Effect of organic substrates on the physiological development of coffee (*Coffea canephora* P.) cuttings

Efecto de sustratos orgánicos en el desarrollo fisiológico de esquejes de café (*Coffea canephora* P.)

Josafhat Salinas-Ruiz[1], **Elías Ortiz-Cervantes**[2], **Marisol Hernández-Lara**[3], **Aristarco Aguas-Atlahua**[3], **Joel Velasco-Velasco**[1*]

[1]Colegio de Postgraduados Campus Córdoba. Km 348. Carretera Federal Córdoba-Veracruz. CP. 94946. Congregación Manuel León, Amatlán de los Reyes, Veracruz.
[2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Km 16. Carretera Tezonapa-El Palmar, Tezonapa, Veracruz.
[3]Instituto Tecnológico Superior de Zongolica Campus Tezonapa. Antiguo Recinto Ferial, Carretera hacia El Palmar. Tezonapa, Veracruz.

*Corresponding author: joel42ts@colpos.mx

Scientific article
Received: june 04, 2019
Accepted: january 7, 2021

How to cite: Salinas-Ruiz J, Ortiz-Cervantes E, Hernández-Lara M, Aguas-Atlahua A, Velasco-Velasco J (2021) Effect of organic substrates on the physiological development of coffee (*Coffea canephora* P.) cuttings. Ecosistemas y Recursos Agropecuarios 8(1): e2329. DOI: 10.19136/era.a8n1.2329

ABSTRACT. The effect of three substrates made from vermicompost and one based on compost was evaluated on the development of physiological variables of cuttings from three coffee clones: INIFAP 00-28, INIFAP 00-24, and INIFAP 97-14 of robusta coffee (*Coffea canephora* P.) and their interactions. Twelve treatments were studied, arranged in a strip plot block design with factorial structure in the treatments (substrate x clone) with four repetitions; ANOVA and Tukey's mean comparison ($p \leq 0.05$) was performed. In the substrate, the variables pH, electrical conductivity (EC), organic matter (OM), apparent density (Bd), cation exchange capacity (CEC), macro and micronutrients were evaluated; while in the coffee cuttings clones, mortality percentage (M), axillary bud yield percentage (S), rooting percentage (R), outbreak length (Ol), number of internodes (Ni), number of leaves (Nl), number of roots (Nr) and root length (Rl). In the INIFAP substrate, the different types of robusta coffee clones showed the highest physiological development. Among types of clones, clone INIFAP 00-28 presented the highest physiological development of the photosynthesis part (axillary bud yield, outbreak length, number of internodes, number of leaves); however, it showed the least root development. The lowest development was observed in the clone INIFAP 97-14, and the highest root development was observed in the clone INIFAP 00-24 (percentage of rooting, number of roots and root length) while the clone INIFAP 00-28 showed the highest development of the photosynthesis part.
Key words: *Coffea canephora* P., compost, cuttings, vermicompost.

RESUMEN. Se evaluó el efecto de tres sustratos elaborados a base de vermicompost y uno a base de compost en el desarrollo de variables fisiológicas de esquejes de tres clones de café: INIFAP 00-28, INIFAP 00-24, e INIFAP 97-14 de café robusta (Coffea canephora P.) y sus interacciones. Se estudiaron 12 tratamientos, alojados en un diseño de bloques en tiras divididas (strip plot) con estructura factorial en los tratamientos (substrato x clon) con cuatro repeticiones, se realizó ANOVA y comparación de medias de Tukey ($p \leq 0.05$). En el substrato se evaluaron las variables pH, Conductividad eléctrica (EC), materia orgánica (OM), densidad aparente (Bd), capacidad de intercambio catiónico (CEC), macro y micronutrientes; mientras que en los clones de esquejes de café se evaluaron porcentaje de mortalidad (M), porcentaje de prendimiento de yemas axilares (S), porcentaje de enraizamiento (R), longitud de brote (Ol), número de entrenudos (Ni), número de hojas (Nl), número de raíces (Nr) y longitud de la raíz (Rl). En el sustrato INIFAP, los diferentes tipos de clones de café robusta tuvieron el mayor desarrollo fisiológico. Entre tipos de clones, el clon INIFAP 00-28 presentó el mayor desarrollo fisiológico de la parte fotosintetizadora (prendimiento de yema axilar, longitud de brote, número de entrenudos, número de hojas); no obstante, mostró el menor desarrollo radicular. El clon INIFAP 97-14 mostró el menor desarrollo, y el clon INIFAP 00-24 mostró el mayor desarrollo radicular (porcentaje de enraizamiento, número de raíces y longitud de raíz).
Palabras clave: *Coffea canephora* P., compost, esquejes, vermicompost.

INTRODUCTION

In Mexico, coffee has been considered one of the most economically, socially and culturally important crops (Escamilla *et al*. 2005). The state of Veracruz is the second largest coffee producer in the country, with coffee being the state's third most important agricultural product after maize (*Zea mays*) and sugarcane (*Saccharum officinarum* L.) in terms of cultivated area and the first in terms of the value of crop exports (SIAP 2015).

The species *Coffea canephora* var. robusta is a cross-pollinated plant, which implies a high variability in the type and production of plants obtained by seed. To adequately multiply robusta coffee one option is to use the asexual method, using vegetative parts obtained from selected mother plants (Abrego 2012). The advantages of this technique are: 1) production of homogeneous plants from selected genotypes with high-yield robusta coffee materials improved by up to 30%; 2) uniform plantations with good productive potential of smaller size that make the harvest and crop tasks easier, 3) more plants per area unit, and 4) shortening of the plantation's pre-productive period (INIFAP 2010). The cutting is a separate portion of the plant, endowed with cauline buds and leaves and induced to form roots and shoots through chemical, mechanical and/or environmental manipulations. The acquisition of new plants through cuttings is possible thanks to two characteristics of the plant cell, which are totipotency and dedifferentiation (Hartmann and Kester 1997).

One of the most important factors influencing asexual growth of seedlings is the choice of substrate since it is the rooting medium whose functions are to keep the cuttings fixed in place, provide moisture to their base and act as a buffer in chemical and pH reactions. The substrate's support function is essential to provide good characteristics such as water-holding capacity, bulk density, electric conductivity, neutral pH, macro and micronutrients (Dumroese *et al*. 2011); the most notable organic substrates are peat-moss, compost and vermicompost; the last two are obtained by biological processes that transform organic remains of different materials into a relatively stable organic matter with chemical, physical and microbiological characteristics for the reproduction of plants in a nursery (Velasco-Velasco *et al*. 2011, Quintero *et al*. 2003). Similarly, Mendoza-Hernández *et al*. (2014) mention that vermicompost outperformed compost and peat for rooting cuttings, the presence of hormone-like substances in the vermicompost might be behind this effect; the same authors mention that the vermicompost-based substrates gave acceptable results for growing plants, though none performed as well as the control.

