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Arbuscular mycorrhizal interaction associated with a botanical garden in the tropics of Mexico

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ABSTRACT

Botanical gardens, areas for vegetation conservation, have become important reservoirs of beneficial soil microbiota, mainly as a source of microbial inoculum for agricultural purposes. Shrubby mycorrhizal fungi (AMF), an important genetic resource of tropical soils, have a high potential for agricultural production, generally used as inoculant medium that provides better yield, productivity and physiological response to crops. This research study explores the presence of AMF in a botanical garden, composed of four areas: cactarium collection, epiphytes and ornamental collection, tropical forest area and coastal zone. Each area is composed of plants representative of its ecosystem. For the study, a random systematic model was used, with nine samples per site at a depth of 20 cm. A physicochemical characterization of the soils was developed. The extraction of AMF spores was carried out by wet sieving and centrifugation in 60% sucrose. The spores were identified by taxon. The results indicate a total of 379 AMF spores identified in the study area. The highest spore incidence was recorded in the tropical forest area with a total of 161 (53.67±5.51) spores extracted, followed by the coastal zone and cactus collection area with 78 (26.00±9.64) and 73 (24.33±4.73) spores in total. In the study two taxa were identified, Glomeraceae and Gigasporaceae. The taxon Glomeraceae is also considered to be the most representative (highest abundance and frequency) of the study. In conclusion, botanical gardens, by their extructure (plant composition) and management can be taken into account as important ecosystems reservoirs and source of viable microbial genetic material for the bioprospecting of beneficial soil genotic resources (rhizophiles and endophylls) for use in modern agriculture and sustainable food production systems.

1. Introduction

Botanical gardens are green spaces that play an important role in the conservation of biodiversity; they have a socio-cultural, scientific, educational, ecological (conservation) and landscape purpose (Baldassarri, 2017; Sanders et al., 2018). It consists of various collections of specimens of endemic, native and introduced plant communities (from different regions) and specimens with some degree of protection. They are usually composed of different extracts in a limited environment. They are divided into zones according to the structure of their vegetation or interspersing a great diversity of specimens of productive species such as fruit trees, vegetables, medicinal use and forestry interest (wood). As well as multi-purpose species that comprise tree, shrub, herbaceous and ornamental species (Ren, 2017; Błaszak et al., 2019). Due to their socio-ecological importance, botanical gardens promote various environmental services to the ecosystem, with a perspective of biodiversity conservation, hydrological services (Ren and Antonelli, 2023), sustainable land use and carbon sequestration, as measures to mitigate the effects of climate change (Ratnayake et al., 2020). Recently, botanic gardens have expectations towards the sustainable use of their natural resources for implementation in food systems, have specifically sought to explore bioprospecting studies of soil microbial resources as a source of plant-beneficial inoculum, directly involved in the biogeochemical cycles and influencing the dynamics of soil nutrients such as phosphorus (Sharma and Dangar, 2016; Faraji and Karimi, 2022).

A genetic resource present in soil microbiota are arbuscular mycorrhizal fungi (AMF), symbiotic microorganisms binding a great diversity of plants, which constitute an important component of the soils of tropical ecosystems (Brundrett and Tedersoo, 2018). For agriculture, AMF play an important role in the molecular, biochemical and physiological response of plants. It also directly influences the mineral nutrition and hormone production of most plants of productive interest, derived from its use as a microbial inoculant with biostimulating effect and biofertilizer (Igiehon and Babalola et al., 2017). Previously, the

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diversity of AMF communities associated with different collections of tree species (Zubek et al., 2005; Walker, 2013), medicinal plants (Sinegani and Yeganeh, 2017) and ornamental species has been documented in botanical gardens. (Xie et al., 2020). However, the incidence and different patterns of diversity of arbuscular mycorrhizal fungi are influenced by ecosystem aspects such as plant community dynamics, ecosystem processes and soil parameters (Zhang et al., 2021). Therefore, the present research aims to be an exploratory work for the bioprospection of arbuscular mycorrhizal fungi in a botanical garden in the tropics of Mexico.

