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Effect of Nitrogen, Phosphorus and Potassium on regional organic substrates in *Agave salmiana* production in Huichapan, Hidalgo, Mexico

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Abstract. Mexico has 159 species of *Agave* spp. In the agri-food industry stand out are *Agave tequilana*, *A*. *angustifolia*, and *A*. *salmiana*. A limitation to producing maguey seedlings is the low availability of organic substrates that favor plant adaptation in the field. The objective was to evaluate the effect of nitrogen (N), phosphorus (P) and potassium (K) found in substrates in response to vegetative and root growth of *A*. *salmiana* in agricultural areas of Huichapan, Hidalgo, Mexico. The treatment consisted of earthworm humus (EH) and leaf compost (LC) substrates with materials from the region with different percentages of EH (100, 75, 50%), LC (5, 10%), and sand (20, 40%). The treatments were applied with 14 random replications in two phases in seeds and 40-day-seedlings. The variables evaluated were NPK amount and pH in substrates. The physiological variables measured were plant height, leaf number, stem diameter, root length, and volume. Significant differences ($P \leq 0.05$) were observed in seedling physiology due to the effect of the treatment. The best agronomic responses (plant growth and root length/weight) of Agave seedlings were **T5** (75% earthworm humus + 20% sand + 5% leaf-soil) and **T6** (50% earthworm humus + 40% sand + 10% leaf-soil); in both treatments, the NPK percentages were different from the control (Haplic Phaeozem soil) group. The final concentration of NPK in **T6** was N = 0.04%, P = 398.13 mg Kg^{-1} and K = 11.88 meq $100g^{-1}$. The results infer that NPK availability in soil and progressive acidification (initial pH = 8.6, final pH = 7.4) of the substrate can favorably influence the plant response. The interactions between NPK availability in the substrate and their use for a better response in maguey seedling adaptability open up new lines of research on the productive systems in the region of Huichapan, Hidalgo, Mexico.

Keywords: *maguey: nutritional quality: plant physiology: productive soils: seedling.*

Introduction

Mexico is the center of origin of Agave species, commonly known as "maguey" an icon of Mexican culture in the world (Narváez-Suárez *et al.*, 2016). In the American Continent, 211 species of the *Agave* spp. exist, of which 75% are distributed in Mexico with 57% of endemic species, mainly in two subgenres *Agave* and *Littaea* (Garcia-Mendoza *et al.*, 2019). Agave as a subgenre has 12 groups with 83 species, while *Littaea* has eight groups with 53 species. However, according to the Code of Botanical Nomenclature, the delimitation of these group is not in a taxonomic sense but useful in a practical way (Turland *et al*., 2018). In Mexico, Cactaceae and Agavaceae represents one of the most important natural resources with strong socioeconomic and sociocultural implications (Cortés-Zárraga and Basurto-Peña, 2021; Gutiérrez-Rojas et al., 2022). Since pre-Hispanic times agaves have always been highly valued by the Mexican population, either as a food resource or medicine, elaboration of handicrafts, fuel, agro-industrial use, livestock feed, and fertilizer (Narváez-Suárez *et al.*, 2016). In addition, many Agavaceae are an icon in the arid and semi-arid landscaping regions of Mexico and are highly appreciated as ornamental plants (Cortés-Zárraga and Basurto-Peña, 2021). One of the most important contributions of agave is an intangible heritage of humanity due to its use in the elaboration of alcoholic beverages based on distillation as tequila (*A. tequilana*) and mezcal (*A. angustifolia*) and those based on fermentation, such as pulque (*A. salmiana*) (Bonilla-Barrientos *et al.*, 2019). In addition, agave is also highly appreciated for its use in preparing one of the most typical dishes of southern Mexico, "barbacoa", which consists of lamb meat baked in a hole-in-the-ground and wrapped in maguey leaves (AGARED, CIATEJ, and CONACYT, 2017).

The main agave producing areas in Mexico are Hidalgo (~112,000 t), Tlaxcala (~37,400 t), Puebla (~11,100 t), and Mexico State (~4,600 t) (SIAP, 2020). However, one of the main problems is obtaining seedlings that are well adapted to production systems. For example, in many agave-producing areas, the agronomic practice for the propagation of these plants consists of detaching the suckers from the mother plant from June to September, and by transplanting, they establish directly in the same primary production lot. However, this practice frequently generates a low percentage of adaptation and survival (Martínez-Jiménez *et al.*, 2019). *In vitro* culture techniques for obtaining desirable clones, the seedlings in a field still show many adaptation problems (Vázquez *et al.*, 2011). In contrast, obtaining agave plantlets from seed is a slow procedure where agronomic viability may take from three to five years (Álvarez-Duarte, 2018). However, these production techniques have the advantage of obtaining greater genetic variability that favors field adaptability and the possibility of entering federal programs for the preservation of native germplasm of national importance (Abanto-Rodríguez *et al.*, 2016).