The raw material used to make compost and vermicompost has a direct impact on the final concentration and behavior of the nutrients, so the final quality of these products is expected to be very different from each other, such as their effect on the soil and crops after application (Duran and Henríquez 2007). In relation to *Coffee canephora* propagation, different results have been published; on the one hand Guisolfi *et al*. (2020) studied physiological variables by using different proportions of agricultural residues as part of the substrate compared to the commercial substrate in the production of Conilon coffee seedlings, and these authors did not found significant differences between them. On the other hand, Quartezani *et al*. (2019) observed that proportions of composted urban waste higher than 50% added to the soil substrate promoted the highest plant growth rates; hence the use of stabilized organic matter in substrates for clonal plant propagation is essential to promote favorable conditions for the development of both shoots and roots. Therefore, there is an opportunity area for studying different types of compost and vermicompost made from local raw materials, and evaluate them as potential material in the clonal *C. canephora* propagation.

The aim of this study was to determine the effect of four types of substrates based on vermicompost and compost made from different types of organic matter, three robusta coffee clones (INIFAP 00-28, INIFAP 00-24 and INIFAP 97-14) produced by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) and their interactions on the development of physiological variables of robusta coffee (*Coffea canephora* P.) cuttings.

E. ISSN: 2007-901X

MATERIALS AND METHODS

Description of the study area

The experiment was carried out in a greenhouse at the INIFAP "El Palmar" Experimental Station, located at 18° 32' LN and 96° 47' LW, at an elevation of 180 masl in the municipality of Tezonapa, Veracruz. This region is characterized by a warm humid climate, summer rains, annual precipitation of 2,885 mm, and a mean annual temperature of 24.4 °C, with a minimum and maximum of 16.1 °C and 35.4 °C. The municipality of Tezonapa is located in the central region of Veracruz and its physiography is characterized by flat lands and rolling hills with slopes of 5 to 20%. The soils of the region are red laterites, deep with excellent natural drainage, clayey-sandy loam texture and pH 4.8 (INIFAP 2009).

Study factors

This research had two study factors: a) type of substrate and b) type of robusta coffee clone. Three substrates based on vermicompost were evaluated: 1) Rabbit manure vermicompost + sand at a ratio of 70:30 v/v (RaVc), 2) Sheep manure vermicompost + sand at a ratio of 70:30 v/v (ShVc), 3) Cow manure vermicompost + sand at a ratio of 70:30 v/v (CoVc) and 4) Compost made on the basis of sugarcane filter cake + coffee husk + sand at a ratio of 33:33:33 v/v (INIFAP); the last-mentioned substrate is what INIFAP usually uses to grow robusta coffee cuttings. With regard to plant material, three types of robusta coffee clones were used: INIFAP 00-28, INIFAP 00-24 and INIFAP 97-14. Table 1 shows the list of treatments resulting from the combination of both factors.

Preparing the rooting chamber

A rooting chamber measuring 6.0 m x 1.3 m equipped with a sprinkler irrigation system was designed to house the coffee clone cuttings. The preparation of the seedbed within the rooting chamber is described below in chronological order: 1) 10 cm of sand was placed, 2) 10 cm of gravel were placed on the sand layer, and 3) 15 cm of the substrate to be evaluated were placed over the entire chamber surface. Divisions perpendicular to the length of the chamber were made with blocks lined with transparent polyethylene to prevent the flow of water and nutrients between divisions where a clone (30 cuttings) was placed. The substrates that include vermicompost were mixed with sand at a 70/30 ratio (vermicompost/sand v/v), except the one corresponding to INIFAP (Table 1). Before sowing the cuttings a prolonged irrigation was applied to field capacity following the method stated in NOM-021-SEMARNAT-2000 (SEMARNAT 2002), and then a fungicide was applied to prevent the growth of any fungus.

Collection, sowing and acclimatization of cuttings

The cuttings were collected in a clonal coffee garden located at the INIFAP "El Palmar" Experimental Station on September 11, 2015 at 6:30 h. The methodology used by INIFAP for selecting and sowing cuttings was the "rooted cuttings" technique proposed by Méndez *et al*. (2015). Young shoots of good size, preferably primary branches and without disease, were selected. The shoots were separated from the mother plant with a bevel cut made from the base with pruning shears, the lateral axillas were removed and the leaves were cut back to three-quarters. The bevel cut was made to shoots of 3 to 4 internodes at 1 cm above and 5 cm below the internode. Once the cuttings were obtained, they were treated with an antioxidant solution (15 L of water mixed with 150 mL L^{-1} of ascorbic acid, 100 mL L^{-1} of citric acid and 30 g L^{-1} of sugar) and then immersed in a commercial fungicidal solution (Amistar 1.5 g L^{-1}). Initially, irrigation was applied to the substrate up to field capacity, following the method mentioned above, and then the water applied was done as it was required. The cuttings were inserted into the substrate and spaced 5 cm apart. After the sowing work, the rooting chamber was covered with 600-gauge clear plastic, which was secured into place. The irrigation and weeding were conducted three times a week by adding water through a pipe with an ending sprayer and weeding manually. The cuttings underwent acclimatization for three months inside the rooting chamber.

Evaluated variables

Physical and chemical analysis of the

Table 1. Evaluated treatments.

