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PRODUCTION OF *Pinus durangensis* Mart. UNDER DIFFERENT SUBSTRATE AND MYCORRHIZAL INOCULATION CONDITIONS IN NURSERY

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ABSTRACT

Substrate and mycorrhization are important aspects when producing plants. The objective of the study was to determine the effect of three substrates inoculated with ectomycorrhizal fungal spores on the morphological characteristics of nursery-grown *Pinus durangensis* as well as the inherent costs of the substrate. A randomized block experimental design with a 3 x 3 factorial arrangement was used. The substrates evaluated were: 1) peat, vermiculite, and perlite (50:25:25) [PVP]; 2) peat, composted bark, and raw sawdust (50:25:25) [PBS]; and 3) peat and bark (50:50) [PB]. The mycorrhizal inoculants were: 1) *Pisolithus tinctorius*; 2) *Laccaria laccata*; and 3) control. The plant was produced in black polyethylene tubes (160 mL) and evaluated at 13 months. The substrate factor presented statistical differences ($p \leq 0.05$) in the variables evaluated, with higher values in PVP, followed by PBS and PB, the latter being similar to each other. The mycorrhizal inoculation factor showed differences ($p \leq 0.05$) in root and total biomass, Dickson's quality index, and mycorrhizal colonization, obtaining higher values when *P. tinctorius* was inoculated, except in the last variable, where the application of *L. laccata* stood out. In the substrate-mycorrhizal inoculation interaction, the most favorable combination was PVP and *P. tinctorius*. The PVP substrate was 28.4 and 34.6 % more expensive than PB and PBS, respectively. The combined effect of PVP and *P. tinctorius* produced the best growth, although the substrate was more expensive.

Keywords: growth media, controlled mycorrhization, plant quality, morphological characteristics, reforestation.

INTRODUCTION

Deforestation is directly related to the loss of biodiversity and the decrease in the efficiency of environmental services provided by forests and jungles, which has contributed to global warming. These ecosystems, in addition to being reservoirs of biodiversity, are a source of consumer goods such as timber, fuelwood, and other non-timber forest products (FAO, 2018). In Mexico, due to the deterioration and decrease of forest resources, with an average annual rate of gross deforestation of 212 834 ha between 2001 and 2019, there is a need to implement strategies that favor the restoration of forest ecosystems (CONAFOR, 2021).

Using nursery-produced plants allows the generation of new forest stands, which in the future will contribute to re-establish forests and obtain economic gains in a sustainable way (Buamscha *et al*., 2012; Scholz and Morera, 2016); however, the propagation material must have quality, which is fundamental in reforestation programs (Grossnickle and MacDonald, 2018). Plant quality is the result of nursery production practices and the management of propagated material until planting (Villar-Salvador *et al*., 2021). After the nursery culture process, the initial survival and growth potential of individuals is related to their morphological and physiological attributes, as well as their eco-physiological response to site environmental conditions (Grossnickle, 2018).

The main cultural procedures that plants receive in nursery include irrigation, fertilizer, mycorrhization, pest and disease control, and environmental management (Sanchún and Obando, 2016; Grossnickle, 2018). Other important aspects include the correct selection of substrates and the proportions for mixing them, as well as the type of container. These factors are related to the formation of the root ball and the growing space for the root system of the plants (Landis, 1990). The importance of the substrate lies in the functions it performs in anchoring the root system to support the plants as a temporary store and diffuser of water and nutrient solutions that are absorbed by the root hairs. The physical and chemical characteristics of substrates are directly related to the morphology and functional performance of plants and, therefore, influence their growth and survival in the field (Villar-Salvador *et al*., 2021).

There is a search for materials to replace the substrate based on peat (50–60 %), perlite (20–25 %), and vermiculite (20–25 %), known as a base mix, whose main disadvantages are its high cost and immediate unavailability. These reasons limit their use for most nurserymen (Aguilera-Rodríguez *et al*., 2016). Therefore, it is necessary to look for alternative materials with an ecological approach (Mateo-Sánchez *et al*., 2011; Gayosso-Rodríguez *et al*., 2016). Pine bark and sawdust have favorable physical and chemical characteristics for the development of seedlings of the genus *Pinus* (Hernández-Zárate *et al*., 2014); in addition, they are by-products of forestry activity with relative abundance (Fregoso-Madueño *et al*., 2017), and it is desirable to find a potential use for them.