In commercial agave production, the use of substrates is increasingly in demand to obtain higher quality seedlings and easier handling in production systems (Magdaleno-Villar *et al.*, 2006). Commonly, the quality of organic substrates implies the proper selection of those that have a balance between nutrients, organic matter, and rhizosphere microbiota and ensure seedling survival in the field, especially in transplanting, which is when the regeneration processes of new roots are the most critical (Durán and Henriquez, 2007; Lincoln *et al.*, 2015). The inclusion of sand in the substrates has multiple benefits in agave production. For example, it improves some parameters, such as aeration, moisture retention capacity, cation exchange capacity, pH, and electrical conductivity (Gayosso-Rodríguez *et al.*, 2018; Acevedo-Alcalá *et al.*, 2020). Substrate quality is related to the chemical characteristics that compose it, mainly the balance and availability of macronutrients, especially those derived from nitrogen (N), phosphorus (P), and potassium (K) (Hernández *et al.*, 2018). In addition, not only is the amount of NPK in the substrates important but also are the concentrations and availability critical in the plants (Sánchez *et al.*, 2020; Torres-Lozada *et al.*, 2021). For example, after agave transplantation or crop establishment, the available nutrient and mineral elements in the soil contribute to plant survival, growth, root regeneration capacity, biomass distribution, and leaf structure, and eventually, NPK deficiency can cause numerous negative effects (Lincoln *et al.*, 2015). One of the disadvantages of the use of substrates in agave seedling production is the high cost of some of their components, especially those derived from composting or vermicomposting processes (Cruz *et al.*, 2010).

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Therefore, the quality of the substrates used in the production may vary depending on the origin of the parts that compose it and the percentages used in the mixture (Raviv *et al.*, 2005). One of the viable and sustainable alternatives is the use of native compostable materials and always taking care of the most adequate NPK concentrations, which result in obtaining plants of higher quality and better adapted to the transplant processes. Therefore, the objective of this research is to evaluate the NPK percentage in substrates for *Agave salmiana* C.V. Xhamini seedling growth in Huichapan, Hidalgo, Mexico.

Material and Methods

Study Area

This investigation was carried out in the 2020 March-September, spring-summer (S-S) and 2020-2021 October-March, fall-winter (F-W) agricultural cycles at the experimental field of the Instituto Tecnológico Superior de Huichapan (ITESHU), located in Saucillo Municipality of Huichapan, Hidalgo, Mexico (20° 19' 09" N and 99° 42' 29" W) at 2172 m de altitude. The average annual temperature for these cycles is 15.7 °C with an average annual rainfall of 516.4 mm. The soils are Haplic Phaeozem type (FAO, 1988) with a dark-colored surface horizon and organic matter content of about 5%, C/N ratio of 10-12 and pH values of 5-7. The characteristics of the soils are medium texture, shallow topsoil, stony and unstable (World Reference Base for Soil Resources, 2014).

Germplasm Collection

The germplasm collection consisted of native seeds of *A. salmiana* C.V. Xhamini, from the communal germplasm collection of Union de Cooperativas de Maguey in the community of Daboxtha, Hidalgo (20° 32' 41" N and 99° 04' 08" W). The seeds were kept in airtight containers at room temperature, isolated, and labeled with the collection date and germplasm origin. The agaves were collected in 2019, with the technical support of SADER (Secretaría de Agricultura y Desarrollo Rural), Hidalgo, Mexico.

Substrate Preparation

The substrates were prepared in equal parts of leaf soil compost, earthworm humus, medium grass, and Haplic Phaeozem type soil. The compost was prepared with excreta from free-grazing sheep in medium open grazing areas where cattle feed mainly on blue gramma (*Bouteloua gracilis* H.B.K. [Lag]), huizache (*Acacia farnesiana* L. [Willd]), mesquite (*Prosopis laevigata* [Humb. et Bonpl. ex Willd]) and cardon (*Pachycereus pringlei* [S.Watson] Britton & Rose). The leaf soil compost was prepared with decomposing leaf litter of huizache (*Vachellia farnesiana*) and mesquite (50:50), collected in the municipality of Tecozautla, Hidalgo (20° 32' 01" N and 99° 38' 05" W). The earthworm humus was obtained from the waste process of *Eisenia foetida* worm from the cooperative NaNa Ya from the locality of Tlaxcalilla, Hidalgo. The diet of *E. foetida* consisted of vegetable residues and dehydrated lamb excreta. The sand was obtained at a construction materials store in Huichapan, Hidalgo. At the ITESHU experimental field, in 1.0 m^2 of agricultural soil, the 5 cm surface layer was removed, and soil was extracted to a depth of 20 cm. Prior to the application of the treatments, the substrates were passed through a 4 mesh sieve with a 4.75 mm opening (MARG, Shennai, India). The standardization of particle size was according to the protocols for organic substrate mix (Morales-Maldonado and Casanova-Lugo, 2015).

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Experimental Design

One experimental unit was established per treatment (one seed of *A. salmiana* per container) with 14 randomly distributed replicates. Irrigation at field capacity was performed at intervals of five days with drinking water. The experimental unit consisted of 25 x 35 cm (1 kg) black plastic containers with 750 g of each substrate. As a control, soil from the region was used, characterized as Haplic Phaeozem type. The treatments were prepared as substrates mixed with different percentages of compost, sand, leaf soil, and earthworm humus (Table 1).