Treatments	Identification	Description
TRT1	RaVc/00-28	Rabbit manure vermicompost + sand (70:30 v/v)/clon INIFAP 00-28.
TRT2	RaVc /00-24	Rabbit manure vermicompost + sand (70:30 v/v)/clon INIFAP 00-24.
TRT3	RaVc /97-14	Rabbit manure vermicompost + sand + arena (70:30 v/v)/clon INIFAP 97-14.
TRT4	ShVc /00-28	Sheep manure vermicompost + sand (70:30 v/v)/clon INIFAP 00-28.
TRT5	ShVc /00-24	Sheep manure vermicompost + sand (70:30 v/v) /clon INIFAP 00-24.
TRT6	ShVc /97-14	Sheep manure vermicompost + sand (70:30 v/v) / clon INIFAP 97-14.
TRT7	CoVc/00-28	Cow manure vermicompost + sand (70:30 v/v) / clon INIFAP 00-28.
TRT8	CoVc /00-24	Cow manure vermicompost + sand (70:30 v/v) / clon INIFAP 00-24.
TRT9	CoVc /97-14	Cow manure vermicompost + sand (70:30 v/v) /clon INIFAP 97-14.
TRT10	INIFAP/00-28	Compost made on the basis of sugarcane filter cake + coffee husk + sand (33:33:33 v/v) / clon INIFAP 00-24.
TRT11	INIFAP/00-24	Compost made on the basis of sugarcane filter cake + coffee husk + sand (33:33:33 v/v) / clon INIFAP 00-28.
TRT12	INIFAP/97-14	Compost made on the basis of sugarcane filter cake + coffee husk + sand (33:33:33 v/v)/ clon INIFAP 97-14

substrates was carried out in the soil science department at the central laboratory of the Universidad Autónoma Chapingo. The evaluated variables were: pH: potentiometer in suspension (sample:water ratio 1:5); Electrical conductivity (EC): conductivity bridge in suspension (sample:water ratio 1:5); Organic matter (OM): Walkley and Black. Nitrogen (N): extracted with 2N potassium chloride and determined by steam distillation; Assimilable phosphorus (P): (P): Bray P1, Potassium and Sodium (K, Na): extracted in 1.0 N ammonium acetate, pH 7.0, 1:20 ratio and determined by flame emission spectrophotometry; Calcium, Magnesium (Ca, Mg): extracted in 1.0 N ammonium acetate, pH 7.0, a 1:20 ratio and determined by atomic absorption spectrophotometry; Iron, Copper, Zinc, Magnesium (Fe, Cu, Zn, Mg): extracted with a 1:4 DTPA ratio and determined by atomic spectrophotometry; Boron (B): extracted with 1.0M $CaCl_2$ calcium and determined by photocolorimetry with azomethine-H; Bulk density (Bd) test-tube method; Carbon: Nitrogen (C:N) and CIC: estimated by calculation. The methodology for analyzing these variables was carried out in accordance with Mexican Official Standard NOM-021-SEMARNAT-2000, which was used to interpret the results.

Robusta coffee clones

The physiological variables evaluated in each of the cuttings were: mortality percentage, axillary bud and rooting, axillary bud length (cm) from the base of the cutting to the terminal part of the shoot, number of shoots, number of internodes, number of leaves, number of roots at the end of the trial and taproot length (cm) from ground level to the root tip. All variables were measured after three months of the experimental period.

Rooting chamber

Maximum and minimum temperatures were recorded every other day during the three-month cutting acclimatization period, as well as the relative humidity inside the rooting chamber. These variables were measured using a datalogger Thermohygrometer TFA Dostmann 30.5000.02.

Experimental design and data analysis

We used a strip-plot design with a factorial structure in the treatment (substrate x clone) distributed in a randomized block design. ANOVA and Tukey's mean comparison ($p \leq 0.05$) was performed. Thirty robusta coffee cuttings were used in each treatment. The model that describes this design is as follows:

$$y_{ijk} = \mu + b_k + \alpha_i + \omega_{ik} + \gamma_j + \varphi_{jk} + (\alpha\gamma)_{ij} + \varepsilon_{ijk}$$

$$i = 1,2,3, \quad j = 1,2,3,4, \quad k = 1,2,3,4$$

where y_{ijk} is the *ij*-th response observed in block *k*, μ is the overall mean, b_k is the random effect of block *k* assuming $b_k \sim i.i.d.N(0,\sigma_b^2)$, α_i is the fixed effect due to clone *i*, ω_{ik} is the random effect due to clone *i* within block k assuming $\omega_{ik} \sim i.i.d.N(0,\sigma_\omega^2)$, γ_j is the fixed effect of substrate *j*, φ_{jk} is the random effect due to substrate *j* within block *k* assuming $\varphi_{jk} \sim i.i.d.N(0,\sigma_\varphi^2)$, $(\alpha\gamma)_{ij}$ is the effect of the

E. ISSN: 2007-901X

interaction due to clone *i* and substrate *j*, and ε_{ijk} is the experimental error assuming $\varepsilon_{ijk} \sim i.i.d.N(0,\sigma^2)$. To compare the length of shoots (Sl) and roots (Rl) and the number of internodes (Ni), shoots (Ns), leaves (Nl) and roots (Nr), two types of statistical models were used. One was a mixed linear model for a continuous response (Sl and Rl). The linear predictor for Sl and Rl is $\eta_{ijk} = \mu + \alpha_i + \omega_{ik} + \gamma_j + \varphi_{jk} + b_k$; where η_{ijk} is the response observed in the *ijth* clone - substrate in the *k* block, and μ, α_i, ω_{ik}, γ_j, φ_{jk} and b_k defined above. Sl and Rl have a normal distribution, that is $y_{ijk}|\omega_{ik}$, φ_{jk}, $b_k \sim N(0,\sigma^2)$. The other was a generalized linear mixed model with Poisson response for Ni, Ns, Nl, and Nr whose linear predictor is $\eta_{ijk} = \mu + \alpha_i + \omega_{ik} + \gamma_j + \varphi_{jk} + b_k$. The response variables Ni, Ns, Nl, and Nr have a Poisson distribution $y_{ijk}|\omega_{ik}$, φ_{jk}, $b_k \sim N(\lambda_{ijk}$ with link function η_{ijk} = log (λ_{ijk}) and the inverse is $\lambda_{ijk} = e^{(\eta_{ijk})}$

RESULTS

Physical and chemical analysis of substrates

The highest pH values were found in the vermicompost substrates; according to NOM-021 the substrate with vermicompost based on cow manure (CoVc) showed a moderately alkaline pH, the RaVc and ShVc substrates showed neutral pH, and the substrate based on INIFAP compost had a moderately acidic pH (Table 2). In the case of EC, all substrates were found in a very slightly saline range with values from 1.17 to 1.94 dS m^{-1}. The OM content in the RaVc and CoVc substrates was found in the middle content, while the ShVc and INIFAP substrates were in the low content. The N content in all substrates was in a range from 2.0 to 3.7%, this was probably due to the different types of organic materials used in each of the substrates. The available P content in each of the substrates was high, according to Official Mexican Standard NOM-021-SEMARNAT-2000, with values ranging from 357.14 to 647.24 mg Kg^{-1} (Table 2). The K and Na content results were high in the substrates analyzed according to the standard used. The available content of micronutrients such as Ca, Mg, Fe, Cu, Zn and Mn were found suitable for use in each of the substrates analyzed. However, the Fe, Cu and Mn micronutrients obtained a higher content in the INIFAP substrate than the other substrates (Table 2).

