La Granja: Revista de Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
https://doi.org/10.17163/lgr.n42.2025.05

Scientific paper/ Artículo científico

Agricultural Sciences

Choice of trap plant and substrate for Mycorrhizal Inoculum production

Elección de planta trampa y sustrato para la producción de Inóculo Micorrízico

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Received on May 25th, 2021. Accepted, after revision on September 27th, 2022.

Abstract

Arbuscular mycorrhizal trap plants can be cultivated or wild species. In addition to withstanding anthropogenic pressure, these are excellent hosts for massive multiplication of arbuscular mycorrhizae. The objective of this work is to select the most suitable trap plant and substrate for the massive propagation of arbuscular mycorrhizal fungi. Four species were evaluated (*Cajanus cajan, Cynodon dactylon, Tagetes patula,* and *Plectranthus tomentosa*), two types of substrates (Substrate 1: sand, rice husk and vermiculite; Substrate 2: sand, rice husk and peat) and two phosphate sources (tricalcium phosphate and rock phosphate). At 120 days after inoculation, the percentage of mycorrhization and sporulation was evaluated. As a result, it was identified that the species *Plectranthus tomentosa* in substrate 2 was the most suitable, since it obtained a total mycorrhization of 79.7% at a concentration of 1000 ppm of tricalcium phosphate, while in substrate 1 it had 67.5% at the same concentration of tricalcium phosphate. This species also presented a higher number of spores (638 spore / 100 g soil) in substrate 1 at a concentration of 1000 ppm of tricalcium phosphate. In conclusion, the trap plant and substrate composition had a direct influence on the production of mycorrhizal inoculum.

Keywords: Endomycorrhizae, Sporulation, Host, Mycorrhization, Substrates.

Resumen

Las plantas trampas de micorrizas arbusculares pueden ser especies cultivables o silvestres. Además de soportar la presión antropogénica, éstas son excelentes huéspedes para la multiplicación masiva de las micorrizas arbusculares. El objetivo de este trabajo es seleccionar la planta trampa y el sustrato para la propagación masiva de micorrizas arbusculares. Se evaluaron cuatro especies (*Cajanus cajan, Cynodon dactylon, Tagetes patula,* y *Plectranthus tomentosa*), dos tipos de sustratos (Sustrato 1: arena, cascarilla de arroz y vermiculita; Sustrato 2: arena, cascarilla de arroz y turba) y dos fuentes fosfatadas (fosfato tricálcico y roca fosfórica). A los 120 días después de la inoculación se evaluó el porcentaje micorrización y esporulación. Como resultado se identificó que la especie *Plectranthus tomentosa* en el sustrato 2 fue la más idónea, ya que obtuvo una micorrización total de 79,7% a una concentración de 1000 ppm de fosfato tricálcico, mientras que en el sustrato 1 tuvo 67,5% a la misma concentración de fosfato tricálcico. Esta especie también presentó un mayor número de esporas (638 esporas / 100 g suelo) en el sustrato 1 a una concentración de 1000 ppm de fosfato tricálcico. En conclusión, la planta trampa y composición del sustrato tiene influencia directa en la producción de inóculo micorrízico.

Palabras clave: Endomicorrizas, Esporulación, Huésped, Micorrización, Sustratos.

Suggested citation: Naranjo-Morán, J., Olivo-Fernández, K., Barcos-Arias, M., and Oviedo-Anchundia, R. (2025). Choice of trap plant and substrate for Mycorrhizal Inoculum production. *La Granja: Revista de Ciencias de la Vida.* [Accepted version] https://doi.org/10.17163/lgr.n42.2025.05.

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1 Introduction

Trap plants, environmental characteristics, and limited dispersion are factors that influence the mass multiplication of arbuscular mycorrhizal fungi (AMF) (Ramalho da Silva et al., 2014). The symbiotic associations between AMF communities and trap plants are complex, as mycorrhizal hyphal networks connect various rhizosphere systems and regulate nutrient flows, communication, and competitive interactions within the ecological niches of each plant. Coexistence with the plant community has led to interspecific specializations, such as the establishment of seedlings in new territories (Tedersoo et al., 2020; Van Geel et al., 2018).

Under natural conditions, most plants are colonized by AMF; however, the presence of these fungi can be affected by anthropogenic factors such as the excessive use of fertilizers, fungicides, and herbicides, leading to a decline or even disappearance of mycorrhizal biodiversity in agroecosystems (Davison et al., 2020). AMF are highly relevant in agriculture due to the multiple benefits they provide to plants, including phosphorus solubilization in the soil and the sustainability of agricultural production systems (Deepika and Kothamasi, 2015).

Trap plants serve as efficient hosts for AMF. One promising host plant is maize (*Zea mays* L.), as it allows for the production of one or more AMF species. Other plant species have also been used as trap plants to propagate AMF spores; these species belong to families such as *Solanaceae, Fabaceae, Cucurbitaceae, Amaryllidaceae,* and *Lamiaceae*, among others (Koske and Gemma, 1995; Salas and Blanco, 2000). Their biological cycles facilitate adaptation to the pot cultivation method, reducing production times to as little as 1.5 months (Salas and Blanco, 2000).