Measurements of Nitrogen-Phosphorus-Potassium in Substrates

The variables analyzed were the quantification of NKP according to USDA (2000), AOAC (2016), and NOM-021-SEMARNAT-2000 protocols. The chemical analysis of the substrates was carried out at the beginning and end of the experiment. To determine N, the criteria of total nitrogen was used (tN) and determined by the Labconco® (Missouri, USA) brand and the Kjeldahl method (Bremmer, 1960).

Table 1. Mixture of substrates by treatment for the development of *Agave salmiana* in Huichapan, Hidalgo, Mexico.

Treatments	Composition (%)
T1(C/100)	100 compost
T2 (C/75-20-5)	75 compost + 20 sand + 5 leaf soil
T3 (C/50-40-10)	50 compost + 40 sand + 10 leaf soil
T4 (EH/100)	100 earthworm humus
T5 (EH/75-20-5)	75 earthworm humus + 20 sand + 5 leaf soil
T6 (EH/50-40-10)	50 earthworm humus + 40 sand + 10 leaf soil
T7 (FHS)	Haplic Phaeozem soil (control)

To determine the available phosphorus (mg kg^{-1}), the criteria proposed by the Olsen method was used (USDA, 2000). Potassium was determined by the exchangeable potassium method (meq $100g^{-1}$). To determine pH in each treatment the ammonium acetate extraction method was used with HANNA® (Hannapro, Mexico) brand potentiometer, taken based on 1:2 dilutions.

Measurement of Physical Variables

The variables of seedling height, root length, and stem diameter were made according to Vigo (2020) measured with a Mitutoyo® (Japan) digital caliper. The height of the plant was measured from the root neck or crown base to the tip of the tallest leaf; radicle length was from root neck to taproot tip and stem diameter from the root neck thickness. The number of leaves of each agave was counted visually. Following Lemus-Soriano (2021), the root volume was obtained by separating the neck from the root using a scalpel. Subsequently, the root was submerged in a test tube (ISOLAB®, Laborgeräte, GmbH) with a capacity of 250 mL, where the volume of water displaced above the gauging line was considered the root volume (1 cm^3).

Statistical Analyses

A completely randomized design was performed. The statistical software INFOSTAT® version 2013 (Di Rienzo *et al.*, 2013) was used. Normality and homogeneity of variances were determined with the

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Shapiro-Wilks test. The analysis of variance (ANOVA) was applied and the differences between treatments of NPK concentration, pH, and physiological quality were calculated with Tukey's test (P≤0.05).

Results and Discussion

Chemical substrate analysis

The response of agave plantlets to the different mixtures of the substrates was favorable in all the treatments compared to the control (Haplic Phaeozem soil). According to the NPK content analyses in substrates and plant response, significant differences (P≤0.05) were observed where the best treatments were based on earthworm humus and corresponded to the mixtures of 75% earthworm humus + 20% sand + 5% leaf soil (**T5**) and 50% earthworm humus + 40% sand + 10% leaf soil **T6** (Table 2).

Table 2. Content of nitrogen-phosphorus-potassium (NPK) in substrates in the region per treatment for the development of *Agave salmiana* in Huichapan, Hidalgo, Mexico.

T[1]	NPK content[2]					
	Cycle 2020 (Initial amount)			Cycle 2021 (Final amount)		
	N%	P mg kg^{-1}	K meq 100g^{-1}	N%	P mg kg^{-1}	K meq 100g^{-1}
T1	0.65±0.01^{a}	777.14±0.01^{c}	42.72±0.01^{a}	0.09±0.01^{a}	477.75±0.01^{ab}	21.46±0.01^{b}
T2	0.41±0.01^{bc}	558.57±0.01^{d}	27.80±0.01^{b}	0.06±0.01^{c}	278.69±0.01^{d}	19.60±0.01^{b}
T3	0.03±0.01^{d}	922.86±0.01^{c}	24.22 ±0.01^{c}	0.03±0.01^{d}	318.50±0.01^{cd}	19.87±0.01^{b}
T4	0.57 ±0.01^{ab}	1554.29±0.01^{a}	42.90±0.01^{a}	0.07±0.01^{b}	537.47±0.01^{a}	25.91±0.01^{a}
T5	0.62±0.01^{a}	1311.43±0.01^{b}	19.06 ±0.01^{d}	0.05±0.01^{c}	477.75±0.01^{ab}	19.04±0.01^{b}
T6	0.41±0.01^{bc}	1214.29±0.01^{b}	15.35±0.01^{e}	0.04±0.01^{d}	398.13±0.01^{bc}	11.88±0.01^{c}
T7	0.08 ±0.01^{d}	25.26±0.01^{e}	1.74±0.01^{f}	0.01±0.01^{e}	0.74±0.01^{e}	1.62±0.01^{d}

1. T = treatments. Control = T7.
2. Different letters in the same column indicate significant differences, according to Tukey's (P≤0.05) test.

