as well as Na, an element that is not essential for higher plants and that in high concentrations may cause toxicity (Luan *et al.* 2009).

Substrata	Ν	Р	К	Ca	Mg
Substrate	%	 g kg⁻¹			
SBTZ	0.030	0.019	0.053	0.114	0.067
CHTZ	0.094	0.223	1.472	0.053	0.160
FCTZ	0.094	0.026	0.180	0.263	0.198
Substrato	Fe	Zn	Mn	В	Na
Substrate			mg kg⁻¹		
SBTZ	0.606	0.127	1.179	0.287	67.537
CHTZ	3.230	2.012	3.604	1.998	102.892
FCTZ	0.755	0.281	0.814	0.135	25.375

Table 4. Nutrient Concentration in the Saturation Paste Extract of the Substrates

 Used for the Germination and Growth of Sweet Pepper (*Capsicum annuum* L.)

In contrast to the concentration results obtained here with the reference levels for organic substrates (in mg kg⁻¹ DM: N, 20 to 200, P: 6 to 10; K, 15 to 25; Ca, above 200; Mg, above 70; Fe, 30 to 300; Mn, 20 to 300; Zn, 030 to 300; and B, 5 to 500) (Zapata *et al.* 2005), it is notable that the FCTZ substrate had appropriate values of both macro and micronutrients, while the CHTZ substrate exceeded the nutrient concentration reference ranges in all cases, except for the Ca concentration, which is classified as deficient. The SBTZ substrate was deficient in potassium and zinc.

Table 5 presents the values of exchangeable cations and cation exchange capacity (CEC) of the substrates under study. A CEC value considered sufficient (between 15 and 25 cmolc kg⁻¹) is necessary to cushion sudden pH and nutrient concentration changes in organic substrates (Argo 1998). In this regard, differences were observed among the substrates; the SBTZ substrate had the lowest CEC and the lowest value of each exchangeable cation, whereas the FCTZ substrate had the highest values of these parameters. Typically, the CEC in soils is positively associated with the ions in solution, which is more evident in the case of Ca and Mg in the FCTZ substrate (Table 5).

Table 5. Exchangeable Cations and Cation Exchange Capacity (CEC) in the
Substrates Used for the Germination and Growth of Pepper (Capsicum annuum
L.)

Substrate -	K+	Ca ²⁺	Mg ²⁺	Na⁺	CEC
	cmol _c kg ⁻¹				
SBTZ	0.75	7.73	0.08	1.16	9.72
CHTZ	16.82	2.59	0.03	0.47	19.90
FCTZ	3.54	20.20	0.13	2.22	26.08

Germination

The process for producing high-quality seedlings involves various aspects, including seed germination (Caldeira *et al.* 2000), which is particularly important in pepper, as it requires a high emergence percentage and uniformity in the size of seedlings for transplant, goals which are not always achieved due to differences in vigor among commercial seed lots (Demir *et al.* 2008). Also, there is wide variation in both germination times and germination percentage among varieties of the species, as shown in Fig. 2.

The lowest germination percentage (20%) was obtained in the California variety (Fig. 2A), regardless of the substrate used, compared to the other varieties evaluated.

For its part, the Sven F1 variety recorded its lowest germination percentage (76.6%) in the CHTZ substrate, at 27 das. Germination percentages of 86.6 and 89.6% were obtained in the SBTZ and FCTZ substrates, respectively (Fig. 2B).

The Sympathy variety had a 79.98% germination percentage in an average period of 26 days, with the highest average in the seeds sown in tezontle (93.3%), followed by those established in the FCTZ substrate, with a value of 83.3%, and in the SBTZ substrate, with 73.3%; finally, the lowest germination (70%) was recorded in the CHTZ substrate (Fig. 2C).



Fig. 2. Germination percentage of five varieties of pepper in different substrates, evaluated between 13 and 39 days after sowing. A = California; B = Sven F1; C = Sympathy; D = Yolo Wonder; E = Zidenka F1.

In regards to the Yolo Wonder variety (Fig. 2D), the germination percentage in descending order was found with the FCTZ, SBTZ, and CHTZ substrates, with values of 72.4, 66.2, and 43.3%, respectively. The average germination of the Zidenka F1 variety was 87.33% in a period of 26.5 days. The highest germination percentage was recorded in the FCTZ substrate, followed by the SBTZ substrate, with percentages of 72.4 and 66.2%, respectively (Fig. 2E).

On the other hand, the substrates under study had differential effects on germination percentage, as shown in Fig. 3; this is because the physical and chemical properties of the substrates have an influence on seed germination and seedling development (Nascimento *et al.* 2003).