Leek (*Allium ampeloprasum* L.) is an ineffective species for inoculum production from AMF, as its performance may be influenced by environmental conditions. This suggests the hypothesis that plant diversity both stimulates and hinders spore production (Liu and Wang, 2003). The selection of trap plants is primarily based on the quantification of spore production per gram of soil or reference substrate. A limiting factor in spore production is the level of phosphorus (P) added to the substrate, as this element can interfere with spore formation in plants such as chili pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), leek (*Allium ampeloprasum* L.), and French marigold (*Tagetes patula* L.).

Other limiting factors associated with AMF propagation include the initial plant material, climatic factors, and the genetic diversity of AMF in the substrate composition (Koske and Gemma, 1995; Salas and Blanco, 2000; Schmidt et al., 2010). The substrate composition also influences the multiplication of mycorrhizal inoculum, particularly in the absorption of nutrients by the trap plant, such as P, Mg, and Ca (Gao et al., 2019). In particular, phosphorus dosage in the nutrient source has a differential effect on the benefits provided by AMF (Alarcón, 2003), impacting spore production efficiency in trap plants due to the origin of the phosphate source. Phosphate fertilization can either enhance or reduce the endogenous potential for mycorrhizal colonization (Covacevich et al., 2006).

For this reason, the objective of this study is to select a suitable trap plant and an optimal substrate for the mass propagation of arbuscular mycorrhizal fungi.

2 Materials and Methods

2.1 Systems conditions

This research was conducted in the greenhouse of the Biotechnology Research Center of Ecuador (CIBE) at the Escuela Superior Politécnica del Litoral (ESPOL), located at km 30.5 of the Perimeter Road on the Gustavo Galindo Campus (Latitude: 2° 9'3.12" S, Longitude: 79°57'13.03" W).

The average climatic conditions recorded in the greenhouse during the study period were 26 °C temperature, 65% relative humidity, and constant luminosity between May and August. The plant material used as trap plants included *Cajanus cajan, Cynodon dactylon, Tagetes patula,* and *Plectranthus tomentosa*, all obtained from wild seeds, except for *P. tomentosa*, which was propagated using 10 cm cuttings. To obtain homogeneous plants for the experiment, seedlings were initially grown in plastic seed trays. Once the seedlings developed two true leaves, they were transplanted into 2 kg polypropyle-

ne pots filled with different substrates according to the treatments and inoculated with 20 g of a native AMF consortium (purified native inoculum), comprising *Acaulospora sp.*, *Ambispora sp.*, *Diversispora sp.*, *Entrophospora sp.*, *Funeliformis sp.*, and *Glomus sp.* at a concentration of 300 total spores per 100 g of substrate (50 spores per genus). Notably, the AMF inoculum was obtained from the CIBE–ESPOL arbuscular mycorrhizal fungi (AMF) bank.

The study was designed using a two-factor experimental design, where Factor A (phosphate source and substrate) included four phosphate concentration levels, and Factor B consisted of the four plant species studied. The substrates used were:

- S1: Sand, rice husk, and vermiculite.
- S2: Sand, rice husk, and peat.

The phosphate sources tested were tricalcium phosphate (TP) and phosphate rock (PR). The four plant species examined in this experiment were *C. cajan*, *C. dactylon*, *T. patula*, and *P. tomentosa*. Phosphate treatments were applied at three doses (150, 500, and 1000 ppm), along with a control (0 ppm), with three replicates per treatment. All substrates and materials were strictly sterilized before the experiment to prevent cross-contamination with pathogenic agents. Agronomic parameters were measured to assess species selection attributes and physiological root mass characteristics, including plant height, leaf number, dry biomass, chlorophyll content index, mycorrhization percentage, and spore count at 120 days post-inoculation. In addition to phosphate fertilization, the plants were supplemented with 10 mL of modified Steiner solution, applied three times per week (Galindo Pardo et al., 2014).

2.2 Measurement of agronomic and physiological parameters

In this study, the following parameters were measured: plant height, recorded from the plant collar to the terminal bud; number of leaves emitted by each species, counted up to the last fully extended leaf; total dry biomass, determined using an oven at a temperature of 80 °C until a constant weight was obtained (López-Hidalgo et al., 2018); chlorophyll content index, analyzed at 120 days, for which the third youngest fully developed leaf was selected, and the measurement was taken between 10:00 and 11:00 AM, under optimal luminosity conditions, using a CCM-200 PLUS meter, Opti-Science (Redha et al., 2019).