2. Materials and methods

2.1. Description of the study area

The area corresponds to a conservation site called "Regional Botanical Garden". The study area is located at Tecnología Nacional de México, Campus Conkal, Yucatán, México (coordinates UTM 16 Q 0240189; 2,332,947). The area is divided into two collections (cactarium, epiphyte-ornamental) and two areas aimed at conserving plant communities of a coastal ecosystem and a tropical forest (Table 1). An inventory of the plant component (trees and shrubs) of each area of the study site was carried out by surveying each area in situ, recording each established plant species. Their identification was carried out taxonomically (Jiménez-Pozo et al., 2024). All plant species (native and endemic) belong to the Biotic Penisula Province of Yucatán, except for the introduced species.

2.2. Study design

A systematic random sampling design was used on an area of no more than 500 m^2, to achieve greater heterogeneity among the characteristics of the study sites. The sample size per study site consisted of 36 simple soil samples at a depth of 20 cm, collecting approximately 1 kg of bulk soil sample per sample. Sampling was carried out during the dry season (March-April).

2.3. Sample processing

To determine the sample size, visual identification of the size was made from comparisons of the Munsell graph (Munsell color, 1994). The physicochemical characterization of the soil consisted in determining the hydrogen potential (pH 1:2) and electrical conductivity (EC ds/m), using the potentiometer-conductometer method (Rhoades 1996) with a LOHAND® potentiometer, model PHS-550. Soil texture variables were determined by the Bouyoucos method (1962), percentage porosity and bulk density by the test tube method (Flint and Flint, 2002). For organic matter, the calcination method of Schulte and Hopkins (1996) was used. The technique to determine soil mineral content was performed by X-ray fluorescence (μ-XRF) method using an M4 Tornado 100 XFlash®6 (Chi-Sánchez et al., 2020).

Table 1
Description of the attributes of the study site.

Site ID	System ID	Purpose	Type of vegetation
Site 1	Cactarium Collection (CC)	Cultivation of ornamental cactus species	thorny lowland forest Native and introduced specie
Site 2	Epiphytic and Ornamental Collection (EOC)	Cultivation of ornamental and epiphytic species	Ornamental cultivars and collection of epiphytic specie
Site 3	Tropical Forest Zone (TFZ)	Agroforestry design	Low and médium tropical vegetation. Combination of deciduous and subevergreen vegetation
Site 4	Coastal Zone (CZ)	Cultivation of coastal species	Native and introduced coastal vegetation

2.4. Determination of mycorrhiza

To characterize the presence of shrubby mycorrhizal fungi in the study areas, wet screening and centrifugation (Gerdemann and Nicolson, 1963) were used in a 60% sucrose gradient (Jenkins and Taylor, 1967). The extracted spores were visualized by a VELAB® stereomicroscope at a vision of 40X. The extracted spores were classified by taxon, using morphological parameters for their identification (Weber et al., 2019).

2.5. Biological parameters

To determine the ability of each taxon to sporulate, the following formulas were used: relative abundance (Ai%) and frequency of isolation (Fi%) (Hernández-Zamudio et al., 2018): Ai%=Total spores per taxon/Number of spores in 10 g soil, X 100 and Fi%=ji/K X 100, where ji is the number of samples in which the taxon has been produced and K is the total number of samples.

2.6. Statistical analysis

The data were statistically analyzed using an analysis of variance (ANOVA), with a significance range of $P<0.05$. The analyses were developed using the InfoStat/L 2020 software (Di Rienzo et al., 2008).

3. Results and discussion

3.1. Plant component

Sixty-nine plant species were identified among native, endemic and introduced species. The cactarium collection area (CC) consists of 12 species, mainly composed of succulent or crass species. The epiphytic-ornamental (EOC) and coastal zone (CZ) collection consisted of 13 and 14 plant species. For the tropical forest zone (TFZ), 30 species of woody trees were identified, mainly belonging to the fabaceae family. This description of the plant component of the botanical garden does not include annual herbaceous plants (Table 2).