Table 2. Chemical and physical characteristics of substrates.

Parameters	Types of substrates			
Chemical and physical	RaVc	ShVc	CoVc	INIFAP
pH	7.15	7.29	7.70	6.07
EC dSm^{-1}	1.638	1.949	1.778	1.172
N%	2.7	3.5	2.2	2.0
P $mgKg^{-1}$	489.00	647.24	357.14	610.19
K $mgKg^{-1}$	3552	3792	4236	2412
Na $mgKg^{-1}$	1632	1572	1452	576
Ca $mgKg^{-1}$	3272	4065	2516	2516
Mg $mgKg^{-1}$	1610	2021	1154	940
CEC Cmol (+)kg^{-1}	21.8	25.7	21.1	33.8
Fe $mgKg^{-1}$	21.64	35.96	21.78	124.36
Cu $mgKg^{-1}$	9.18	9.62	4.87	13.23
Zn $mgKg^{-1}$	30.20	17.22	13.05	9.65
Mn $mgKg^{-1}$	5.54	9.22	5.64	21.34
B $mgKg^{-1}$	4.34	1.75	3.34	3.43
Bd g/cm^3	0.95	0.98	1.14	1.08
OM%	1.68	1.28	2.69	0.67
C:N	2.8	4.7	1.4	5.1

Physical and chemical variables: pH (hydrogen potential), EC (Electrical conductivity), N (Nitrogen), P (Phosphorus), K (Potassium), Na (sodium), Mg (magnesium), CEC (cation exchange capacity), Fe (Iron), Cu (Copper), Zn (Zinc), Mn (Magnesium), B (Boron), Bd (Bulk density), OM (Organic matter) and C: N (Carbon Ratio, Nitrogen).

As regards CEC, RaVc and CoVc obtained an average value, while the ShVc and INIFAP substrates showed a high one. This variable increases notably with the presence of organic matter and it could be the basis of the substrate fertility. The highest Bd values were found in the CoVc substrate and the lowest in the RaVc one, it worth to mention that no statistical analysis was done on this variable.

Effect of substrate type on the physiological development of coffee cuttings

Substrates based on vermicompost and compost had significantly different effects on the physiological development of some variables, as shown in Table 3. Based on the analysis of variance, the average percentages for plant mortality (M), sprouting (S) and rooting (R) did not show significant differences in the different types of substrates used (p = 0.53,0.91 and 0.22, respectively). However, it should be mentioned that the INIFAP substrate showed the highest average percentages in sprouting

Table 3. Means and standard errors of the physiological variables of coffee cuttings evaluated in the different types of substrates.

sustrato	M (%)	S (%)	R (%)	Ol (cm)	Ni (cm)	Nl	Nr	Rl (cm)
RaVc	$4.54 \pm 1.7^{a*}$	$82.0 \pm 3.5^{a*}$	$54.7 \pm 4.48^{a*}$	$1.88 \pm 0.15^{ab*}$	$0.89 \pm 0.06^{a*}$	$1.71 \pm 0.12^{a*}$	$1.71 \pm 0.09^{ab*}$	$11.72 \pm 0.32^{a*}$
ShVc	6.9 ± 2.6^{a}	80.2 ± 3.4^{a}	49.6 ± 4.19^{a}	1.84 ± 0.15^{ab}	0.76 ± 0.05^{ab}	1.43 ± 0.10^{a}	1.56 ± 0.10^{b}	11.21 ± 0.35^{ab}
CoVc	8.3 ± 2.9^{a}	80.0 ± 4.1^{a}	53.8 ± 5.1^{a}	1.46 ± 0.15^{b}	0.63 ± 0.05^{b}	115 ± 0.09^{b}	1.95 ± 0.12^{a}	11.40 ± 0.36^{a}
INIFAP	5.3 ± 2.0^{a}	83.0 ± 3.4^{a}	63.1 ± 4.28^{b}	2.01 ± 0.11^{a}	0.83 ± 0.05^{a}	1.52 ± 0.11^{a}	1.72 ± 0.09^{ab}	10.96 ± 0.28^{b}

* Averages and standard deviation in columns with the same letters are statistically equal ($p \leq 0.05$). M (Percentage of mortality), S (Axillary bud yield percentage), R (Percentage rooting), Ol (Outbreak length), Ni (Number of internodes), Nl (Number of leaves), Nr (Number of roots) and Rl (root length). VcCo (70% vermicompost rabbit dung + 30% sand); VcBo (70% vermicompost of sheep manure + 30% sand); VcVa (70% vermicompost cow dung + 30% sand) and INIFAP (33% coffee husk + 33% sugarcane compost + 33% sand.

and rooting, with 83.02 and 63.1% respectively, while the highest mortality percentage was observed in the CoVc substrate. In terms of the average outbreak length (Ol) in cuttings, the INIFAP substrate showed the greatest average length with 2.01 cm, which was significantly different ($p \leq 0.05$) from the other substrates, whereas the RaVc and ShVc substrates had average lengths of 1.88 and 1.84 cm, respectively, which were statistically equal; the shortest average length was observed in the CoVc substrate, with 1.46 cm.

Regarding the number of internodes (Ni), the different types of substrates showed highly significant differences (p = 0.005). In the RaVc and INIFAP substrates the cuttings showed the highest average number of internodes (0.89 and 0.83 respectively), followed by the ShVc substrate (0.76) and lastly the CoVc one (0.63) per cutting. For the number of leaves, analysis of variance showed highly significant differences (p = 0.003) among the substrates used, with the RaVc and INIFAP substrates having the highest number of leaves with 1.71 and 1.52 respectively, while the CoVc substrate had the lowest number of leaves with 1.15 (Table 3). As for the number of roots, the cuttings showed no significant differences among the different types of substrates used (p = 0.09). In the root length of the cuttings, there were also no significant differences (p = 0.08); however, the RaVc and INIFAP substrates had the longest length with means of 11.86 and 11.86 respectively, while the ShVc substrate showed the shortest root length with 11.31 (Table 3).