To measure the total mycorrhization percentage in the roots of the trap plants, they were subjected to water stress for one week, during which irrigation and fertilization were suspended. After this period, a combined sample of 10 g of secondary roots from the three replicates of each treatment was collected. The roots were washed with potable water to remove impurities, then cut and placed in an amber container, applying 1 mL of 10% KOH for 10 minutes in an autoclave (125 °C and 15 psi pressure). After this time, the KOH was removed, washed with water, and 1% HCl was applied for 3 minutes to acidify the samples. The HCl was then removed without washing the roots, and finally 0.05% trypan blue was applied and sterilized for 15 minutes. Once sterilized, the samples were allowed to cool, and 10 rootlets were placed on each microscope slide. A drop of lactoglycerol was then added, and a cover slip was placed to observe the infective structures and determine the mycorrhization percentage (McGonigle et al., 1990).

For spore count determination, a 100 g substrate sample was taken in triplicate from each treatment. The samples were evaluated using the wet sieving method, where sieves of 45, 106, and 710 μm were used, and then centrifuged in a tween 20 plus sucrose gradient for 5 min at 2000 rpm (Gerderman and Nicholson, 1963). The final supernatant was dissolved in water to wash the spores (Furlan et al., 1980). The final content was poured into a segmented Petri dish where counting was performed using a stereoscope. The criterion for determining spore density across all combined species was the same: low density corresponds to < 1 spore/g of substrate; medium density corresponds to $1 - 10$ spores/g of substrate; and high density corresponds to > 10 spores/g of substrate (Sieverding, 1983).

2.3 Statistical Analysis

The results were subjected to an analysis of variance (ANOVA) and Tukey's test with p-values < 0.05 for the studied variables, using the Infostat V.1.2.0 software (2017).

3 Results and Discussion

3.1 Growth parameters of trap plants with tricalcium phosphate (TF) and rock phosphate (RF) phosphate sources

Regarding plant height, in *C. cajan* plants, it was observed that the interaction between S1 and the phosphate source at 1000 ppm of FT resulted in heights of 79 cm, compared to the control, which reached 59 cm (Table 1). Meanwhile, at the same concentration in S2, the height of *C. cajan* was 59 cm, compared to the control, which reached 51 cm. With the RF phosphate source, plants reached a height of 71 cm at the maximum concentration, compared to 67 cm in the control in S1. In this substrate, 47 leaves were recorded, compared to 41 leaves in the control (Table 1). Similarly, a comparable pattern was observed in S2. Regarding the number of leaves in S1, a count of 51 leaves was recorded at a concentration of 1000 ppm of FT.

In *C. dactylon* plants, both S1 and S2 treatments at 1000 ppm of FT resulted in a height of 25 cm. The highest leaf count was observed in S2, where 50 leaves were obtained at 1000 ppm of FT, compared to 73 leaves in the control in S2. In S1, a chlorophyll content index (CCI) of 13 units was recorded at 1000 ppm of RF, compared to the control, which had 5 CCI units. For this species, no significant differences were observed in the evaluated variables in S1 and S2.

In *T. patula* plants, those grown in S1 reached a height of 6 cm in the 1000 ppm FT treatment, compared to 8 cm in the control. It is worth mentioning that this species showed minimal variation in S2, maintaining a pattern similar to the other species studied. On the other hand, in the 1000 ppm RF treatment, the height reached 9 cm, compared to 11 cm in the control in S1, while in S1 and S2, differences were not significant. The species *P. tomentosa* reached a height of 12 cm in S2 under the 1000 ppm RF treatment, compared to 17 cm in the control (Table 1).

Regarding chlorophyll content, no significant differences were observed in *C. cajan, T. patula,* and *P. tomentosa* in either substrate (Table 1). However, *C. dactylon* in S1 had 14 CCI units in the 1000 ppm FT treatment, compared to 7 CCI units in the control. *T. patula* plants in S2 recorded 11 CCI units in the 1000 ppm FT treatment, compared to 13 CCI units in the control. No other species exhibited statistically significant differences in either substrate, indicating that these species better adapt to the substrates and can maintain higher photosynthetic capacity and tolerance to water stress.

The experimental data revealed significant differences in plant growth with S2, which showed the highest adaptability across nearly all evaluated plant species. S1 had the greatest influence on *P. tomentosa* plants. These results indicate that trap plant selection plays a fundamental role in the production of mycorrhizal inoculum. Recent studies have demonstrated that the trap plant plays a crucial role in assembling the mycorrhizal community present in the inoculum (Van Geel et al., 2018), as plant species and fungal communities from specific sites enhance ecological restoration (Wu et al., 2020).

3.2 Mycorrhization of Trap Plants

The highest mycorrhization in S1 with both phosphate sources was observed in *P. tomentosa* plants, with mycorrhization levels exceeding 60%, while the lowest mycorrhization was found in *C. cajan*, reaching approximately 25% for both phosphate sources (Table 2). The species *C. dactylon* and *T. patula* showed similar percentages, with notable increases at 150 ppm of FT and RF, where both species achieved higher mycorrhization values.

In S2, *P. tomentosa* exhibited the highest mycorrhization percentage, reaching 71% and 74% in the 1000 ppm treatment with FT and RF, respectively, compared to the controls, which reached 68% and 63%. This indicates a higher affinity of the mycorrhizal consortium for the selected substrate and plant species compared to the other studied plant species.