In the case of the compost-based treatments, when the percentage was above 75%, no significant differences were observed in nitrogen content. In the case of earthworm humus-based treatments, when the percentage was less than 75%, the statistical differences in nitrogen percentage were marked with respect to treatments with a lower percentage of earthworm humus. In none of the treatments based on leaf soil or sand was a significant contribution of nitrogen observed. At the end of the evaluation, the N percentage showed significant differences due to the effect of the treatments; the difference was observed in **T1**, showing higher N content (0.09%). The data from this research differ from that reported by Cetre *et al.* (2020), who found that in substrates for vitro-plants of pineapple (*Ananas comosus* L.) based on cocoa soil and fertile manure compost, the percentage of total N was 1.8% and 1.48%, respectively. In addition, the data from this research contrasts with the N values reported by Rosas-Patiño *et al.* (2021), who showed that the N percentage was not affected by the mixture of soil + dolomite lime for cocoa growth with 15.06%. In the results of this research, N consumption per agave seedling expressed different levels between the substrates. In Figure 1,

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differences (91.94%) were observed in **T5**: 75% earthworm humus + 20% sand + 5% leaf soil. Otherwise, in **T2**: 75% compost + 20% sand + 5% leaf soil, where lower N consumption was observed (85.37%). The differences may be due to the degradation and integration of organic matter. That is, in this investigation, the effect of the treatments on the Agave seedlings was reflected in Plant height, stem diameter, number of leaves, root length, and root mass due to the use of the mineral elements available in **T5** due to the availability of nitrogen, similar data was observed in **T1**, where the percentage of nitrogen contributed by the degradation of organic matter through vermicomposting, the agave seedlings benefited. in the development of vegetative structures. Similar data agree with Ruiz-Luna *et al*. (2012), who observed greater use of N (1.12%) in leaves of *Agave angustifolia* Baya.

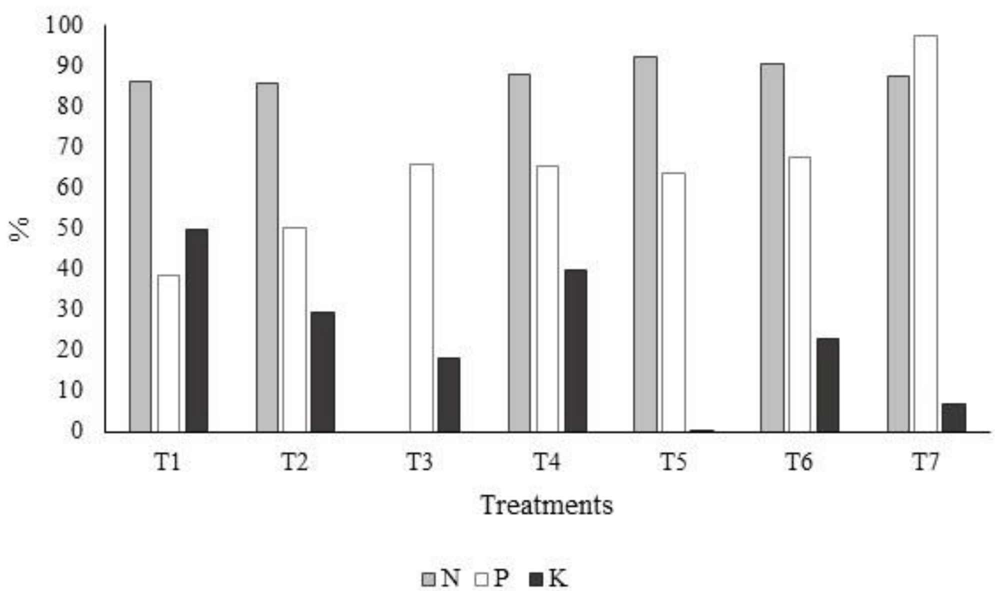

Figure 1. Percentage of nitrogen-phosphorus-potassium (NPK) displacement in the substrate base of initial and final quantification, observing the eventual fixation of N in **T3** and low K tendency through time, while phosphorus (P) displacement increased from the start and end of the experiment.

Nitrogen is one of the most important components of amino acids, proteins, chlorophyll, and nucleic acids for agave plant nutrition. The most assimilable forms are NO_3^- and NH_4^+, thus the deficiency of this mineral element in the plant is expressed with initial chlorosis in the oldest leaves, and progressively in the youngest ones (Miguel-Zarate *et al*., 2021). In plants, N is a fundamental part of metabolic activity, in the case of agave, it contributes to the synthesis of proteins, nucleic acid components, coenzymes, chlorophyll, and alkaloids (Ramírez-Santiago *et al*., 2012).

In the results based on P disposition in the substrates, the highest P concentration was found in the treatments based on earthworm humus (**T4**, **T5,** and **T6**) with the amounts of 1554.29, 1311.43, and 1214.29 ppm, respectively, in contrast to the 25.26 ppm observed in the control (native Haplic Phaeozem soil) group. The differences in phosphorus at the beginning and end of the experiment based on the best seedling response were 1016, 834, and 816 ppm, corresponding to **T4**, **T5,** and **T6**, respectively, while in control a difference of 24.5 ppm was observed.