Effect of the type of robusta coffee clone on the physiological development of cuttings

In general, significant differences were observed in the physiological development of the different cuttings from robusta coffee clones (Table 4). Analysis of variance of the mortality percentage showed statistically significant differences in at least one clone (p = 0.025). The highest mortality (M) percentage was observed in the INIFAP 97-14 clone (8.76%) followed by the INIFAP 00-24 clones with 7.59%, both clones were different statistically to INIFAP 00-28 (3.35%). In the percentage of axillary bud (S), the coffee clones showed significant differences (p = 0.031), with the INIFAP 00-28 clone obtaining the highest average percentage (89.2), followed by the INIFAP 00-24 and INIFAP 97-14 clones with statistically equal means (77.8 y 74.2, respectively). Significant differences (p = 0.008) were observed among the clones in rooting percentage: the INIFAP 00-24 and INIFAP 97-14 clones showed the highest percentage (70.60 and 63.80%, respectively) and were statistically equal to each other, while the INIFAP 00-28 clone had the lowest percentage (30.60). In outbreak (Ol), the robusta coffee clones showed statistical differences (p = 0.001), with the INIFAP 00-28 clone with a mean of 2.10 cm obtaining the greatest outbreak length compared to the rest, and the INIFAP 97-14 clone having the shortest average length of 1.49 cm. Regarding the number of internodes (Ni) the clones showed significant differences (p = 0.019). The INIFAP 00-28 clone had the highest number of internodes (0.87) and the INIFAP 97-14 clone the lowest (0.69). In the number of leaves (Nl), analysis of variance showed that the clones had significant differences among

E. ISSN: 2007-901X

Table 4. Means and standard deviations of physiological variables of coffee cuttings in different types of substrates.

Robusta coffee clones	M (%)	S (%)	R (%)	Ol (cm)	Ni	Nl	Nr	Rl (cm)
INIFAP 00-28	3.35 ± 1.1^{b}	89.2 ± 2.3^{a}	30.6 ± 6.57^{b}	2.1 ± 0.091^{a}	0.87 ± 0.05^{a}	1.63 ± 0.11^{a}	1.5 ± 0.1^{b}	9.57 ± 0.39^{b}
INIFAP 00-24	7.59 ± 2.2^{a}	77.8 ± 3.36^{b}	70.6 ± 6.57^{a}	1.79 ± 0.093^{b}	0.77 ± 0.05^{ab}	1.47 ± 0.10^{ab}	1.78 ± 0.07^{ab}	12.48 ± 0.24^{a}
INIFAP 97-14	8.76 ± 2.5^{a}	74.2 ± 3.6^{b}	63.8 ± 6.57^{a}	1.49 ± 0.095c	0.69 ± 0.04^{b}	1.25 ± 0.09^{b}	1.95 ± 0.08^{a}	11.99 ± 0.25^{a}

Columns with the same letters are statistically equal ($p \leq 0.05$). M (Percentage of mortality), S (Axillary bud yield percentage), R (Percentage rooting), Ol (Outbreak length), Ni (Number of internodes), Nl (Number of leaves), Nr (Number of roots) and Rl (root length).

them (p = 0.02). The INIFAP 00-28 clone had the highest average number of leaves (1.63) compared to the leaf mean observed in the INIFAP 00-24 and INIFAP 97-14 clones (1.47, and 1.25, respectively). For the number of roots (Nr), the clones showed significant differences (p = 0.026); the INIFAP 97-14 clone cuttings showed the highest average number of roots (1.95), followed by the INIFAP 00-24 and INIFAP 00- 28 clones which had a lower average number of roots (1.78 and 1.50, respectively). Finally, the clones showed significant statistical differences in average root length (Rl) (p = 0.002), with the INIFAP 00-24 and INIFAP 97-14 clones having an average root length (12.48 and 11.99, respectively) statistically equal to each other but different from that of the INIFAP 00-28 clone (9.57).

Interaction of substrate and coffee clone on the physiological development of cuttings

Mortality

Cutting mortality was statistically different ($p \leq 0.05$) among the substrate/coffee clone combinations used (Table 5). Higher cutting mortality percentages were observed in the ShVc and CoVa substrates than in the RaVc substrate, while the INIFAP 00-28 clone showed better adaptability in the substrates tested.

Sprouting

The axillary bud percentages were significantly different ($p \leq 0.05$) among the different treatments (Table 5). Results show that the INIFAP 00-28 clone in combination with the different substrates showed the highest sprouting, followed in descending order by the INIFAP 00-24 and 97-14 clones.

Rooting

The rooting percentages in the substrate-clone interaction were significantly different ($p \leq 0.05$); the cuttings from the INIFAP 00-24 and 97-14 robusta coffee clones (Table 5) showed a greater number of roots in all substrates, while fewer roots were observed in the INIFAP 00-28 clone.

Outbreak length

In the substrate-clone interaction the cuttings showed a significantly different ($p \leq 0.01$) average outbreak length in the different combination of factors (Table 5). The highest average length in cuttings was observed in those planted in the INIFAP substrate followed by the RaVc and ShVc substrates, while the shortest length observed was in the cuttings planted in the CoVc substrate. It is important to note that the INIFAP 00-28 clone showed the greatest average length in each of the substrates used and the INIFAP 97-14 clone showed the shortest length in most substrates except the INIFAP one.

Internodes

In the variable number of internodes, highly significant differences ($p < 0.01$) were found in the different substrate-clone combinations (Table 5). Better interaction of the two factors on the number of internodes was found in the cuttings planted in the RaVc, ShVc and INIFAP substrates, whereas a lower interaction effect was observed in the cuttings planted in the CoVc substrate.

Number of leaves

Similarly, in the number of leaves, significant differences ($p < 0.01$) were obtained in the different treatments evaluated (Table 5). Cuttings sown in the RaVc substrate showed the highest number of leaves

Table 5. Comparison of physiological variables between treatments.