Several reports have indicated that phosphate sources may affect the mycorrhizae in the initial inoculum. In this regard, *P. tomentosa* was the species that responded best to the highest phosphate source concentrations (FT and RF), which may be due to P availability influenced by pH (Lu et al., 2019). P absorption patterns can vary depending on its availability in the substrate or soil (Stewart et al., 2005).

Our results demonstrate that RF provides P and other essential elements for both plants and the mycorrhizal community, as these interactions are always modulated by the trap plant habitat conditions (Hu et al., 2019; Li et al., 2018, 2020). In the substrate, relative abundance changes occur in certain AMF species (Gigasporaceae, Glomeraceae, Diversisporaceae, and Acaulosporaceae), which can be attributed to nutrient fertility in the substrate's micro-ecosystem for continuous monitoring (Xiang et al., 2016).

Table 1. Growth of trap plants in substrates S1 and S2 and phosphate sources FT and RF (at concentrations of 0, 150, 500, and 1000 ppm): height (cm), number of leaves, chlorophyll content (CCI units).

Trap plants	Treatment (ppm)	S1			S2		
		Tricalcium Phosphate (FT)					
		Height (cm)	**Number of leaves**	**Chlorophyll (U/CCI)**	**Height (cm)**	**Number of leaves**	**Chlorophyll (U/CCI)**
Cajanus cajan	0 (control)	*59 ± 6.5 ^{a}	*36 ± 1.8 ^{a}	44 ± 1.1 ^{a}	51 ± 1.7 ^{a}	35 ± 0.5 ^{a}	47 ± 2.2 ^{a}
	150	*66 ± 1.4 ^{b}	*43 ± 1.0 ^{b}	45 ± 0.7 ^{a}	59 ± 4.4 ^{a}	40 ± 2.9 ^{a}	51 ± 1.4 ^{a}
	500	*61 ± 4.3 ^{b}	*46 ± 2.7 ^{b}	39 ± 3.0 ^{a}	56 ± 2.0 ^{a}	38 ± 3.4 ^{a}	44 ± 3.9 ^{a}
	1000	*79 ± 1.4 ^{b}	*51 ± 2.3 ^{b}	48 ± 4.1 ^{a}	59 ± 1.5 ^{a}	39 ± 3.2 ^{a}	51 ± 0.9 ^{a}
Cynodon dactylon	0 (control)	23 ± 1.7 ^{a}	65 ± 4.0 ^{a}	*7 ± 0.3 ^{b}	27 ± 1.2 ^{a}	*73 ± 0.3 ^{b}	9 ± 0.8 ^{a}
	150	19 ± 1.2 ^{a}	55 ± 3.7 ^{a}	*6 ± 1.8 ^{a}	28 ± 1.1 ^{a}	*64 ± 8.0 ^{b}	19 ± 5.1 ^{a}
	500	23 ± 2.0 ^{a}	61 ± 3.6 ^{a}	*14 ± 3.6 ^{b}	26 ± 0.6 ^{a}	*51 ± 2.6 ^{a}	16 ± 1.0 ^{a}
	1000	25 ± 1.1 ^{a}	63 ± 5.3 ^{a}	*14 ± 4.8 ^{b}	25 ± 1.0 ^{a}	*50 ± 3.4 ^{a}	15 ± 0.5 ^{a}
Tagetes patula	0 (control)	8 ± 0.5 ^{a}	22 ± 1.1 ^{a}	12 ± 0.9 ^{a}	9 ± 0.3 ^{a}	25 ± 2.0 ^{a}	*13 ± 0.0 ^{b}
	150	8 ± 1.0 ^{a}	22 ± 1.3 ^{a}	14 ± 4.0 ^{a}	10 ± 0.5 ^{a}	22 ± 0.6 ^{a}	*11 ± 0.2 ^{b}
	500	7 ± 1.8 ^{a}	20 ± 0.6 ^{a}	16 ± 5.0 ^{a}	8 ± 0.6 ^{a}	23 ± 1.3 ^{a}	*12 ± 1.1 ^{b}
	1000	6 ± 0.0 ^{a}	20 ± 0.6 ^{a}	19 ± 3.1 ^{a}	9 ± 0.5 ^{a}	24 ± 1.1 ^{a}	*11 ± 1.1 ^{a}