Fig. 3. Germination percentage as a function of the substrate used, evaluated between 13 and 39 days after sowing; A = SBTZ; B = FCTZ; C = CHTZ

Independently of the pepper variety, in the evaluation period (from 13 to 39 days), the lowest germination average (55.3%) was recorded in the CHTZ substrate (Fig. 3B), followed by the FCTZ substrate, with a germination percentage of 67.66% (Fig. 3C), and by the SBTZ substrate (Fig. 3A), with a percentage average of 69.22%. It has been observed that germination and growth in some crops are associated with substrate properties. Among the chemical properties, pH and EC are the most influential. In this regard, it is interesting to note that the substrate where the lowest germination was recorded was CHTZ, which presented a pH within the optimum range (5.2 to 6.3), which implies greater nutrient availability in this substrate (Abad *et al.* 2005). However, this substrate presents high EC values (3.16 dS m⁻¹, Table 4) and a high concentration of soluble Na (102.89 mg L⁻¹) in the saturation paste extract (Table 5), factors that adversely affect germination.

The average time in which the maximum germination percentage was reached was variable for each substrate. Average maximum germination was reached in less time in the FCTZ substrate, at 28.6 days; on the contrary, in the CHTZ substrate, the maximum germination was recorded at 32.4 days (Fig. 3). Coinciding results have been reported in other varieties of *Capsicum annuum* L., such as Demre, Cetinel 150, and Ilica 256, as increases in the concentration of NaCl gradually reduce the germination percentage and lengthen the time it takes to reach it (Yilmaz *et al.* 2004). In addition, the CHTZ substrate had a TP value of 72%, which is within the appropriate range. However, this substrate had a greater water-holding capacity, which normally results in excess moisture buildup (Latshaw *et al.* 2009) and delayed germination (Naasz *et al.* 2009).

Plant Height

Due to the substrate effect, the greatest plant height (3.1 cm) was recorded in the FCTZ substrate, whereas the plants grown in SBTZ and CHTZ attained smaller sizes. In terms of varieties, Sympathy and Zidenka F1 reached the greatest height (3.0 cm), while Sven F1 placed second, and California and Yolo Wonder were the smallest (Fig. 4).



Fig. 4. Pepper seedling height as a function of substrate used (A) and variety (B) at 69 days after sowing. Means \pm SD with different letters in each column are significantly different (Tukey, $P \le 0.05$).

Shoot Dry Weight

Due to the substrate effect (Fig. 5A) and associated with the variable height, it was observed that seedlings grown in the FCTZ substrate recorded the greatest shoot biomass weight, followed by those established in CHTZ and, finally, those that grew in SBTZ. Also, the California and Yolo Wonder varieties had the lowest shoot dry biomass weight, and

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this was significantly different from that of the other varieties; the highest values in regard to shoot biomass were recorded in Sympathy and Zidenka F1 (Fig. 5B).



Fig. 5. Shoot dry weight of pepper seedlings as a function of substrate used (A) and variety (B) at 62 days after sowing. Means \pm SD with different letters in each column are significantly different (Tukey, $P \le 0.05$).

Root Dry Weight

As was the case with shoot biomass, seedlings grown in the FCTZ substrate had the highest root dry weight (Fig. 6A). On the other hand, the Zidenka F1 variety had the highest root dry biomass, followed by Sven F1 and Sympathy, while California and Yolo Wonder had the lowest values for this variable (Fig. 6B).



Fig. 6. Root dry weight of pepper seedlings as a function of substrate used (A) and variety (B) at 62 days after sowing. Means \pm SD with different letters in each column are significantly different (Tukey, $P \le 0.05$).



Fig. 7. Root/shoot ratio of pepper seedlings as a function of substrate used (A) and variety (B) at 62 days after sowing. Means \pm SD with different letters in each figure are significantly different (Tukey, $P \le 0.05$).

Root/Shoot Ratio

As for the substrate effect, no significant differences were observed in terms of the root/shoot ratio (Fig. 7). Instead, it was the variety factor that showed significant differences, with the Yolo Wonder variety recording the greatest value for this variable, which means that with this variety, the root continued to grow as shoot growth weakened.

CONCLUSIONS

- 1. In general, the mixtures of lignocellulosic materials derived from agricultural residues that were tested in this experiment (sugarcane bagasse, filter cake from sugarcane processing, and coffee husk, in combination with the volcanic rock tezontle) exhibited physical characteristics suitable for use as substrates for the germination and growth of plants under greenhouse conditions; however, none of them shows a readily available water level that can be considered ideal. As for chemical characteristics, in general, the substrate composed of filter cake and tezontle stands out, as it shows appropriate EC, CEC, and nutrient content levels.
- 2. When testing the effect of these substrates on biological indicators of different varieties of pepper, it was observed that the substrates have differential effects on both seed germination percentage and time, as well as on the biomass production of growing plants. In general, the substrate containing filter cake (FCTZ) had the best effects on these biological indicators. Differences were also observed among the varieties, with the California and Yolo Wonder varieties having the lowest germination and growth values, regardless of the substrate used.

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