An important component in plant production is mycorrhizal inoculation through the application of spores of wild ectomycorrhizal fungi (HSECM) (Escobar-Alonso and Rodríguez-Trejo, 2019), which promotes plant-fungus symbiosis. This condition is desirable in species of the genus *Pinus* in nursery and field to favor plant survival and growth (García-Rodríguez *et al*., 2017).

The objective of this study was to determine the effect of three substrates and inoculation with spores of two species of ectomycorrhizal fungi and a control on the morphological characteristics of nursery-grown *Pinus durangensis* Mart. Similarly, it was sought to determine the costs inherent to the type of substrate. It was hypothesized that at least a combination of substrate and ectomycorrhizal fungus favors the survival, growth, and mycorrhizal colonization of the plant in a nursery at the lowest cost due to the components of the substrate.

MATERIALS AND METHODS

Location

The experiment was carried out in the forest nursery of the Experimental Field Valle del Guadiana, of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP), located at Carretera Durango-El Mezquital km 4.5, Durango, Durango, Mexico (24° 01′ N and 104° 44′ W), with an altitude of 1860 m and average annual rainfall of 504 mm.

Plant production

The plant was produced under three environmental conditions, with the following sequence: a) from October 17, 2019 (sowing) until seven months later, a saw-type greenhouse was used, 50 m long and 30 m wide, with plastic film cover caliber 720 μm, milky white color, treated against UV rays, and 50 % black color shade mesh, with an average temperature of 22 °C, maximum of 46 °C, and minimum of -2 °C; b) from May 8, 2020, for four months, using 50 % shade mesh, with an average temperature of 23 °C, maximum of 39 °C and minimum of 9 °C; and c) from September 3, 2020, for two months, under outdoor conditions, without sun protection, with an average temperature of 20 °C, maximum of 42 °C, and minimum of 2 °C. The production cycle lasted 13 months. Temperature was monitored with an Elitech data logger (Elitech Technology, San Jose, CA, USA).

The plants were produced in black rigid containers mounted on 98-cavity racks. Each tube was 3.8 cm in top diameter, 19 cm long, 160 mL in capacity, and had internal root guides. Prior to sowing, the seed was soaked in water for 24 hours to activate germination; subsequently, it was dried. To avoid possible damage by pathogenic damping-off fungi, it was impregnated with Tecto 60® fungicide (thiabendazole, 2-(4-thiazolyl) benzimidazole).

Experimental design and treatments

Nine treatments were evaluated, considering three substrate mixtures based on peat, composted pine bark, raw pine sawdust, vermiculite, and perlite, and two spore inocula from wild ectomycorrhizal fungi (HSECM): a) *Laccaria laccata* (Scop.) Cooke, native inoculum made from HSECM fruiting bodies collected in mid-2019 from cold temperate forest stands in the Iztaccíhuatl-Popocatépetl National Park and surroundings in Central Mexico, dehydrated fruiting bodies were ground in a Willye type mill, sieved through a 1 mm mesh, and stored at 5 °C until inoculation; b) *Pisolithus tinctorius* (Pers.) Coker et Couch, commercial spore inoculum Ecto-Rhyza®; and c) control (no inoculum) (Table 1). A randomized, complete block experimental design with a 3 x 3 factorial arrangement was used. Each treatment consisted of four replicates and 49 plants per experimental unit.

Table 1. Substrate composition and inoculum of ectomycorrhizal fungi in *Pinus durangensis* Mart. plant production in nursery conditions.