Plectranthus tomentosa	0 (control)	13 ± 2.0 ^{a}	64 ± 2.4 ^{a}	13 ± 2.8 ^{a}	14 ± 1.1 ^{a}	*70 ± 0.0 ^{a}	15 ± 4.4 ^{a}
	150	13 ± 1.7 ^{a}	68 ± 4.3 ^{a}	16 ± 0.3 ^{a}	11 ± 1.1 ^{a}	*74 ± 0.6 ^{b}	17 ± 0.5 ^{a}
	500	9 ± 0.6 ^{a}	58 ± 4.0 ^{a}	11 ± 1.9 ^{a}	11 ± 0.8 ^{a}	*80 ± 1.7 ^{c}	15 ± 3.6 ^{a}
	1000	12 ± 2.0 ^{a}	60 ± 7.6 ^{a}	15 ± 2.9 ^{a}	12 ± 0.5 ^{a}	*76 ± 0.8 ^{c}	18 ± 0.1 ^{a}
		Phosphate Rock (RF)					
Cajanus cajan	0 (control)	67 ± 1.1 ^{a}	41 ± 1.2 ^{a}	44 ± 1.1 ^{a}	63 ± 4.3 ^{a}	41 ± 1.2 ^{a}	46 ± 1.1 ^{a}
	150	60 ± 1.8 ^{a}	42 ± 1.4 ^{a}	45 ± 0.9 ^{a}	52 ± 0.6 ^{a}	36 ± 1.4 ^{a}	46 ± 1.0 ^{a}
	500	58 ± 6.0 ^{a}	43 ± 4.5 ^{a}	43 ± 1.7 ^{a}	65 ± 2.5 ^{a}	42 ± 4.5 ^{a}	49 ± 2.0 ^{a}
	1000	71 ± 4.9 ^{a}	47 ± 3.7 ^{a}	42 ± 1.3 ^{a}	61 ± 0.3 ^{a}	41 ± 3.7 ^{a}	50 ± 0.4 ^{a}
Cynodon dactylon	0 (control)	20 ± 1.7 ^{a}	*68 ± 4.0 ^{a}	*5 ± 1.1 ^{a}	30 ± 0.0 ^{a}	67 ± 2.6 ^{a}	13 ± 0.2 ^{a}
	150	23 ± 1.2 ^{a}	*49 ± 0.6 ^{a}	*20 ± 2.7 ^{b}	28 ± 0.6 ^{a}	64 ± 2.6 ^{a}	21 ± 8.7 ^{a}
	500	22 ± 0.3 ^{a}	*44 ± 0.8 ^{b}	*13 ± 2.0 ^{b}	27 ± 1.3 ^{a}	68 ± 4.9 ^{a}	19 ± 3.1 ^{a}
	1000	22 ± 2.0 ^{a}	*63 ± 2.6 ^{b}	*13 ± 1.7 ^{b}	28 ± 1.0 ^{a}	59 ± 6.2 ^{a}	16 ± 2.5 ^{a}
Tagetes patula	0 (control)	11 ± 2.0 ^{a}	21 ± 1.7 ^{a}	18 ± 1.9 ^{a}	9 ± 0.6 ^{a}	20 ± 0.6 ^{a}	*14 ± 0.8 ^{b}
	150	11 ± 0.8 ^{a}	20 ± 1.1 ^{a}	14 ± 1,0 ^{a}	9 ± 0.5 ^{a}	22 ± 1.7 ^{a}	*13 ± 0.9 ^{b}
	500	10 ± 3.1 ^{a}	22 ± 1.1 ^{a}	16 ± 1.6 ^{a}	8 ± 1.0 ^{a}	22 ± 1.1 ^{a}	*12 ± 0.5 ^{b}
	1000	9 ± 3.0 ^{a}	22 ± 1.3 ^{a}	17 ± 1.7 ^{a}	9 ± 1.3 ^{a}	24 ± 1.3 ^{a}	*15 ± 0.4 ^{b}
Plectranthus tomentosa	0 (control)	13 ± 1.2 ^{a}	66 ± 4.0 ^{a}	12 ± 1.3 ^{a}	*17 ± 0.8 ^{b}	*73 ± 1.6 ^{a}	18 ± 2.2 ^{a}
	150	10 ± 1.2 ^{a}	59 ± 3.3 ^{a}	12 ± 4.0 ^{a}	*13 ± 0.8 ^{a}	*79 ± 2.4 ^{b}	18 ± 2.5 ^{a}
	500	10 ± 0.3 ^{a}	63 ± 6.6 ^{a}	9 ± 1.1 ^{a}	*15 ± 1.1 ^{b}	*84 ± 0.6 ^{b}	15 ± 1.3 ^{a}
	1000	10 ± 1.0 ^{a}	58 ± 3.0 ^{a}	7 ± 1.5 ^{a}	*12 ± 0.5 ^{a}	*72 ± 3.0 ^{a}	18 ± 0.9 ^{a}

Substrates: S1 = sand, rice husk, and vermiculite; S2 = sand, rice husk, and peat; FT = Tricalcium phosphate; RF = Phosphate rock. Mean values ± standard error, comparisons are made according to the phosphate source used. *Different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

Table 2. Mycorrhization percentages in trap plant roots, in substrates S1 and S2, and phosphate sources FT and RF (at concentrations of 0, 150, 500, and 1000 ppm).