3.2. Soil characterization

The soils have a dark/reddish hue according to the method of Munsell (Solis et al., 2022) with a texture cataloged in loamy-loam to silty-loamy soils, characteristic of slightly grassy soils, free of sediments and clay. The physical parameters of the soils were classified as thin, with light apparent density (<0.13 g/cm) and average total porosity (levels above 60%), typical of thin soils, with rapid surface drainage. On the other hand, chemical characterization determined that the study sites showed a neutral-moderately alkaline pH (6.6–7.3), a slightly saline electrical conductivity (<65 d/sm), without salinity problems. Also, a high percentage of organic matter was recorded above 9%, which means very high levels of organic matter in the soil (Borges-Gómez et al., 2008; Estrada-Medina et al., 2016) (Table 3). Authors such as Rozanova et al (2016) and Chupina (2020) indicate that the physicochemical profiles of a soil in a botanical garden are directly influenced by the composition and structure of vegetation (deciduous and herbaceous plants). Generating high contributions in organic matter and increased humification in the first horizons of the soil.

In the study, the chemical composition indicated soils with high levels of minerals such as phosphorus, iron, zinc, manganese and copper (Table 4). However, values between 300–4600 ppm of phosphorus, the main key mineral in mycorrhizal processes, can be inferred from non-assimilable phosphorus for plants (Borges-Gómez et al., 2008; Estrada-Medina et al., 2016). Musielok et al. (2018) and Chupina (2020),

Table 2
Description of the plant component of the study site.

Site ID	System ID	Predominant vegetation
Site 1	Cactarium Collection (CC)	*Cereus jamacaru* DC. *Epiphyllum hookeri* (Link & Otto) Haw. *Euphorbia láctea* Haw. 1812, *Euphorbia trigona* Mill., 1768 *Nopalea gaumeri* Britton & Rose *Nopalea inaperta* Schot ex Griffiths *Opuntia stricta* (Haw.) Haw. *Nopalea cochenillifera* (Linn) Mill. *Acanthocereus tetragonus* (Linn.) Hummelinck. *Stenocereus laevigatus* (Salm-Dyck) Buxb. *Selenicereus grandiflorus* (Linn.) Britton & Rose ssp. *Pilosocereus gaumeri* (Britton & Rose) Backeb.
Site 2	Epiphytic and Ornamental Collection (EOC)	*Ficus obtusifolia* Kunth *Lasiasis rucsifolia* (Kunth) Hitchc. ex Chase. *Erythroxylum areolatum* Linn. *Enterolobium cyclocarpum* Griseb. *Philodendron jacquinii* Schott. *Zamia prasina* W. Bull. *Alocasia cucullata* (Schott) G.Don, *Bougainvillea glabra* Choisy 1849 *Crinum amabile* Donn ex Ker Gawl. *Callisia fragrans* (Lindl.) Woodson. *Anthurium schiechtendalii* Kunth ssp. Schlechtendalii. *Lophiaris oertedii* (Rchb. f.) R. Jiménez, Carnevali & Dressler. *Hechtia schottii* Baker.
Site 3	Tropical Forest Zone (TFZ)	*Brosimum alicastrum* Sw. 1788 *Plumeria obtusa* Linn. *Neea psychotrioides* Donn. Sm. *Pilocarpus racemosus* Vahl var. *yucatanus* Kaastra, *Bursera simaruba* (Linn.) Sarg. *Cordia alliodora* (Ruiz & Pav.) Oken. *Diospyros albens* C. Presl. *Acacia pennatula* (Schltdl. & Cham.) Benth. *Piscidia piscipula (Linn.) Sarg.* *Caesalpinia yucatanensis* (Britton & Rose) Greenm. *Ceiba pentandra* (Linn.) Gaertn. *Havardia albicans* (Kunth) Britton & Rose. *Lysiloma latisiliquum* (Linn.) Benth. *Mimosa bahamensis* Benth. *Acacia cornígera* (Linn.) Willd. *Acacia collinsii* (Saff.) Seigler & Ebinger *Gymnopodium floribudum* Rolfe *Guazuma ulmifolia* Lam. *Hamelia patens* Jacq. *Tecoma stans* (Linn.) Juss. ex Kunth var. stans. *Bromelia karatas* Linn. *Canna indica* Linn. *Cresentia cujete* Linn. *Maclura tinctoria* (L.) D. Don ex Steud. *Pouteria glomerata* (Miquel) Radl. ssp. Glomerata. *Senna racemosa* (Mill.) H.S. Irwin & Barneby var. *racemosa* *Tabebuia rosea (Bertol.) DC.* *Sapindus saponaria* Linn. *Amphitecna latifolia* (Mill.) A.H. Gentry.
Site 4	Coastal Zone (CZ)	*Sabal yapa* C. Wright. ex Becc. *Sabal mexicana* Mart. *Suriana marítima* Linn.*, 1753* *Thrinax radiata* Lodd. ex Schult. & Schult. f. *Cocos nucifera* Linn, 1753 *Ambrosia híspida* Pursh *Acoelorraphe wrightii* (Griseb. & H. Wendl.) H. Wendl. ex Becc. *Pseudophoenix sargentii* H. Wendl. ex Sarg. *Coccothrinax readii* H. J. Quero R. *Coccoloba uvifera* Linn. *Cakile lanceolata* (Willd.) O. E. Schulz ssp. *Cordia sebestena* Linn. *Scaevola plumieri (*Linn.*)* Vahl. *Hymenocallis littoralis* (Jacq.*)* Salisbury.