Treatment	Mortality	Sprouting	Rooting	Outbreak length (cm)	Number of		Number of roots	Root length (cm)
					internodes	leaves		
RaVc/00-28	2.5^{d}	91.4^{ab}	30.9^{de}	1.9^{a}	0.9^{a}	2.2^{abc}	1.6^{bcd}	9.8^{d}
RaVc/00-24	6.2^{abcd}	74.2^{c}	63.9^{abc}	1.8^{ab}	0.9^{a}	2.6^{a}	1.6^{bcd}	13.2^{a}
RaVc/97-14	5.9^{abcd}	75.8^{bc}	67.5^{ab}	1.4^{bc}	0.8^{a}	2.6^{a}	1.8^{abc}	12.5^{a}
ShVc/00-28	4.1^{dc}	86.2^{abc}	20.7^{e}	1.9^{a}	0.9^{a}	2.2^{abc}	1.4^{cd}	9.9^{cd}
ShVc/00-24	7.1^{abcd}	80.3^{abc}	72.7^{ab}	1.7^{ab}	0.8^{ab}	2.3^{ab}	1.3^{d}	12.4^{a}
ShVc/97-14	11.3^{ab}	72.5^{c}	56.7^{bc}	1.3^{c}	0.5^{c}	2.1^{bc}	2.1^{ab}	12.0^{abc}
CoVc/00-28	5.4^{bcd}	86.1^{abc}	31.1^{de}	1.8^{ab}	0.8^{a}	2.2^{abc}	1.7^{abcd}	10.0^{bcd}
CoVc/00-24	7.1^{abcd}	76.1^{abc}	64.2^{abc}	1.3^{c}	0.6^{bc}	2.2^{abc}	2.3^{a}	12.1^{ab}
CoVc/97-14	14.4^{a}	76.7^{abc}	65.0^{abc}	1.1^{d}	0.5^{c}	2.1^{bc}	1.9^{abc}	12.3^{ab}
Inifap/00-28	2.2^{d}	91.8^{a}	42.0^{dc}	2.1^{a}	0.8^{a}	2.0^{c}	1.3^{d}	8.7^{e}
Inifap/00-24	10.6^{abc}	80.4^{abc}	77.1^{a}	1.8^{ab}	0.1^{ab}	2.3^{ab}	2.0^{ab}	12.2^{ab}
Inifap/97-14	5.9^{abcd}	71.7^{c}	67.1^{ab}	1.8^{ab}	0.9^{a}	2.4^{ab}	1.9^{abc}	12.0^{abc}

Columns with the same letters are statistically equal ($p \leq 0.05$). Treatments: Rabbit vermicompost 00-28 clon (RaVc/00-28), Rabbit vermicompost 00-24 clon (RaVc/00-24), Rabbit vermicompost 97-14 clon (RaVc/97-14), Sheep vermicompost 00-28 clon (ShVc/00-28), sheep vermicompost 00-24 clon (ShVc/00-24), sheep vermicompost 97-14 clon (ShVc/97-14), Cow vermicompost 00-28 clon (CoVc/00-28), Cow vermicompost 00-24 clon (CoVc/00-24), cow vermicompost 97-14 clon (CoVc/97-14), INIFAP Compost 00-28 clon (Inifap/00-28), INIFAP Compost 00-24 clon (Inifap/00-24), INIFAP Compost 97-14 clon (Inifap/97-14).

and those planted in the CoVc substrate produced the smallest number. This result correlates with the variable number of internodes since each internode usually has a pair of leaves.

Number of roots

The number of roots produced by the coffee cuttings showed significant differences ($p < 0.05$) among the different treatments. The INIFAP 00-24 and 97-14 clones produced a higher number of roots in the four substrates, while the INIFAP 00-28 clone obtained a lower number of roots in each of the substrates used (Table 5). This could have been influenced more by the characteristics of the species itself rather than the nutrient content of the substrates.

Root length

The average root length among the different substrate-clone combinations was significantly different ($p = 0.05$). It can be seen that the INIFAP 00-24 clone cuttings showed the greatest root length in all substrates tested, followed by the INIFAP 97-14 clone and the INIFAP 00-28 one, which produced the shortest root length (Table 5).

Temperature and relative humidity inside the rooting chamber

Figure 1 shows the maximum and minimum temperatures as well as the relative humidity inside the rooting chamber during the three-month cutting acclimatization period. The minimum temperature ranged from 18 to 34 °C, and the maximum from 26 to 48 °C; these temperature ranges together with the applied irrigations caused the relative humidity to vary between 80 and 98%.

DISCUSSION

In addition to pH and nutrient availability, the most important chemical properties of a substrate are cation exchange capacity (CEC), electrical conductivity (EC), C:N ratio and bulk density (Bd) (Cruz *et al*. 2014). Ginés and Mariscal (2002) state that a slightly acidic pH provides better assimilation of essential elements (N, K, Ca, Mg). Barbaro *et al*. (2014) state that the higher the electrical conductivity the higher the salts concentration, and that low EC facilitates fertilization and prevents crop phytotoxicity problems, since the presence of some ions (such as those of calcium, magnesium and sodium chlorides and sulfates) in the soil solution at certain concentrations causes toxic effects to the plant. High Bd facilitates substrate compaction and root compression, also affecting irrigation efficiency and fertilization (Abad *et al*. 2004). Martínez and Roca

E. ISSN: 2007-901X

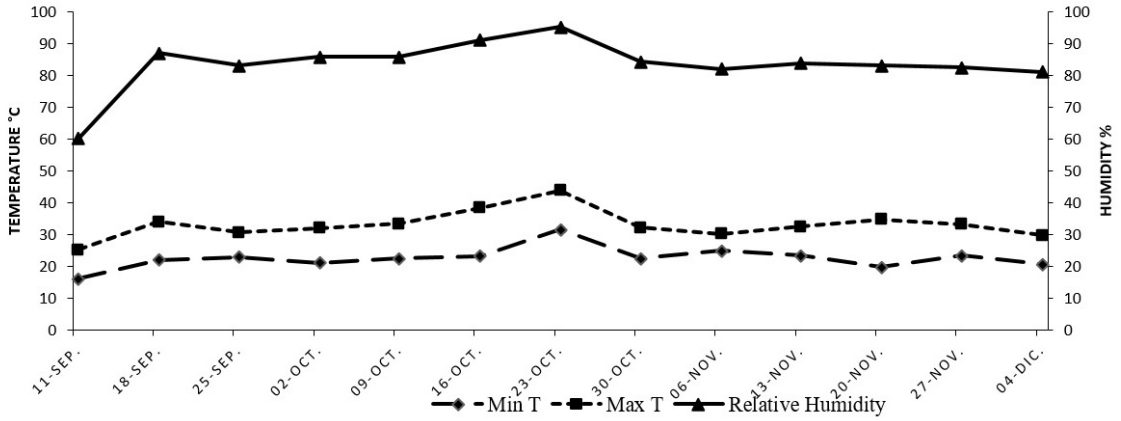

Figure 1. Maximum and minimum temperatures and relative humidity inside the rooting chamber during the three-months of cutting acclimatization period.