Percentage of mycorrhization (%)					
Trap plants	**Treatment (ppm)**	**FT**		**RF**	
		S1	**S2**	**S1**	**S2**
Cajanus cajan	0	27± 2.5 ^{c}	25±2.8 ^{c}	35±1.3 ^{f}	33±2.5 ^{d}
	150	25±1.7 ^{b}	32±10 ^{d}	41±3.5 ^{g}	18±0.6 ^{a}
	500	31±2.0 ^{e}	23±1.3 ^{b}	29±2.2 ^{d}	31±3.3 ^{d}
	1000	22±0.9 ^{a}	31±4.2 ^{d}	24±0.7 ^{b}	32±0.7 ^{d}
Cynodon dactylon	0	39±1.9 ^{f}	39±2.7 ^{d}	42±1.7 ^{g}	38±0.0 ^{e}
	150	46±3.6 ^{i}	47±2.4 ^{e}	43±1.7 ^{c}	34±2.8 ^{d}
	500	42±1.3 ^{g}	34±2.8 ^{d}	34±2.4 ^{f}	42±3.2 ^{e}
	1000	45±2.4 ^{h}	31±4.2 ^{e}	38±2.6 ^{f}	48±0.7 ^{f}
Tagetes patula	0	63±1.7 ^{l}	42±1.1 ^{g}	60±1.8 ^{l}	51±0.0 ^{e}
	150	71±3.2 ^{l}	50±1.6 ^{f}	62±5.2 ^{l}	45±2.0 ^{e}
	500	45±5.4 ^{h}	45±1.3 ^{e}	55±3.5 ^{k}	45±3.8 ^{e}
	1000	46±5.5 ^{i}	46±1.8 ^{e}	49±1.9 ^{j}	40±4.0 ^{e}
Plectranthus tomentosa	0	68±4.9 ^{l}	68±3.1 ^{h}	67±2.4 ^{l}	63±0.6 ^{h}
	150	63±1.3 ^{l}	79±1.9 ^{h}	61±1.6 ^{l}	75±2.4 ^{h}
	500	65±3.5 ^{l}	77±2.5 ^{h}	67±2.6 ^{l}	79±3.1 ^{h}
	1000	69±1.3 ^{l}	71±6.1 ^{h}	69±4.2 ^{l}	74±1.0 ^{h}

Substrates: S1 = sand, rice husk, and vermiculite; S2 = sand, rice husk, and peat; FT = tricalcium phosphate; RF = phosphate rock. Mean values ± standard error, comparisons are made according to the phosphate source used. *Different letters in the same row indicate significant differences according to Tukey's test ($p<0.05$)

3.3 Spore Production in the Substrates

This study found that different trap plants can adapt to a mycorrhizal inoculum and substrate composition, forming a specific nesting by affinity during the plant's life cycle. This was supported by the differences observed in spore production. The fact that we used several mycorrhiza-dependent trap plants and a native inoculum under water stress suggests that these factors may have significantly contributed to the synergistic effects in trap plant development.

In *P. tomentosa* plants grown in S1, the highest spore count was obtained, reaching 637 and 623 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to the controls (T0), which reached 434 and 438 spores per 100 g of soil in FT and RF (Figure 1). On the other hand, the species with the lowest spore count was *C. cajan*, with 195 and 198 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to T0, which reached 211 and 166 spores per 100 g of soil in FT and RF, respectively. The other species exhibited similar trends in treatments with 150 and 500 ppm of phosphate sources.

In *P. tomentosa* plants grown in S2, the highest spore count was obtained, reaching 612 and 623 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to T0, which reached 426 and 506 spores per 100 g of soil in FT and RF (Figure 2).

The species with the lowest spore count was *C. cajan*, with 256 and 238 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to T0, which reached 187 and 133 spores per 100 g of soil in FT and RF, respectively. The other species followed similar patterns in treatments with 150 and 500 ppm of phosphate sources.

The results demonstrate that *P. tomentosa* exhibited higher root mycorrhization by the fifth month, with mycorrhization levels exceeding 60% and 70% in FT and RF, respectively, where the infective structure characteristics of AMF were observed (Figure 3). This suggests an effective complementarity when selecting a trap plant for biotechnological applications or for restoring contaminated soils.