Treatment	Substrate	Mycorrhizal inoculant
1	Peat + vermiculite + perlite (50:25:25) [PVP].	No inoculum [NI].
2	Peat + bark + sawdust (50:25:25) [PBS].	No inoculum [NI].
3	Peat + bark (50:50) [PB]	No inoculum [NI].
4	Peat + vermiculite + perlite (50:25:25) [PVP].	*Laccaria laccata* [Ll]
5	Peat + bark + sawdust (50:25:25) [PBS].	*Laccaria laccata* [Ll]
6	Peat + bark (50:50) [PB]	*Laccaria laccata* [Ll]
7	Peat + vermiculite + perlite (50:25:25) [PVP].	*Pisolithus tinctorius* [Pt]
8	Peat + bark + sawdust (50:25:25) [PBS].	*Pisolithus tinctorius* [Pt]
9	Peat + bark (50:50) [PB]	*Pisolithus tinctorius* [Pt]

After preparing the substrate mixtures for disinfection, they were impregnated with Anibac® PLUS bactericide at a dose of 6.3 mL L^{-1} of water and covered for 15 d with black rubber. During mixing, eight-month-old Multicote® (12N-25P_5O-12K_2O) controlled-release fertilizer was added to each substrate at a rate of 3 g L^{-1}. Seven months after sowing, a second application of fertilizer of the same brand was made, but with a 17N-17P_5O-17K_2O ratio and with four months of release, at a dose of 2 g L^{-1}. The second application was made in each tube, burying the fertilizer 1 cm deep.

To complement the nutrition, fertilization was carried out through irrigation: a) one month after germination and up to seven months after sowing, the soluble fertilizer Master® Finalizer (4N-25P_5O_5-35K_2O) was added at a dose of 1 g L^{-1} of water (except in the period from 3 to 5.5 months of plant age, due to inoculation) (40-109-290 ppm of N-P-K); and b) after seven months of age, Master® Development (20N-7P_2O_5-19K_2O) was used at a dose of 0.5 g L^{-1} of water (100-15-79 ppm of N-P-K). During the fall and winter, water-soluble fertilizers were applied twice a week, and in the spring and summer, fertilizer was applied three times a week.

For each substrate mixture, total porosity, aeration porosity, and moisture holding capacity were estimated (Table 2) based on the methodology developed by Landis (1990). For mycorrhizal inoculants, the spore concentration of each inoculum was determined in a Neubauer chamber. On each plant, 5 mL of spore suspension were applied, equivalent to 1 643 750 spores on average. Two inoculations were performed, the first at 3.5 months, and 30 days later (4.5 months), the second application was performed using the same dose.

Table 2. Total porosity, aeration porosity, and moisture holding capacity of the substrate mixtures used in the production of *Pinus durangensis* Mart. plants in nursery conditions.

Substrate mixture	Total porosity (%)	Aeration porosity (%)	Humidity retention capacity (%)
PVP	58.3	31.7	26.6
PBS	57.2	28.9	28.3
PB	63.4	31.4	32.0
Recommended value[+]	60 a 80	25 a 35	25 a 55

[+]Landis (1990). PVP: peat + vermiculite + perlite (50:25:25); PBS: peat + bark + sawdust (50:25:25); PB: peat + bark (50:50).

Variable evaluation and statistical analysis

The percentage of survival was evaluated thirteen months after sowing. To analyze morphological growth, 10 plants per experimental unit were randomly taken, and the height (measured using a PILOT® model graduated ruler in millimeters), root collar diameter (using a CALDI-6MP model digital vernier; Truper, Mexico), and wet and dry biomass of the aerial part and the root were recorded. Dry biomass was obtained by placing the plants at 70 °C in a forced ventilation oven (model FE-291D; Felisa, Mexico) for 72 hours. The samples were weighed on an analytical balance (model PA1502, accurate to 0.001 g; Ohaus, NJ, USA). Similarly, the dry biomass ratio of the aerial part, the dry biomass of the root, and the lignification index (Equation 1) were calculated (Villalón-Mendoza *et al*., 2016), as well as the Dickson quality index (DQI) (Equation 2) (Dickson *et al*., 1960).

$$\text{Lignification index} = \left(\frac{\text{Total dry biomass (g)}}{\text{Total wet biomass (g)}}\right) \times 100 \qquad (1)$$

$$\text{DQI} = \frac{\text{Total dry biomass (g)}}{\dfrac{\text{Height (cm)}}{\text{Diameter (mm)}} + \dfrac{\text{Aerial dry biomass (g)}}{\text{Root dry biomass (g)}}} \qquad (2)$$