(2011) mention that a Bd < 0.75 g cm^{-3} is an optimal substrate level. In this work, high values of Bd ranging from 0.95 to 1.14 g cm^{-3} could affect the evaluated variables such as percentage of mortality (M); for example the substrate CoVc had an M percentage value of 8.3 (Table 3), at the same time this substrate presented 1.14 of Bd (Table 2), and the RaVc had the lowest Bd value (0.95 g cm^{-3}) and it has the lowest mortality percentage. According to Abad *et al*. (2004), a C:N ratio <30 in organic substrates is considered suitable for cultivation, and is an index of a mature and stable material. Therefore, the C:N ratios obtained by the different types of substrates were all found in optimal ranges.

According to the results, the vermicompost substrate made from rabbit manure (RaVc) and the compost substrate made from coffee husk and sugarcane filter cake compost (INIFAP) showed higher physiological development than the sheep manure vermicompost (ShVc) and cow manure vermicompost (CoVc) substrates. This effect could be attributed to several characteristics of the study substrates; pH can be one of them, which were INIFAP (6.07) and RaVc (7.15), EC could be another characteristic that could affect positively on the physiological variables, other two are Bd and CEC; however, it is not clear which one had greater influence on the physiological variables. In this regard, Santamaría *et al*. (2001) mention that most studies on the use and efficacy of vermicompost at nursery level in fruit and ornamental species show better results than the implementation of other composted organic materials, although they may have similar chemical and biological characteristics. Nevertheless, Contreras *et al*. (2008) evaluated the effect of vermicompost application and mineral fertilization on coffee, observing higher dry weight in plants with mineral fertilization, followed by treatments with 20 and 30% vermicompost. It has been observed in coffee cultivation that vermicompost induces a better response in the vegetative development of the coffee tree as compared to bokashi (Mosquera *et al*. 2016). Some results may differ, considering that the quality of the substrate is related to the source material and handling during the preparation process, having variation in the content of nutrients and microorganisms. In general, in coffee cultivation, a positive and significant effect has been observed with the application of various organic fertilizers at the nursery stage (Julca *et al*. 2002).

Overall, in this work, the INIFAP 00-28 clone exhibited the greatest physiological development of the photosynthesis part (axillary bud, outbreak length, number of internodes, number of leaves), the INIFAP 00-24 clone obtained the least development, and the INIFAP 97-14 clone had the greatest root development. In accordance with the above, in many growing plants a balance between the photosynthesizing surface area and the root area is maintained. This confirms what was stated by Raven *et al*. (1999), who

mention that in seedlings the adsorption (root) area greatly exceeds the photosynthesizing area. This behavior is very important, since if the sprouting of the buds occurs before root emission, they would compete and could deplete the water and nutritional reserves of the cutting (Ruiz and Mesén 2010). In the same way, Alfenas *et al*. (2009) argue that the root formation process may be influenced by the genetic constitution of the matrix plant. In this sense, some species present great differences in rhizogenic capacity and it is probable that the rooting capacity of robusta coffee is genotypically conditioned. Nonetheless, Alves *et al*. (2016) mention that the organic substrate is a favorable alternative that stimulate biometric characteristics compared to the soil and commercial substrate in the formation of clonal coffee canephora seedlings during the formation stage in the nursery. This factor is important for future cloning work, where the genotype/clone to be propagated must be taken into account (Abanto *et al*. 2014).

Sáenz *et al*. (2010) mention that the root length is the most important quality characteristic that allows predicting the survival of the plant in the field, which is why these two variables are key for deciding which clone should be propagated. Additionally, Rodriguez *et al*., (2017) state that some physiological variables had significant effects on the initial development of coffee seedlings with substrates using cattle manure with 5.25 kg m^{-3} of liThoThamnium compared to filter cake with 1.75 kg m^{-3} of liThoThanmium. On the other hand, plants grow continuously, producing new cell organs from their meristems; consequently, postembryonic development is a continuous process in plants that depends on environmental factors and the type and quality of the substrate, resulting in a high degree of phenotypic plasticity because the plant cells are unable to escape from their environment, and are forced to cope with changing and often unfavorable growth conditions. Thus, mechanisms regulating tissue cells can facilitate stable changes in gene activity and adjust gene expression patterns, allowing plants to survive and reproduce successfully in an unpredictable and changing environment.

CONCLUSIONS

Vermicompost made from rabbit manure (RaVc) and compost based on coffee husk and sugarcane filter cake (INIFAP) showed a greater effect on the physiological development of coffee cuttings compared to substrates based on cow manure vermicompost (CoVc) and sheep manure vermicompost (ShVc). pH and EC were the substrates characteristics that affected the physiological variables and reduce the mortality percentage of coffee cuttings, and Bd and CEC had not clear influence on the physiological variables. The INIFAP 00-28 clone showed the greatest physiological development of the studied coffee cuttings. Therefore, according to this research, the physiological development of the cuttings depended on the origin of the organic residue used in making the vermicompost and compost, and the genotypic characteristics of the propagated clone. The 00-28 coffee clone had less mortality percentage, the highest axillary bud yield percentage, high outbreak length, high number of internodes and number of leaves.

ACKNOWLEDGEMENTS

The main author thank to the Colegio de Postgraduados Campus Córdoba and to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for the support granted during this work.

LITERATURE CITED

Abad BM, Noguera MP, Carrión BC (2004) Los sustratos en los cultivos sin suelo. En: Urrestarazu Gavilán. Cultivo sin suelo. Mundi Prensa Madrid. España. pp: 113-158.

Abanto CR, Chagas EA, Choy SJ, Andrade dos Santos V, Lozano RM, Ríos GS (2014) Capacidad de enraiza-

E. ISSN: 2007-901X

miento de plantas matrices promisoras de *Myrciaria dubia* (Kunth) Mc Vaugh en cámaras de subirrigación. Ceres, Viçosa 61: 134-140.

Abrego JCO (2012) Manual para la producción orgánica de café robusta. Agricultura orgánica. Ministerio de Desarrollo Agropecuario. 51p. https://www.mida.gob.pa/upload/documentos/librosdigitales/PIDCAC/Manual_Cafe_Robusta/manual_cafe_robusta.pdf. Date of consultation: december 11, 2020.

Alves ER, Schmidt R, Moreira R, Dias JRM, Maia E (2016) Biometry and gas exchange in clonal cuttings coffee submitted to different substrates. Coffee science 11: 435-443

Alfenas AC, Zauza EAV, Mafia RG, Assis TF (2009) Clonagem e doenças do eucalipto. 2ª Ed. Viçosa, Editora Universidad Federal de Vicosa. Brazil. 500p.