point out that the nature of soils in a botanical garden are the result of a combination between an agrosoil (influenced by agricultural activities, e.g. fertilizers, rods, etc.) and an urban soil (modified by anthropogenic materials such as asphalt, concrete, etc.). In addition, the soil modification that can be generated in this type of soil characteristic of a botanical garden, causes greater stress on plant communities established in the system, is derived from low mobility of nutrients in the Fe-bearing soil that limits the dynamics of other minerals and infuses piogenic processes within the same system (Silva et al., 2015). Ideal conditions to promote symbiotic interaction between shrub mycorrhizal fungi and plant roots, as the greater the stress on the soil, The greater the possibility of forming a mutual relationship with the rhizosphere and the more effective the establishment of the mycorrhizal arbuscular relationship with communities. of plant species (Giovannetti et al., 2017; Jacott et al., 2017). Also, high concentrations of Ca, commonly observed in Yucatán, were recorded due to the karstic characteristics and heterogeneity of its natural soils and the direct anthropogenic influence of agrotechnical practices that modify the nature of the soils (Estrada-Medina et al., 2016).

3.2. Incidence of AMF in the soil

In total, 379 AMF spores (31.6±15.00) were counted in the 36 samples collected. The tropical forest zone (TFZ) is considered to be the most abundant area of AMF spores, with a total of 161 spores (53.67 ±5.51). The coastal zone (CZ) and the cactus collection (CC) recorded 78 (26.00±9.64) and 73 (24.33±4.73) spores extracted, respectively. Areas composed of ornamental and epiphyte species reported abundance of spores from 67 spores (22.33±7.02) (Fig. 1). In previous studies, have reported the same assertions. by determining that deciduous and evergreen rainforest vegetation is considered the most relevant natural ecosystem for AMF. Polo-Marcial et al., (2021), reported in the tropical forests of the Gulf of México that symbiotic interaction mycorrhizal, plays a key role in the establishment and development of plant communities. Reporting more than 30% of the species identified in Mexico, mainly in the structural woody species of this type of ecosystem. Which implies the importance of this tropical ecosystem as a key reservoir for the diversification of the symbiotic interaction of an AMF with the rhizosphere of plant communities, especially interacting with tree species of the Fabaceae family (Marinho et al., 2018).

