BIODIVERSITAS
Volume 25, Number 4, April 2024
Pages: 1570-1579

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250425

Richness and community structure of arbuscular mycorrhizal fungi associated with *quelites* in the Sierra Sur region of Oaxaca, Mexico

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Manuscript received: 8 January 2024. Revision accepted: 15 April 2024.

Abstract. *Villagómez-González BB, Robles C, Carballar-Hernandez S. 2024. Richness and community structure of arbuscular mycorrhizal fungi associated with* quelites *in the Sierra Sur region of Oaxaca, Mexico. Biodiversitas 25: 1570-1579.* Wild plant species that are collected and consumed by local populations are called "*quelites*". In Mexico, and particularly in the state of Oaxaca, the culture of collecting and consuming *quelites* is widely preserved. The interactions of wild plants with Arbuscular Mycorrhizal Fungi (AMF) promote the successful establishment of plants in ecosystems and allow their survival. The aim of this work was to characterize the richness, diversity, and population structure of mycorrhizal interaction in five species of *quelites*. The structure of AMF communities was different among the five quelite species. A total of 42 AMF morphospecies grouped into five families and 12 genera were identified. The composition of AMF communities in the five species of *quelites* studied was significantly different. The Acaulosporaceae family recorded the highest number of morphospecies (42% of the total), followed by the Glomeraceae and Ambisporaceae families (33 and 9.5% respectively). *Funneliformis geosporum* and *Claroideoglomus claroideum* were the predominant AMF morphospecies. The AMF communities with greater diversity and equity indexes were recorded in the *quelites Alloispermum* sp. and *Solanum americanum*. The Jaccard similarity index separates quelite species into three groups, since the composition of AMF species was significantly different between these groups. The pH and available phosphorus of the soil affected the distribution and abundance of AMF species associated with the plant species studied.

Keywords: AMF, mycorrhizal symbiosis, population structure, quelite

INTRODUCTION

"*Quelites*" are native plants that have been used as food in Mesoamerica for many years. The word quelite comes from the Nahuatl "quilitl", which means grass, legume, or edible vegetable (Mateos-Maces et al. 2020). These plants are a source of vitamins, minerals, fiber, proteins, carbohydrates, and functional compounds (Mateos-Maces et al. 2020; Sahu et al. 2020; Arumugam et al. 2023). In Mexico, native and/or peasant communities use *quelites* as complementary food or as the basic diet of families. The consumption of these plants constitutes a strategy to combat hunger and malnutrition in these areas (Mateos-Maces et al. 2020).

In San Agustin Loxicha (Sierra Sur region of Oaxaca, Mexico), *quelites* that grow in temperate forests, backyards, gaps, or in the "milpa" system are collected and consumed (Vásquez-Dávila et al. 2023). The climate of the region is temperate humid, with an average annual temperature of 12 to 18°C, with rain in summer and presence of an intra-summer dry period; annual precipitation is 600 to 1000 mm. The soil in the *quelites* collection area has a dark surface horizon rich in organic matter, acidic pH, and light texture (Martínez-Domínguez et al. 2022).

Studies of *quelites* have focused on their botanical classification and knowledge of their nutritional properties (Mateos-Maces et al. 2020; Sahu et al. 2020; Arumugam et al. 2023); however, very little is known about the diversity of microorganisms associated with the rhizosphere of these plants. Arbuscular mycorrhizal fungi (AMF) are one of the most important and abundant groups in soil, they are symbiotically associated with most plant species (Chaudhary et al. 2022; Ma et al. 2023) including *quelites*. AMF are essential for the establishment, survival, and productivity of many plants, since they facilitate the uptake of phosphorus, nitrogen, and other nutrients. Furthermore, they increase water absorption and improve soil structure thanks to the extraradical mycelium (Baum et al. 2015; Fall et al. 2022; Boyno et al. 2023; Fasusi et al. 2023). In Oaxaca, we have recorded AMF present in ecosystems and agrosystems, being the state of Mexico with the greatest richness of AMF in the country (Carballar-Hernández et al. 2013; de la Cruz-Ortiz et al. 2020; Álvarez-Lopeztello et al. 2023). It has been determined that soil characteristics affect the composition and diversity of AMF that are associated with a plant species; pH increases the number of spores and the percentage of colonization (Davison et al. 2021; Liu et al. 2021); the content of total nitrogen, available phosphorus, and pH modify the diversity and

composition of AMF communities (Chen et al. 2017; Han et al. 2020; Zhang et al. 2020).

In Mexico, even though *quelites* are an important part of the culture and diet of many communities (Quiñones et al. 2009; Balcázar-Quiñones et al. 2020) and it is known that this group of plants are associated with AMF (Song et al. 2019), the composition and diversity of AMF in these plant species is unknown. Studies of AMF in *quelites* have focused on their use as biofertilizers (Hoseini et al. 2021) and there are very few studies on the composition and richness of AMF (Johnson et al. 2013). Dandan and Zhiwei (2007) analyzed the diversity of AMF in *Chenopodium ambrosioides*, reporting eight morphospecies, of which *Glomus clarum* is the most common. In *Vigna unguiculata* which grows in tropical environments in Africa, 15 AMF species were reported, with predominance of the Glomeraceae family (Johnson et al. 2013).

The aim of this work was to know the composition and structure of AMF communities associated with five species of *quelites* grown in San Agustín Loxicha (Oaxaca, Mexico).