Barbaro LA, Karlanian MA, Mata DA (2014) Importancia del pH y la conductividad eléctrica (CE) en los sustratos para las plantas. Ministerio de Agricultura, Ganadería y Pesca. Instituto de Floricultura, Buenos Aires, Argentina. 11p. https://inta.gob.ar/documentos/importancia-del-ph-y-la-conductividad-electrica-ce-en-los-sutratos-para-plantas. Date of consultation: December 05, 2020.

Contreras J, Acevedo I, Escalona A (2008) Efecto del vermicompost sobre el crecimiento de plántulas de café (*Coffea arabica*). Revista Unellez de Ciencia y Tecnología 26: 14-21.

Cruz CE, Can CA, Bugarín MR, Pineda J, Flores R, Juárez LP, Alejo SG (2014) Concentración nutrimental foliar y crecimiento de chile serrano en función de la solución nutritiva y el sustrato. Revista Fitotecnia Mexicana 37: 289-295.

Dumroese RK, Heiskanen J, Englund K, Tervahauta A (2011) Pelleted biochar: chemical and physical properties show potential use as a substrate in container nurseries. Biomass and Bioenergy 35: 2018-2027.

Duran L, Henríquez C (2007) Caracterización química, física y microbiológica vermicompost producidos a partir de cinco sustratos orgánicos. Agronomía Costarricense 31: 41-51.

Escamilla E; Ruiz O, Díaz G, Landeros C, Platas D, Zamarripa CP, González HVA (2005) El agroecosistema café orgánico en México. Manejo Integrado de Plagas y Agroecología 76: 5-16.

Gines I, Mariscal SI (2002) Incidencia de los fertilizantes sobre el pH del suelo. Fertiberia S.A. de C.V. España. 9p.

Guisolfi LP, Lo Monaco PAV, Krause MR, Meneghelli CM, Almeida KM, Meneghelli LAM, Vieira GHS (2020) Agricultural wastes as alternative substrates in the production of conilon coffee seedlings. Bioscience Journal 36: 792-798.

Hartmann H, Kester D (1997) Propagación de plantas: principios y prácticas. Trad. A. Ambrosio. Ed. Continental. México. 760p.

INIFAP (2010) Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Reporte Anual. Ciencia y Tecnología para el Campo Mexicano. México. www.inifap.gob.mx/Documents/reportes/reporte_anual2010.pdf. Date of consultation: december 11, 2020

INIFAP (2009) Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Reporte Anual. Ciencia y Tecnología para el Campo Mexicano. www.inifap.gob.mx/Documents/reportes/reporte_anual2009.pdf. Date of consultation: December 13, 2020.

Julca-Otiniano A, Solano-Arrue W, Crespo-Costa R (2002) Crecimiento de *Coffea arabica* variedad Caturra amarillo en almácigos con sustratos orgánicos en Chanchamayo, selva central del Perú. Agricultura: Producción Protegida Vegetal 17: 353-366.

Martínez PF, Roca D (2011) Sustratos para el cultivo sin suelo. Materiales, propiedades y manejo. En: Flórez RVJ (ed) Universidad Nacional de Colombia. Colombia. pp. 37-77

Méndez LI, Rodríguez BA, Sánchez HM (2015) Metodología para la producción de plantas de café Robusta por "estacas enraizadas". Desplegable informativo Núm. 22. SAGARPA-INIFAP-CIRPAC-Campo Experimental rosario Izapa, Tuxtla Chico, Chiapas, México. 2p. www.inifap.gob.mx. Date of consultation: December 11, 2015.

Mendoza-Hernández D, Fornes F, Belda RM (2014) Compost and vermicompost of horticultural waste as substrates for cutting rooting and growth of rosemary. Scientia Horticulturae 178: 192-202.

Mosquera AT, Melo M, Quiroga C, Avendaño D, Barahona M, Galindo F, Lancheros J, Prieto S, Rodríguez A, Sosa D (2016) Evaluación de fertilización orgánica en cafeto (*Coffea arábica*) con pequeños productores de Santander, Colombia. Temas agrarios 21: 90-101.

Quartezani WZ, de Sales RA, Pletsch TA, Berilli SS, Mantoanelli E, Hell LR, de Oliveira EC, Neves FL, Neves JDC (2019) Effect of different proportions of urban organic compost on Conilon coffee (*Coffea canephora*) propagation. Australian Journal of Crop Science 13: 821-827.

Quintero CE, Boschetti NG, Benavidez RA (2003) Effect of soil buffer capacity on soil test phosphorus interpretation and fertilizer requirement. Communications in Soil Science and Plant Analysis 34: 1435-1450.

Raven J A, Evans MCW, Kord RE (1999) The role of trace metals en photosynthetic electron transport in O_2-evolving organisms. Photosynth. Research 60: 111-149.

Rodriguez WDM, Evangelista AWP, Junior JA, Honorato MV (2017) Growing of coffee seedlings on different substrates and fertilized with lithothamium. Revista Facultad Nacional de Agronomia Medellin 70: 8177-8182.

Sáenz RJT, Villaseñor FJ, Muñoz FH J, Rueda S A, Prieto R J A (2010) Calidad de planta en viveros forestales de clima templado en Michoacán. SAGARPA-INIFAP-CIRPAC-Campo Experimental Uruapan. Uruapan, Michoacán, México 48p. http://biblioteca.inifap.gob.mx:8080/jspui/handle/123456789/1289. Date of consultation: December 09, 2020.

Santamaría RS, Ferrera-Cerrato R, Almáraz SJJ, Galvis SA, Barois I (2001) Dinámica y relaciones de microorganismos, C-orgánico y N-Total durante el composteo y vermicomposteo. Agrociencia 35: 377- 384.

SEMARNAT (2002) Norma oficial Mexicana NOM-021-SEMARNAT-2000 que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Diario Oficial de la Federación, México. pp. 19-21.

SIAP (2015) (Servicio de Información Agroalimentaria y Pesquera). Anuario estadístico de la producción agrícola. Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. México. https://www.gob.mx/siap/articulos/cierre-estadistico-de-la-produccion-agricola-2015. Date of consultation: december 10, 2020.

Velasco-Velasco J, Parkinson R, Kuri V (2011) Ammonia emissions during vermicomposting of sheep manure. Bioresource Technology 102: 59-64.

E. ISSN: 2007-901X