Solís- Rodríguez et al., (2020), report a direct relationship between the abundance of shrub mycorrhizal fungi and the establishment of arboreal and herbaceous vegetation in tropical forests. The latter corresponds to an extract of the key vegetation in the diversification of AMF communities. This is mentioned by Ramos-Zapata et al. (2013), who reported that the greatest sporulation of AMF in a natural tropical forest ecosystem occurs in vegetation cover, often in annual vegetation, characteristic of ruderal species. The mycorrhizal interaction with the plant cover responds to the annual growth of plant roots, usually of species with creeping habits belonging to the families Asteraceae, Portulacaceae and Poaceae. This type of vegetation acts as a buffer for the biodiversity of soils in recovery, interacting as temporary hosts and diversifying mycorrhizal fungi communities, to enhance their

Table 3
Physical-chemical property of soils in the study.

Sampling Site ID	Apparent density (g/cm^3)	Porosity (%)	Texture	Organic matter (%)	Hydrogen potential	Electric conductivity (ds/m)
Site 1	0.11±0.001	54.63±3.06	Slimy	9.67±0.58	7.29±0.04	67.50±6.34
Site 2	0.04±0.05	49.33±2.31	Silt loam	9.67±1.15	7.29±0.10	61.80±2.60
Site 3	0.19±0.002	68.87±1.15	Slimy	11.00±0.001	7.23±0.04	53.37±4.61
Site 4	0,15±0.04	63.73±4.56	Silt loam	8.00±0.001	7.25±0.06	71.40±2.95
Site 5	0,17±0.02	70.10±1.73	Silty clay	9.33±1.15	7.30±0.06	66.57±7.15

Table 4
Mineral composition of the soils obtained in the study sites.

Site ID	Total minerals (ppm)						
	P	K	Ca	Fe	Zn	Mn	Cu
Site 1	4615.67	79,260.00	332,758.67	486,162.67	2034.00	7702.33	943.33
Site 2	357.33	64,666.67	125,831.67	440,734.67	1305.00	5627.33	464.67
Site 3	834.00	77,248.33	235,212.33	466,899.67	1569.33	7157.00	841.33
Site 4	518.33	62,621.00	228,598.67	430,516.33	982.33	5522.67	295.67
Site 5	308.33	68,743.00	217,225.67	464,611.67	913.00	6181.00	304.67

P: phosphorus; P: potassium; Ca: calcium; Fe iron; Zn: zinc; Mn: manganese and Cu: copper.