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According to Cetre *et al.* (2020), these results differ by 80.5%; they determined that the cocoa soil showed 8000 ppm of phosphorus. The differences observed may be due to the nature, process, and mixture of organic materials. Also, Sánchez-Mendoza *et al.* (2020) determined up to 8000 ppm of phosphorus in the soils where *A. angustifolia* Haw is grown in Oaxaca. Unlike Crespo *et al*. (2018), who reported lower phosphorus values in a compost-based mixture with coconut powder 100%, agave bagasse compost, coconut powder substrate, and peat (2800, 1000, 900, 300 ppm, respectively) in Tequila, Jalisco. In the case of earthworm humus-based treatments, when the percentage was from 50 to 75%, the statistical differences in phosphorus were not significant. However, if the percentage of earthworm humus is greater than 75%, the difference is significant compared to 100% (242.86 ppm). Figure 1 shows the differences in plant response during the two agricultural cycles evaluated, observing that phosphorus in compost-based substrates was lower than that in substrates with 50% humus (**T6**). No significant contribution of phosphorus was observed in any of the treatments based on leaf soil or sand. Phosphorus is involved in energy transfer in the plant and absorbed as dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^-), so the lack of P reduces plant growth, the number of leaves, and root development (Alvarado *et al*., 2009).

In relation to K^+ displacement in the substrates, K^+ was observed in the mixture of substrates significantly higher in **T1** (42.72 meq $100g^{-1}$) and **T4** (42.9 meq $100g^{-1}$) with 100% based on compost and earthworm humus, respectively. At the end of the evaluation, the K^+ content in the substrates showed significant differences ($P \leq 0.05$) between treatments, which was expressed in 100% in the treatment with earthworm humus (25.91 meq $100g^{-1}$). The results of this research differ from those reported by Massone *et al.* (2018), who suggested that as the percentage of N decreases, meq of K^+ becomes highly available from the soil for plants. Potassium acts on stomatal opening and closing to regulate CO_2 absorption and is involved in photosynthesis, and transport of sugars and starches essential for cell function. Deficiency produces chlorosis, followed by necrosis at the tips and edges of the leaves (Lincoln *et al*., 2015).

The pH in the substrate interferes with the availability of its macronutrients and for the use of the maguey plant. The pH below 7.0 indicates acidic substrates, while pH values greater than 7 have a tendency to alkalinity. In this sense, the agave plantation is a type of conventional agrosystem in monoculture (Herrera-Pérez *et al.*, 2013), where agronomic practices and soil management are limited, so it should maintain the substrate pH within a reduced range from 6 to 8 (Ruiz-Corral *et al.*, 2013). The results, at the beginning of this investigation, showed alkaline pH from **T1** to **T6** with intervals from 10.9 to 11.8, unlike the control (8.6) (Figure 2).

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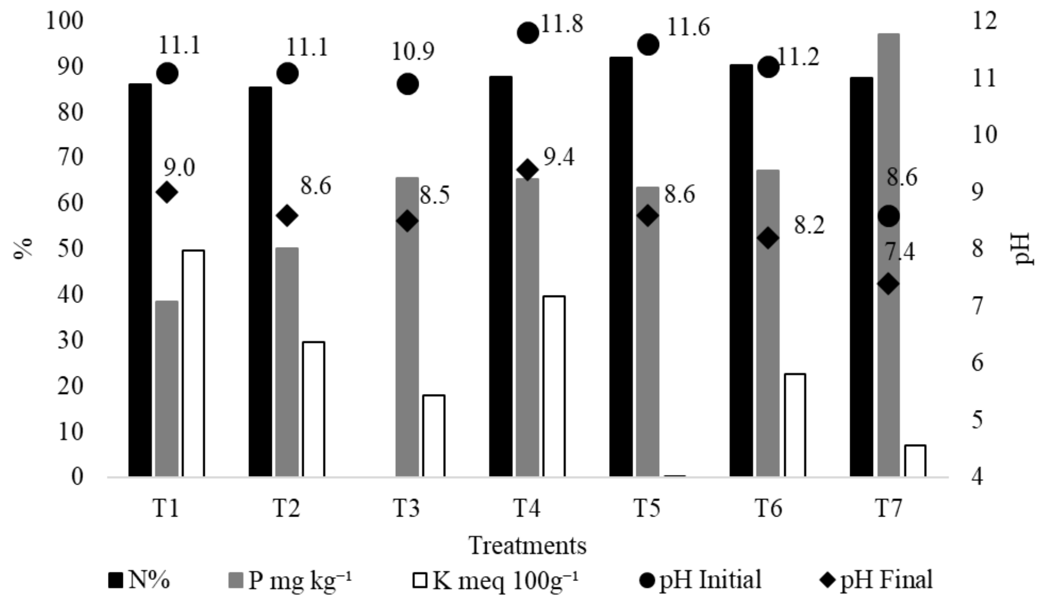

Figure 2. Displacement (%) of nitrogen-phosphorus-potassium (NPK) in the substrates compared with acidification values between treatments at the beginning and end of the experiment for the development of *A. salmiana* seedling cycle 2020 spring-summer (S-S) and 2020-2021 fall-winter (F-W), in Huichapan, Hidalgo, Mexico. The tendency through time at initial (●) and final parts of the experiment (♦) shows an acidification of the substrates 1.2 points in pH values.