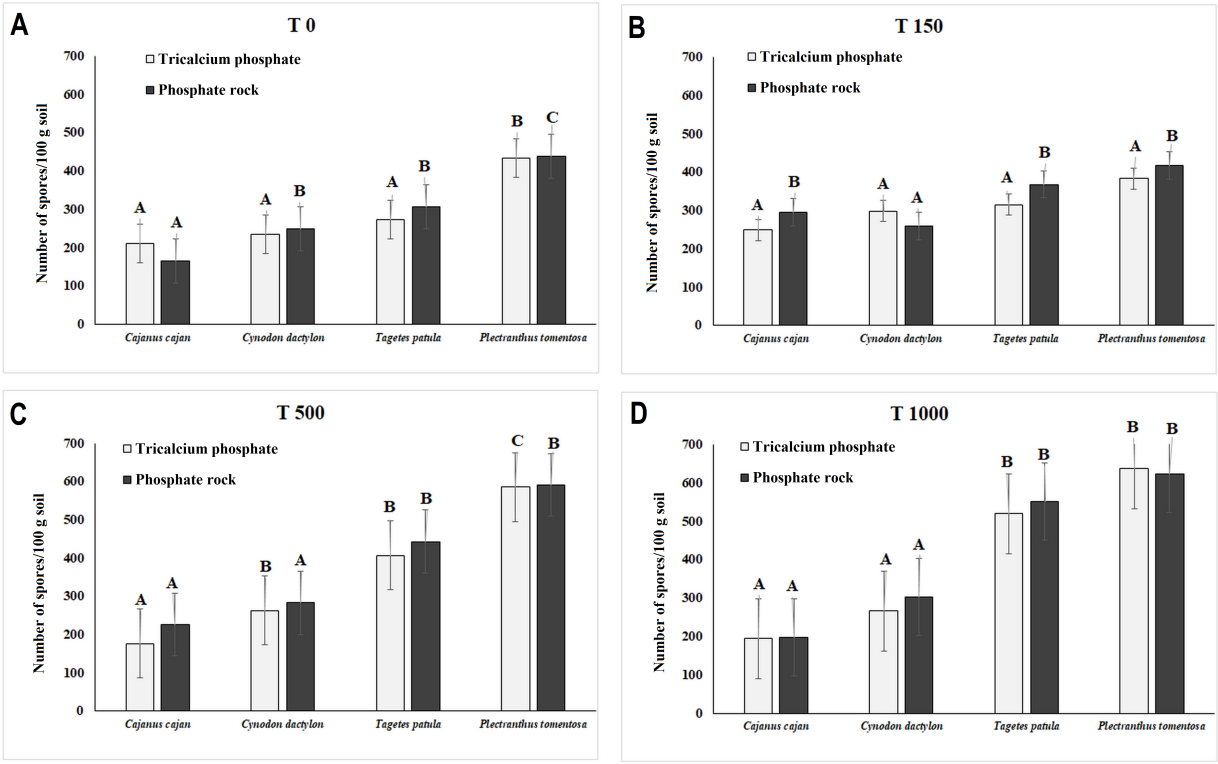

Figure 1. Spores produced in substrate 1; A) Treatment 0 (control), B) Treatment 150 ppm, C) Treatment 500 ppm, D) Treatment 1000 ppm. The bar limits represent the standard error with a 95 % confidence interval. Different letters above the bars indicate statistically significant differences according to Tukey's test ($p<0.05$).

Trap plants that experience multiple stress factors and are inoculated with different mycorrhizal species are more efficient than those not inoculated or those that have not been enhanced with a greater diversity of AMF genera (Crossay et al., 2019).

We found that plant height, chlorophyll content, and biomass in trap plants respond to the type of substrate used and the phosphate source (FT or RF). These results are consistent with reports indicating that mycorrhizal inoculation enhances photosynthesis, root expansion, and water and nutrient uptake (Selvakumar et al., 2018).

This information is crucial for trap plant selection criteria, as an acceptable biomass production performance may stimulate or delay mycorrhizal colonization, which in turn affects spore production (Liu and Wang, 2003).

The density of trap plants is also considered a limiting factor, as plants compete for inorganic P in the substrates, which could enhance spore production (Fabiańska et al., 2020), particularly in tillering plants, such as *C. dactylon*.

Regardless of nutrient variations, the AMF fungal network provides trap plants with a constant P supply, making them effective resource managers (Van't Padje et al., 2021b). Colonization patterns have shown that time and the nutrient requirements of the trap plant play a crucial role, as these factors are highly dynamic and difficult to predict in natural conditions. This represents a challenge in studying host-symbiont relationships that are measurable and reliable within the reality of natural associations (Van't Padje et al., 2021a).