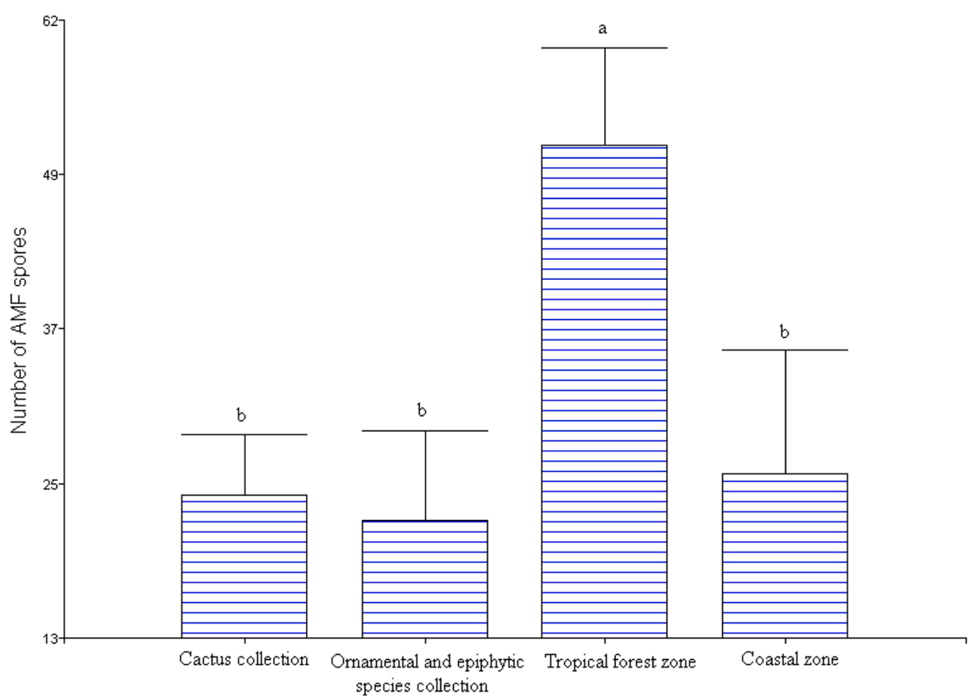

Fig. 1. Number of arbuscular mycorrhizal fungi spores extracted (g^{-10}) from the study sites.
Different literals represent statistical differences by means of the Tukey test ($P<0.05$)

interaction with tall woody species (Ramos-Zapata et al., 2012). In similar studies, Polo-Marcial et al., (2023), determined that AMF communities are directly related to the establishment of woody trees, pioneer species (grasses, herbaceous) and ruderals, characteristics of a recovering ecosystem (secondary vegetation). This implies that, in a biologically recovering ecosystem, the greatest mycorrhizal (spore sporulation and colonization) occurs during the early stages of secondary vegetation. Increases as vegetation cover and tree component regeneration progresses.

Regarding the coastal vegetation zone, other authors have reported the affinity of AMF-mycorrhizal communities in the same species established in the study area. AMF has been reported in *Acoelorraphe wrightii* (Fabian et al., 2018), *Cocos nucifera* (Lara-Pérez et al., 2020), species of the family Arecaceae (*Thrinax radiate, Coccothrinax readii, Pseudophoenix sargentii, Sabal yapa*), scirbus and herbaceous vegetation (*Sesuvium portulacastrum, Amaranthus greggi, Coccoloba uvifera* and *Jacquinia auranthiaca*) characteristic of coastal sand dunes scrubland, aquatic vegetation and low-flood vegetation (petén) (Carmona-Escalante et al., 2013; Solís- Rodríguez et al., 2021).

3.3. Composition of AMF in soil

For this study, two representative taxa (class: Glomerycota), Glomeraceae and Gigasporaceae were identified. Counting a total of 349 (29.08±13.90) spores for Glomeraceae and 30 (2.50±1.83) for Gigasporaceae. The taxon Glomeraceae was significant among the study sites. (Fig. 2). On the other hand, the ecological parameters evaluated determined a relative abundance of 93.22% for glomeraceae with an isolation frequency of 100%. Gigaspoaraceae had an abundance of 7.81% and a frequency of 83.33%. According to these results, the