It is important to note that at the end of the evaluation, the pH of the treatments ranged from 8.2 to 9.4, in contrast to the Haplic Phaeozem soil where the pH stabilized at 7.4. In the case of compost-based treatments, the increase in pH could be due to a percentage equal to or greater than 75%. In what corresponds to substrates above 50% with earthworm humus, the pH was maintained with an average of 10.9. In contrast, with the behavior of the pH at the end of the evaluation, the results showed a pH below 9.5, but above 8.1. In this context, the substrates evaluated in this research indicate that, based on time, the pH could be stable as in **T7**, otherwise, these substrates would not be conducive to the cultivation and development of agave plants (Ruiz-Corral *et al.*, 2013). However, the results of this research differ from those reported by Crespo *et al*. (2018), who observed a pH of 6.60 in 30% agave bagasse compost, 5.70 in coconut powder-based substrate, and 4.40 in Peat in Tequila, Jalisco.

Physiological Response to Substrates

Variables such as seedling height stem diameter, root length, number of leaves, and root volume measured in this investigation showed significant differences ($P \leq 0.05$) due to the effect between treatments. In the seedling height from the emergence until the formation of four true leaves, the phenological development of the agave seedlings was influenced by the mixture of substrates, the results indicated significant differences due to the effect between treatments ($P \leq 0.05$) (Table 3), These differences could be by the percentage of earthworm humus, sand and leaf soil per treatment, in these results, it was observed that from 50% of earthworm humus, the increase in the height of the seedlings was favored from 90 to 100% in **T6** and **T5**, respectively (Figure 3), likewise, when the pH was between 8.2 and 8.6, respectively, unlike the height observed in treatments **T2** (67.4%) and **T1** (70.5%) where the pH was from 8.6 to 9.0, respectively. Martinez *et al*. (2012) published similar data, where optimal growth was observed in a combined effect of physical-chemical characteristics in combination with the soil pH of the regions of Yodoyuxi, Oaxaca. In relation to the stem diameter, the largest stem diameter was observed in agave seedlings, with percentages of compost and earthworm humus lower than 75% (**T3**, **T5**, **T6**, and **T7**), unlike the seedlings that were treated with 100% earthworm humus (**T4**). In the agave, the diameter of the stem is fundamental for the support of the leaves, the physiological development of the root, the mobilization of water and mineral elements that move from the radicle to the apical part, as well as the mobilization of nutrients from the aerial part of the plant to the base of the stem (Lincoln *et al.*, 2015). This is due to the amount, contribution, and availability of NPK, which was used by the agave seedlings.

In relation to the number of leaves, the results in this investigation indicated that the treatments influenced the number of leaves present in the seedlings until the last evaluation (Table 3). **T1**, **T4**, and **T6** showed significant differences ($P \leq 0.05$), compared to the other treatments. These differences could be derived from the content of NPK present in the substrates of each treatment. According to Narváez-Suárez *et al.* (2016) and Bonilla-Barrientos *et al.* (2019), the proliferation of leaves of agave seedlings are the vegetative structures that will provide the commercial value of the plant based on the quality of the leaves for organic, biological, agroecological, culinary and industrial use.

Table 3. Agronomic response of agave plants to different substrates.

T[1]	Plant biometrics[2]				
	Plant height (cm)	**Stem diameter (cm)**	**Number of leaves**	**Root length (cm)**	**Root mass (cm^3)**
T1	32.91±0.01[de]	7.24±0.01[c]	5±0.01[a]	23.55±0.01[c]	0.46±0.01[c]
T2	31.47±0.01[e]	7.82±0.01[bc]	4±0.01[b]	24.62±0.01[c]	0.55±0.01[abc]
T3	36.91±0.01[cd]	9.32±0.01[ab]	4±0.01[b]	38.58±0.01[b]	0.53±0.01[abc]
T4	38.25±0.01[c]	8.73±0.01[abc]	5±0.01[a]	35.40±0.01[b]	0.50±0.01[bc]
T5	46.69±0.01[a]	9.66±0.01[a]	4±0.01[b]	48.89±0.01[a]	0.60±0.01[ab]
T6	42.48±0.01[b]	9.62±0.01[a]	5±0.01[a]	52.69±0.01[a]	0.56±0.01[abc]
T7	35.38±0.01[cde]	9.08±0.01[ab]	4±0.01[b]	56.89±0.01[a]	0.62±0.01[a]