To determine mycorrhizal colonization (MC) (Equation 3), the methodology described by Salcido-Ruiz *et al*. (2020) was used by randomly selecting three plants per experimental unit and extracting 100 cm of secondary roots, which were preserved in FAA fixative (formaldehyde, glacial acetic acid, 96° alcohol, and distilled water) in a 10:5:50:35 ratio, respectively. In each sample, direct observations were made on the roots with an optical stereoscope (Leica S9i, Switzerland), recording the number of apices with and without mycorrhizae.

$$MC = \left(\frac{\text{Mycorrhizal apices}}{\text{Mycorrhizal apices} + \text{Non-mycorrhizal apices}} \right) \times 100 \quad (3)$$

The percentage results of MC and survival, being binomial variables, were transformed with the arcsine and square root functions. Furthermore, for the variables evaluated, analyses of variance with Tukey's multiple comparison of means tests ($p \leq 0.05$) were performed using SAS software (SAS Institute, 2009). To calculate the costs per plant, a volume of 160 mL per cavity was considered, according to the inputs used per substrate mix. The evaluation was carried out based on three quotations for the products in Durango, Durango, Mexico, and the proportions used per mixture.

RESULTS AND DISCUSSION

Substrate factor

The substrate factor showed differences ($p \leq 0.05$) in the variables evaluated; in all cases, the PVP-based treatment stood out, except in nursery survival, where PBS and PB were superiors. Likewise, there was a similarity of results between PBS and PB. For the aerial/root ratio (< 2.5) and robustness index (< 6), the values are in agreement with those suggested by Ritchie *et al*. (2010) (Tables 3 and 4).

Regarding total porosity, aeration porosity, and moisture holding capacity, the percentages are in the ranges suggested by Landis (1990) (Table 2), except for total porosity, where only the mixture composed of peat and bark was in the recommended range, which may have influenced the plants to reach the morphological characteristics stipulated by the Mexican Standard for the Certification of Forest Nursery Operation NMX-AA-170-SCFI-2016 (DOF, 2016).

Mycorrhizal inoculum factor

In the mycorrhizal inoculum factor, only the variables root and total biomass production, Dickson's quality index, and percentage of mycorrhizal colonization presented statistical differences ($p \leq 0.05$); the best results were obtained when Pt or Ll was applied for mycorrhizal colonization, but there were no differences between Ll and T for Dickson's quality index. Regarding the hardiness index (< 6) and aerial/root ratio (≤ 2.5), all treatments presented values in the range recommended by Ritchie *et al*. (2010) (Tables 3 and 4).

Vicente-Arbona *et al*. (2019) reported a similar trend when growing *Pinus greggii* Engelm. ex Parl. on five substrates, generated from combinations with sawdust as the main component (60 to 80 % in each substrate), mixed with bark, peat, compost, vermiculite,

Table 3. Morphological characteristics in nursery-grown *Pinus durangensis* Mart. plants 13 months after sowing.

T	I	S	Survival (%)	Height (cm)	Diameter (mm)	Dry biomass (g)		
						Aerial	Root	Total
	All	PVP	93.9 b	15.7 a	5.6 a	2.5 a	1.7 a	4.2 a
S	All	PBS	98.7 a	14.3 b	5.0 b	1.9 b	1.2 b	3.1 b
	All	PB	96.9 ab	13.5 b	5.0 b	1.9 b	1.3 b	3.2 b
	NI	All	95.8 a	15.1a	5.2 a	2.0 a	1.3 b	3.4 b
I	Ll	All	96.5 a	14.1a	5.2 a	2.0 a	1.4 ab	3.5 ab
	Pt	All	96.6 a	14.3 a	5.3 a	2.2 a	1.5 a	3.7 a
1	NI	PVP	94.3 ab	17.6 a	5.6 ab	2.5 ab	1.6 ab	4.1 ab
2	NI	PBS	99.5 a	14.5 b	5.0 bc	1.8 d	1.2 c	3.0 d
3	NI	PB	93.8 ab	13.3 b	5.0 bc	1.9 d	1.2 c	3.1 d
4	Ll	PVP	92.7 b	14.8 ab	5.5 abc	2.4 abc	1.6 ab	4.0 abc
5	Ll	PBS	98.4 ab	14.2 b	5.1 bc	1.9 cd	1.3 c	3.2 cd
6	Ll	PB	98.4 ab	13.4 b	5.0 bc	2.0 cd	1.3 bc	3.3 cd
7	Pt	PVP	94.8 ab	14.7 b	5.8 a	2.8 a	1.8 a	4.6 a
8	Pt	PBS	98.4 ab	14.5 b	5.1 bc	2.0 cd	1.3 bc	3.3 cd
9	Pt	PB	97.9 ab	13.7 b	5.1 bc	2.0 bcd	1.4 bc	3.4 bcd