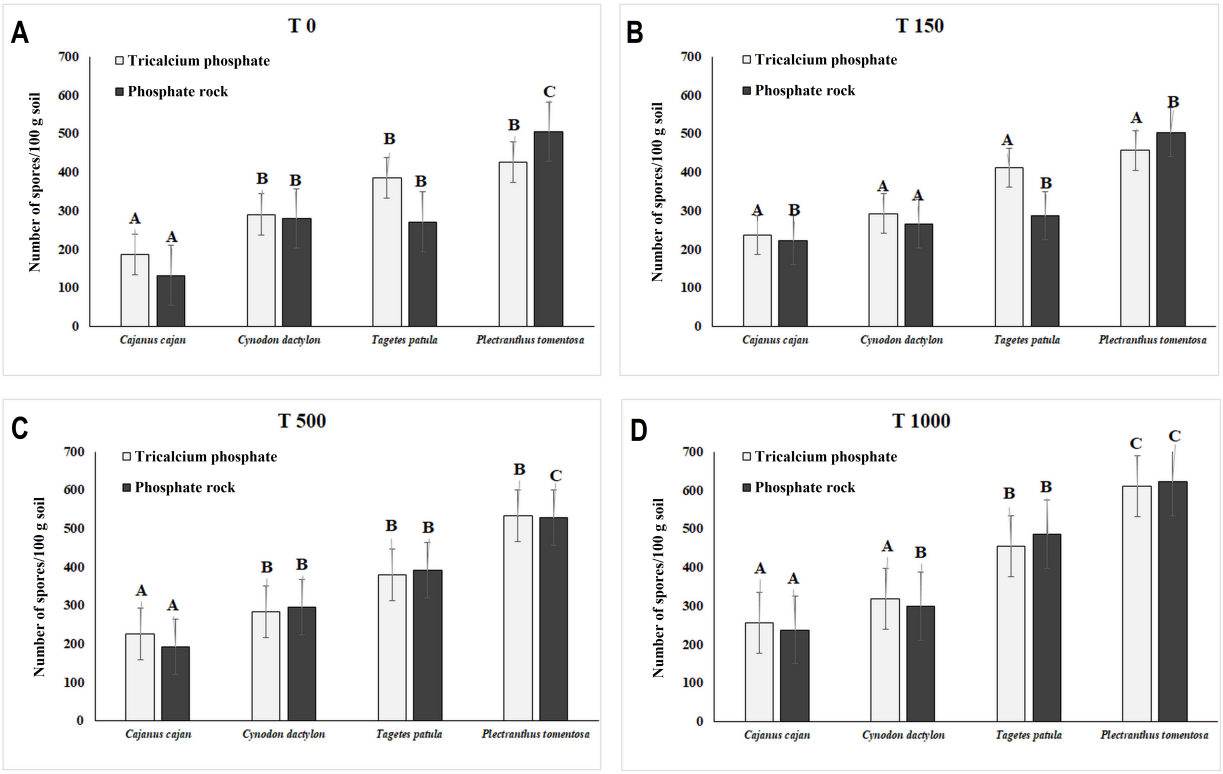

Figure 2. Spores produced in substrate 2; A) Treatment 0 (control), B) Treatment 150 ppm, C) Treatment 500 ppm, D) Treatment 1000 ppm. The bar limits represent the standard error with a 95 % confidence interval. Different letters above the bars indicate statistically significant differences according to Tukey's test ($p<0.05$).

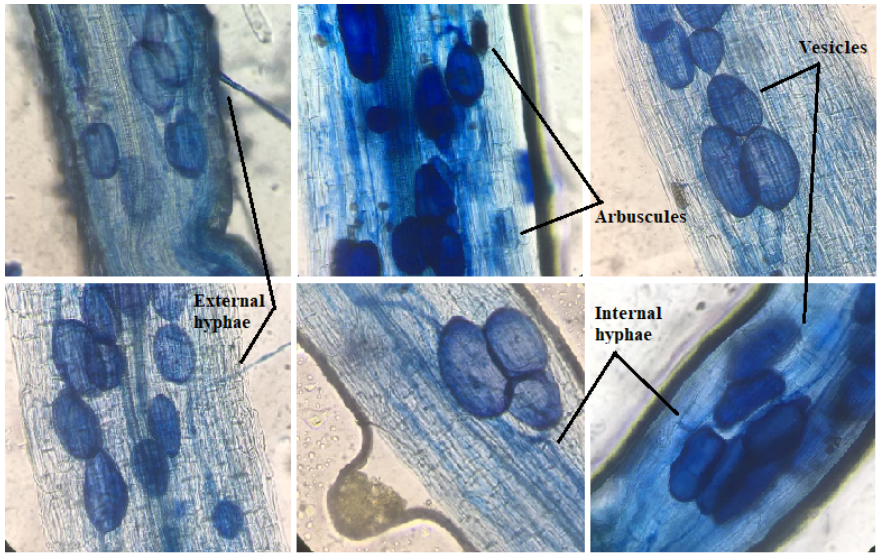

Figure 3. Mycorrhized roots of *P. tomentosa* observed under an optical microscope at 400X magnification, 120 days post-inoculation.

4 Conclusions

Among the four trap plants evaluated, the species that responded best to the mycorrhizal consortium was *Plectranthus tomentosa*. This species exhibits promising traits for mycorrhizal inoculum production, regardless of the substrate type used.

In contrast, *Cajanus cajan* was the least efficient host for AMF spore production.

The dosage of phosphate sources did not affect mycorrhizal colonization or spore production in the studied species, but it did impact their growth.

Acknowledgments

We express our gratitude to the staff at the Biotechnology Research Center (CIBE-ESPOL) for facilitating the execution of this project. We also thank the Ecuadorian Corporation for Research and Academia Development (CEDIA) for its financial support in this project.

Author's contribution

J.N.M.: Conceptualization, Writing- original draft, Methodology. K.O.F.: Research, Data curation. R.O.A.: Writing- review editing, Formal analysis, Resources, Visualization. M.B.A.: Project administration, Supervision, Funding acquisition.

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