1. T = treatments
2. Distinct letters in the same column indicate significant differences (Tukey, $P \leq 0.05$).

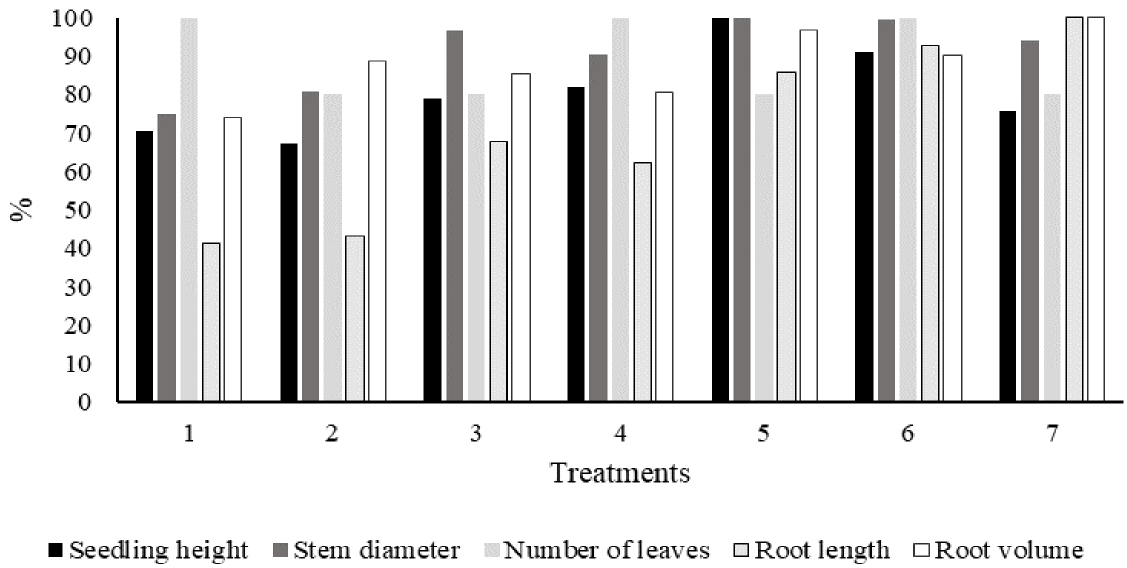

Figure 3. The analysis of variance (Tukey, P≤0.05) showed a percentage increase in the variables of seedling height, stem diameter, number of leaves, length, and volume of roots per treatment.

Likewise, the percentage increase in the number of leaves in seedlings per treatment was favored with **T1**, **T4,** and **T6** (100%), contrary to the behavior observed in **T2**, **T3**, and **T5** (80%). These results may indicate that during this stage, the nutrients contained in the organic matter of the evaluated substrates were not available to the plant, which should be degraded to particles with a smaller diameter to be available to the requirements of the plant during its growth. These data coincide with Enríquez *et al.* (2013) who studied the effect of substrate mixture based on perlite + Steiner solution and sand + Steiner solution on *A*. *americana* var. Oaxacensis *in-vitro*, where the mezcalero agave seedlings obtained five leaves on average. However, the results of Martínez *et al.* (2012) showed that, at this phenological stage, the number of leaves was from six to seven in mezcalero agave seedlings (*A. angustifolia* and *A. potatorum*) fertilized with ammonium sulfate. With respect to root length and volume, the radicle represents the plant point of anchoring, support, and absorption; in the case of agave, length and root volume are essential for the supply of the stem, leaves, and flowers (Martínez-Ramirez *et al.*, 2014). According to Martínez-Jaramillo *et al.* (2019), the substrate structure is the basis of the longitudinal development and volume of the agave radicle. The results obtained in root length of *A. salmiana* seedlings with **T7**, **T6**, and **T5** showed significant differences ($p \leq 0.05$), compared to **T1**, **T2**, **T3**, and **T4** (Table 3). These differences were expressed with a higher percentage (100%) of root length in the Haplic Phaeozem type soil (**T7**) (Figure 4). These data differ from Sánchez-Mendoza *et al.* (2020), who reported a maximum radicle growth of 5.6 cm in *A. angustifolia* seedlings during the acclimatization process in peat + potting soil. The volume occupied by the roots in the substrates also expressed statistical differences and according to root length, in **T7** based on Haplic Phaeozem soil, the average with the highest volume was 0.62 cm^3. This behavior observed could be due to the adaptation of the endemic agave plants in the soils of the region, a site where agaves grow and proliferate naturally. In contrast, Sánchez-Mendoza *et al.* (2020) observed that the minimum root volume in *A. angustifolia* plants fertilized with Multigro (21-14-10 NPK + 2 MgO) was 5.6 cm^3. In this investigation, the substrates with a particle size of 0.5 mm, expressed differences in the growth variables of the agave seedlings. This finding agrees with Morales-Maldonado and Casanova-Lugo

(2015) that the particle size of 0.5 mm in substrates improves nutrient absorption by the root, which is confirmed by *A*. *salmiana* seedlings expressing greater growth.