Different letters in the same column indicate statistical differences (Tukey, $p \leq 0.05$); T: treatment; I: mycorrhizal inoculum; S: substrate; NI: no inoculum; Ll: *Laccaria laccata*; Pt: *Pisolithus tinctorius*; PVP: peat-vermiculite-perlite; PBS: peat-bark-sawdust; PB: peat-bark.

Table 4. Quality indices in nursery-grown *Pinus durangensis* Mart. plants 13 months after sowing.

T	I	S	Aerial/ root ratio	Robustness index	Lignification index (%)	DQI	MC (%)
	All	PVP	1.5 a	2.8 a	30.1 a	1.0 a	27.1 a
S	All	PBS	1.6 a	2.9 a	28.1 b	0.7 b	21.6 b
	All	PB	1.5 a	2.7 a	28.7 ab	0.8 b	21.7 b
	NI	All	1.5 a	2.9 a	28.0 a	0.7 b	15.4 b
I	Ll	All	1.4 a	2.7 a	29.4 a	0.8 ab	28.4 a
	Pt	All	1.5 a	2.7 a	29.5 a	0.9 a	26.6 a
1	NI	PVP	1.6 a	3.1 a	28.8 a	0.9 abc	16.9 bc
2	NI	PBS	1.6 a	2.9 a	27.4 a	0.7 c	14.3 c
3	NI	PB	1.6 a	2.7 a	28.0 a	0.8 bc	15.0 c
4	Ll	PVP	1.5 a	2.7 a	30.1 a	1.0 ab	33.1 a
5	Ll	PBS	1.5 a	2.8 a	29.0 a	0.8 bc	25.9 ab
6	Ll	PB	1.5 a	2.7 a	29.4 a	0.8 bc	26.1 ab
7	Pt	PVP	1.6 a	2.5 a	31.7 a	1.2 a	31.4 a
8	Pt	PBS	1.5 a	2.8 a	28.1 a	0.8 bc	24.6 ab
9	Pt	PB	1.4 a	2.7 a	29.0 a	0.9 bc	23.9 abc

Different letters in the same column indicate statistical differences (Tukey, $p \leq 0.05$). T: treatment; I: mycorrhizal inoculant; S: substrate; DQI: Dickson quality index; MC: mycorrhizal colonization; NI: no inoculum; Ll: *Laccaria laccata*; Pt: *Pisolithus tinctorius*; PVP: peat-vermiculite-perlite; PBS: peat-bark-sawdust; PB: peat-bark.

and perlite, in different proportions, with and without inoculation of *L. laccata* (1.5 g per plant). These authors observed that plant growth due to mycorrhization was not very evident, although the inoculated treatments had 44 to 64 % mycorrhization and the uninoculated individuals had between 14 and 40 % mycorrhization. The same happened with Baltasar-Martínez *et al*. (2007) when inoculating spores of *Rhizopogon roseolus* Corda on *Pinus ponderosa* Dougl. ex C. Laws. plants subjected to three fertilization levels, two inoculation times, and two nursery harvest dates, finding no differences in plant growth or mycorrhizal colonization, with values lower than 39 % in all cases.