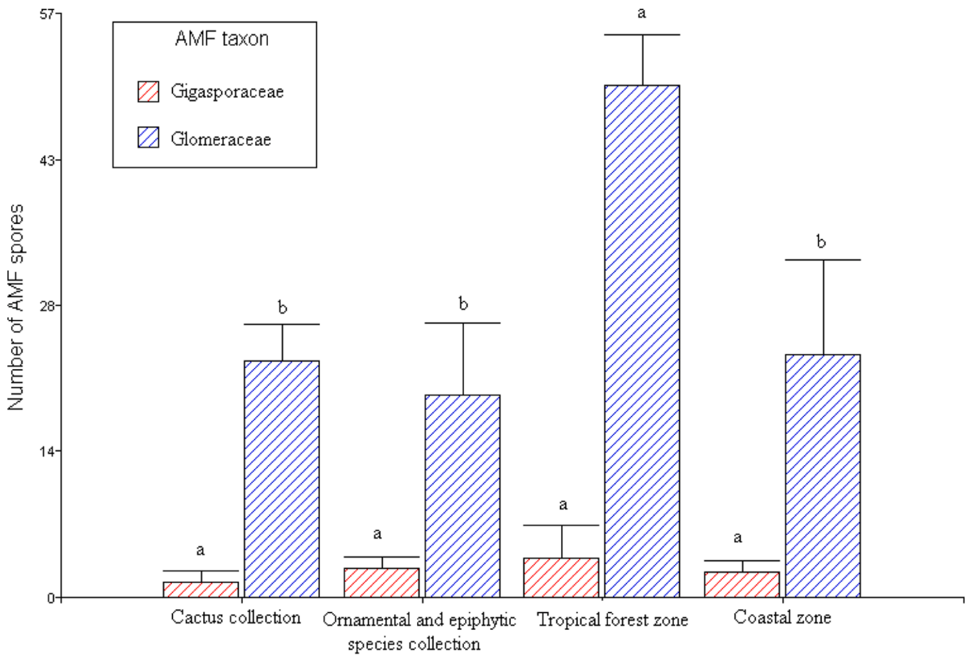

Fig. 2. Taxón of arbuscular mycorrhizal fungi from the study sites.
Different literals represent statistical differences by means of the Tukey test (P<0.05).

glomeraceous taxon is the most representative in each of the study sites. The higher incidence and presence of the taxon Glomeracea in the evaluated ecosystems is due to a greater resilience and lower sensitivity to land use changes (Bharti et al., 2013).

Polo-Marcial et al (2021), points out that in Mexico more than 160 species of the different 13 AMF families have been reported. Identifying the taxon Glomeracea as the most representative (more than 40% incidence) in natural and modified ecosystems (agricultural systems, homegardens and pasturales), resisting the different degrees of anthropogenic disturbances. Noteworthy, Glomeraceae has smaller spores (50–150 μm), well developed internal hyphae and long external hyphae. This generates less energy expenditure for their metabolism (sporulation and formation of an extensive micellar network), favoring a greater early success in establishing a symbiotic interaction with the roots of pioneer plants during the first serial stages of a regeneration biological (Weber et al., 2019). On the contrary, shorter external hyphae, less developed internal hyphae and larger spores (>150 μm) generate greater energy use that reduces their sporulation and limits the initial stage of interaction with plant communities (Weber et al., 2019). Comparing with previous studies, the abundance of AMF spores per taxon is influenced by vegetation structure (Gottshall et al., 2017). Demonstrating that the Glomeraceae concentrates AMF species in early stages of recovery in disturbed ecosystems. The Gigasporaceae is considered less frequent in disturbed systems, until it is associated with plant communities with a longer establishment time (Polo-Marcial et al., 2021; Manrique-Caamal et al., 2024).

4. Conclusions

The results of our study explore the importance of botanical gardens for the bioprospecting of shrubby mycorrhizal fungi, specifically for use in modern agriculture and to improve the production of plant-based nutrients. This study also contributes to the incidence and identification reports of AMF in modified ecosystems in Mexico. Providing data that enrich the composition and diversity of arbuscular mycorrhizal fungi in tropical soils. The botanical gardens, which are key to the conservation of biodiversity in vegetation and soil microbial resources, represent an important reservoir and source of native shrub mycorrhizal inoculum. Where, the taxon Glomeraceae, is recognized as the AMF family with the greatest incidence in the study and in each of the zones that make up it (coastal zone, tropical forest, cactaceae, epiphytes and ornamental). Identifying the composition of this important genetic resource of soils, will allow us to establish strategies for its agricultural management, with expectations to develop native inoculos (Mycorrhizal monosporic consortium) with greater resilience and adaptability to adverse soil conditions in the field.

CRediT authorship contribution statement

José Alberto Gío-Trujillo: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization. **Carlos J. Alvarado-López:** Conceptualization, Methodology, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jose Alberto Gio Trujillo reports a relationship with National Technological Institute of Mexico, Conkal campus that includes: non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Links and Accession Numbers for data and code are available in the manuscript text.

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