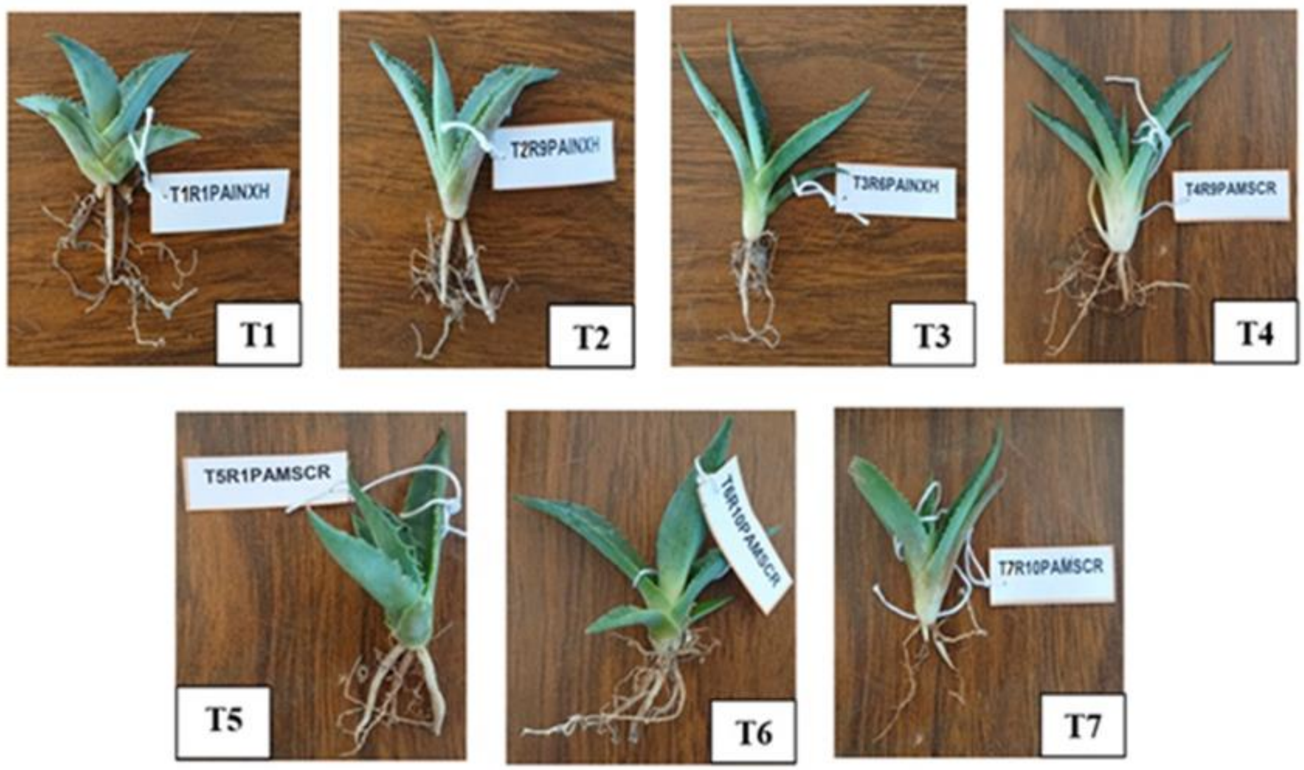

Figure 4. *Agave salmiana* seedlings per treatment at the end of the evaluation (cycle Fall-Winter 2021), observing the maximum seedling growth and greatest length and root weight in **T5** and **T6** treatments, compared with the control group (**T7**).

This result also agrees with Martínez-Jaramillo *et al.* (2019) where the local mixtures of substrates favorably promoted agave seedling growth. In this sense, substrate quality and fertilization are factors that determine plant growth, where the number of leaves that agave seedlings displayed was related to growth. Therefore, the number of leaves per seedling constitutes an appropriate variable to evaluate the effect of fertilization found in substrates (Nobel *et al*., 1988; Cruz *et al.*, 2019). At the end of the evaluation period, the percentage of N^+ recorded in the **T6** substrate was 100%, unlike **T1** and **T4** (86% and 88%, respectively) where the agronomic variables of *A. salmiana* seedlings showed a higher usage percentage.

The best responses related to seedling biometry values (plant height and stem diameter) and roots (length and root volume) were those in the treatments based on earthworm humus as observed in **T5** combinations (75% earthworm humus + 20% sand + 5% leaf soil) and **T6** (50% earthworm humus + 40% sand + 10% leaf soil). These physiological responses agree with the high nitrogen displacement values observed in **T5** (92%) and **T6** (90%) and phosphorus displacement observed in **T6** (67%) and **T5** (63.6%). Undoubtedly, this is an approach to how NPK availability in substrates can influence the production of maguey seedlings to obtain them with desirable agronomic characteristics. Apparently, the substrates based on composts (alone or in a mixture) do not seem to have a favorable response compared to the control group (agricultural soil) and those with earthworm humus. However, more studies are necessary to know NPK availability in the plant and how they have a bearing on plant physiology and morphogenesis. Although no statistical differences were observed between the treatments based on earthworm humus and the control soil, a better general response to the parameters evaluated was observed, which implies that it can be a viable option and a good alternative for commercial production of maguey seedlings.

Conclusions

The agronomic response of *A. salmiana* C.V. Xhamini seedling variables: height, stem diameter, length, and root volume showed significant differences in all the treatments, where the highest response was with earthworm type-substrates (**T5** and **T6**), compared with the control (Haplic Phaeozem soil type). In these treatments, significant differences were observed in NPK displacement (from the beginning to the end of the experiment) with the final values of N = 9.75%, P = 32.8% and K= 80% for **T6** and N = 8%, P = 36.4% and K = 100% for **T5**. Based on these results, mixtures of earthworm humus (50-75%) with different percentages of sand (20-40%) and leaf compost (5-10%) are evidently a good alternative to support the production of *A. salmiana* seedlings. The effect of NPK favored the development of height, stem diameter, length and root volume in seedlings of *A. salmiana* was greater with the percentages of NPK and opens the opportunity to establish new lines of research for the best adaptability of the native lines of maguey in the productive systems in Huichapan, Hidalgo, Mexico.

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