Substrate-mycorrhizal inoculum interaction

The interaction of substrate-mycorrhizal inoculum factors was significant in the variables evaluated ($p \leq 0.05$), with the exception of the of the aerial part/root ratio, robustness index, and lignification index. Survival only showed differences between treatments 2 (PBS-SI) (99.5 %) and 4 (PVP-Ll) (92.7 %) (Table 3). Salcido-Ruiz *et al*. (2020) obtained similar results, with 80 to 96 % survival in *Pinus engelmannii* Carr. plants grown in a substrate based on peat, vermiculite, and perlite (57:23:20) eight months after being inoculated with ectomycorrhizal fungi (HECM). Plant survival in the forest nursery at INIFAP's Guadiana Valley Experimental Field was satisfactory (> 90 %) due to relatively controlled environmental conditions.

In the present study, differences ($p \leq 0.05$) were found in morphological variables. Height ranged from 13.3 to 17.6 cm, with treatments 1 (PVP-SI) and 4 (PVP-Ll) in the top statistical group (although there were no differences between the two, nor between the latter and the rest of the treatments) ($p > 0.05$). Diameter ranged from 5 to 5.8 mm, with the highest value in treatment 7 (PVP-Pt), with no differences with treatments 1 and 4; the rest of the treatments had average data from 5 to 5.1 mm (Table 3). The height variable is important because it is associated with the number of needles produced in the plants, which in turn is related to photosynthetic capacity and transpiration area; meanwhile, the diameter of the collar is related to the robustness of the plants (Ritchie *et al*., 2010).

The values of the morphological parameters evaluated comply with the ranges recommended in NMX-AA-170-SCFI-2016 (DOF, 2016), which establishes a height of 15 to 20 cm and neck diameter greater than 4 mm; both parameters are met in treatment 1 (PVP-SI), while the second variable is met in all treatments evaluated. The most prominent substrate (PVP) is the most widely used in Mexico; however, its cost is higher than the other two evaluated (PBS and PB), which is why the latter are beginning to be widely used in nurseries located in central and northern Mexico (Aguilera-Rodríguez *et al*., 2016).

The production of dry biomass of the aerial part fluctuated from 1.8 to 2.8 g, with the formation of diverse statistical groups, where treatment 7 (PVP-Pt) stood out without statistical differences with treatments 1 (PVP-SI) and 4 (PVP-Ll) and values of 2.8, 2.5, and 2.4 g, respectively; the other treatments produced from 1.8 to 2 g. For root dry biomass, the averages ranged from 1.2 to 1.8 g, with the same behavior as the aerial biomass variable (treatments 7, 1, and 4); total biomass varied from 3 to 4.6 g, and the best results were obtained in the same treatments as for aerial and root biomass (Table 3).

For the aerial/root ratio, the index ranged from 1.4 to 1.6 (Table 4), while the robustness index ranged from 2.5 to 3.1. This implies a robust and balanced plant between root biomass and the aerial part, which favors its quality (Ritchie *et al*., 2010). As for the lignification index, the percentages ranged from 27.4 to 31.7 %, with no differences ($p > 0.05$) between treatments. For the Dickson quality index, values from 0.7 to 1.2 were found, with differences ($p > 0.05$) between treatments. Treatment 7 (PVP-Pt) showed better results, with no significant differences with treatments 4 (PVP-Ll) and 1 (PVP-SI). In general, the PVP substrate stood out (Table 4).

When evaluating *Pinus cooperi* Blanco, produced on four substrates based on S1: peat and bark (54:46); S2: raw sawdust, composted bark, and peat (50:20:30); S3: sawdust, bark, and peat (50:20:30); and S4: sawdust, bark, and peat (50:25:25), González-Orozco *et al*. (2018) reported satisfactory results in S1 in the variables height (15.1 cm), root collar diameter (4 mm), and total biomass (2.8), although S2 and S3 produced plants with acceptable morphological characteristics in height (16.4 and 16.2 cm), diameter (3.8 and 3.7 mm), and total biomass (2.5 and 2.4 g). Castro-Garibay *et al*. (2018) found similar results in plant quality due to the use of sawdust when producing *Pinus greggii* plants on sawdust, bark, and peat-based substrates (60:20:20).

Mycorrhizal colonization (MC) (14.3 to 33.1 %) showed differences ($p \leq 0.05$), forming several statistical groups where treatments 4 to 9 (inoculated with ectomycorrhizal fungi, regardless of the substrate used, and MC from 23.9 to 33.1 %) stood out, although in several cases without differences with the rest of the treatments, which had colonization percentages from 14.3 to 16.9 % (Table 4). Inoculation with spore suspensions with *P. tinctorius* and *L. laccata* allowed mycorrhization in the root systems of *P. durangensis* 13 months after sowing and eight months after inoculation. However, the values were less than 34 %, considered medium to low, including the percentage of undesirable mycorrhization observed in the control treatment (uninoculated). This effect is difficult to avoid in experiments of this nature, since the inoculum can arrive through the air, from the mycorrhizal treatments, or even from outside the experiment; however, it was always significantly lower compared to the inoculated treatments.

The mycorrhizal colonization resulting from this trial is lower than that recorded by Salcido-Ruiz *et al*. (2020), who inoculated *Pinus engelmannii* plants with two mixtures of HECM spores, one composed of *Amanita rubescens* (Pers. ex Fr.) Gray, *Amanita* sp., *Lactarius indigo* (Schwein.), *Ramaria* sp., and *Boletus* sp., and another by a mixture of *P. tinctorius* and *Scleroderma citrinum* Pers; in addition, they added controlled release fertilizer (3 and 6 g L-1 of substrate), obtaining from 15 to 71 % mycorrhization, with an inverse trend in plant quality as a function of the fertilization dose applied. Proper substrate selection is a key element because it favors the production of aerial and root biomass compared to uninoculated plants (Rentería-Chávez *et al*., 2017).

In general, mycorrhizal fungi are aerobic. Saif (1981) indicates that oxygen concentrations between 12 and 16 % are ideal for both mycorrhization and the beneficial expression of this symbiosis in plant growth. In the present study, the lowest MC percentages coincide with the substrates with the highest percentage of moisture retention (PBS and PB) and the highest with the one with the highest aeration porosity (PVP).

This study's mycorrhization percentages were modest, and the mycorrhizal component had little effect on the morphological growth of *Pinus durangensis*. This was similar to Vicente-Arbona *et al.* (2019) on *Pinus greggii* with *L. laccata* and Baltasar-Martínez *et al*. (2007) with *Pinus ponderosa* and *Rhizopogon roseolus*, who found slight differences in plant growth and mycorrhizal colonization due to mycorrhizal inoculum application. Both studies agree that this issue should be further studied in nurseries and plantations. At the latter site, positive effects of mycorrhizal inoculation on initial growth are more likely to be found. This situation was corroborated by Barroetaveña *et al*. (2016), who found that after five years of inoculating *P. ponderosa* plants in nursery conditions with *Suillus luteus* (L) Roussel, *Rhizopogon roseolus*, *Hebeloma mesophaeum* (Louis), and leaf litter, the first two species significantly favored the growth of the plants under study during the period after planting in the field, despite the prevailing conditions of water stress.

The factor with the greatest influence on the results was the substrate, where the PVP combination stood out, but with close values in the other two substrates (PBS and PB). This was largely because the three substrates evaluated, in general, meet the criteria for total porosity, aeration porosity, and moisture holding capacity recommended by Landis (1990). In relation to mycorrhization, although it was not a determining factor in the quality of the plants evaluated, the plant material inoculated with *P. tinctorius* stood out.

Based on the results obtained, treatment 7 (PVP-Pt) stands out with the best values in the variables evaluated, and above all, it complies with the recommendations of NMX-AA-170-SCFI-2016 (DOF, 2016), which establishes the minimum morphological parameters to consider that the plant produced in nursery conditions will have the necessary attributes for adequate survival and initial growth after planting. In the case of the other two substrates evaluated (PB and PBS in interaction with Pt and Ll), they also comply with the minimum values suggested by the standard.

Substrate costs

Based on the price of each component used to prepare the substrates evaluated, the cost per cubic meter was determined (Table 5), with the PVP combination being the highest, followed by PB and PBS (Figure 1). Of the three substrates evaluated, the first was 28.4 % more expensive than PB and 34.6 % higher than PBS, which proved to be the most economical. The difference between the two most economical substrates (PB and PBS) was 8.6 %, with PBS being the least expensive of all.

Table 5. Costs of the materials used in the substrate mixtures evaluated in the production of *Pinus durangensis* in nursery.

Component	Volume presentation (L)	Cost per bulk (USD)¶	Bulks per cubic meter	Cost per cubic meter ($USD)
Peat	160+	45.26	6.25	282.9
Vermiculite	114	35.14	8.77	308.2
Agrolite	100	10.65	10	106.5
Bark	1000	69.22	1	69.2
Sawdust	1000	10.64	1	10.6

+Volume of the bulk once the peat has been decompacted. ¶Dollar costs as of January 31, 2023, based on three quotes in Durango, Durango, Mexico ($18.78 MXN per $1 USD).

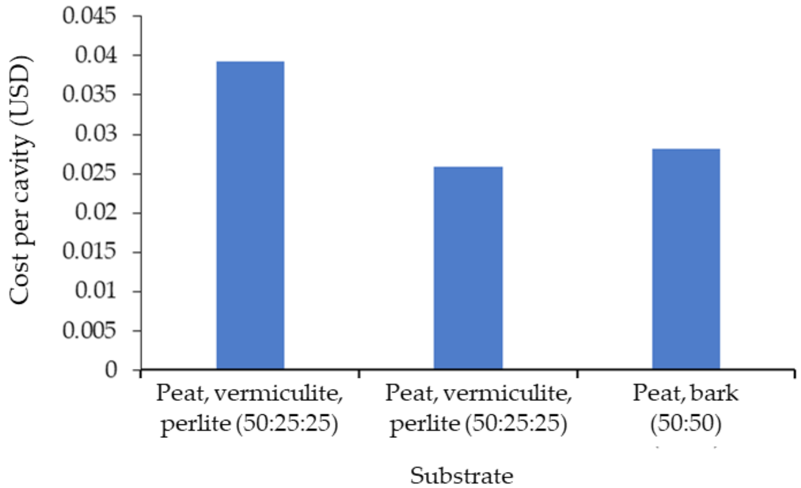

Figure 1. Cost of the evaluated substrates per 160 mL cavity.

Madrid-Aispuro *et al*. (2020) evaluated four substrates based on composted pine bark, raw pine sawdust, and peat, mixed in different proportions, and found that the substrate based on raw sawdust, composted pine bark, and peat is an alternative to produce quality forest plants at a lower production cost, as long as sawdust is incorporated up to a maximum of 50 % in the mixture.

Aguilera-Rodríguez *et al.* (2016) compared in *Pinus montezumae* Lamb. the quality of the plant produced and production costs due to the use of two substrates: composted pine sawdust, composted pine bark, and vermiculite (70:15:15), and peat, perlite, and vermiculite (60:20:20); in both substrates, plant quality was favorable. However, they recommend the former because it is 45.8 % cheaper. González-Orozco *et al*. (2018) compared costs on four substrates: S1: peat and composted pine bark (46:54); S2:

peat, composted bark, and raw pine sawdust (30:20:50); S3: peat, bark, and sawdust (25:25:50); and S4: peat, bark, and sawdust (20:30:50). Although in the first substrate they obtained favorable results in plant quality, S2 and S3 also produced acceptable growth, with lower production costs by 39.8 and 43.1 % in relation to S1.

CONCLUSIONS

The combination of the substrate composed of peat, vermiculite, and perlite (50:25:25) and the inoculation with spores of *Pisolithus tinctorius* had superior values in the morphological characteristics of *Pinus durangensis* plants produced in nursery conditions. The substrate based on peat, vermiculite, and perlite (50:25:25) gave the best results in plant growth; however, the addition to peat of alternative substrates, such as composted bark and raw sawdust (50:25:25), and peat plus composted bark (50:50), also generated good-quality plants, reducing costs by 28. 4 % when using the mixture of peat and bark, and by 34.6 % with the combination of peat, bark, and sawdust. Mycorrhization of *Pinus durangensis* with *Pisolithus tinctorius* produced under nursery conditions generated better quality plants, followed using *Laccaria laccata*.

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