MATERIALS AND METHODS

Study area

The municipality residents of San Agustín Loxicha, Mexico (Figure 1) had great knowledge about wild edible plants. The predominant vegetation type was pine-oak forests.

Soil sampling and physicochemical analysis

Roots and rhizospheric soil samples (0-15 cm) were collected from five species of *quelites* (*Gonolobus* aff. *stenanthus*, GU, *Solanum americanum* Mill., HM, *Eryngium foetidum* L., CE, *Crotalaria longirostrata* Hook and Arn. CH, *Alloispermum* sp., HP). For each plant species, three subsamples were collected in three places in the municipality of San Agustín Loxicha (La Conchuda, Quee lovee, and Río Granada), in the summer of 2021. Each of soil samples was divided into two lots: the first determined the AMF community and the other part analyze the physical-chemical parameters of the soil. Soil samples for the HMA community studies were stored under refrigeration (4-5°C) until processing. The samples for physical-chemical analysis were dried in a greenhouse for 72 hours, ground, and sieved (2 mm mesh). For each soil sample, pH (H_2O) and electrical conductivity (EC) were determined with a digital potentiometer (Hanna® HI98129), moisture content by gravimetry (CH), texture using the Bouyoucos method, and organic matter content (OM) and organic carbon (CO) by wet digestion of Walkley and Black method (Nelson and Sommers 1983), available phosphorus (PO4) by the Bray and Kurtz method (Bray and Kurtz 1945), inorganic nitrogen (N) with the Microkjeldahl method (Lee et al. 2017).

AMF spore density and morphospecies identification

Recovery and counting of AMF spores (at morphospecies level) were performed using the wet sieving and decantation method, followed by sucrose gradient centrifugation (20 and 60%, 2400 rpm) according to the method of Brundrett et al. (1996) with slight modifications. The spores and sporocarps were identified at genera and morphospecies, differentiating the morphospecies by morphological criteria of size, shape, color, number, and type of layers of the spore wall, type of connection of the supporting hypha and histochemical reaction to the Melzer (Sandoval-Pineda et al. 2020). The measurement of spores and their subcellular structure was performed with an optical microscope (Leica DM750 with ICC50W digital camera). The determination of the species was carried out considering specialized descriptions of species from the page of the Mycorrhizal Biology laboratory of the Federal University of Rio Grande do Norte (https://glomeromycota.wixsite.com/lbmicorrizas), from Arthur Schüßler website (http://www.amf-phylogeny.com/), West Virginia University (http://fungi.invam.wvu.edu), and Janusz Blaszkowski website (http://www.zor.zut.edu.pl/Glomeromycota/).

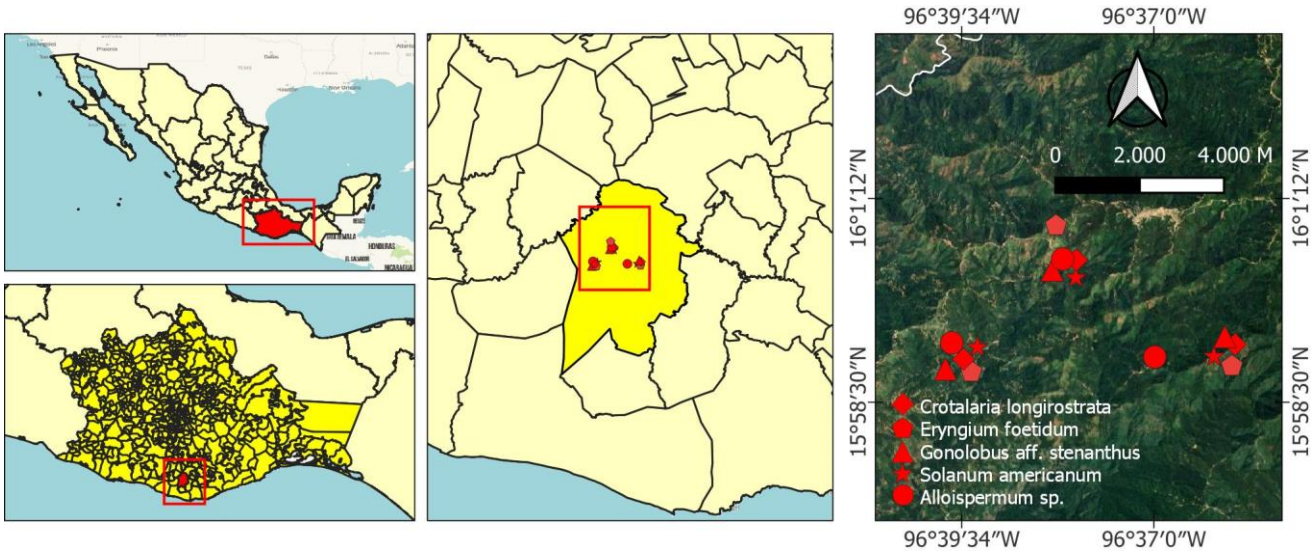

Figure 1. Location of sampling sites of five species of *quelites* in San Agustin Loxicha, Oaxaca, Mexico

Estimation of mycorrhizal colonization

For this determination, secondary and tertiary quelite roots were used. It was carried out using the modified Brundrett et al. method (1996). The roots were washed abundantly in tap water, placed in a 10% KOH solution at room temperature for 48 hours, followed by washing with tap water, covered with a 10% HCl solution for 10 minutes, and stained with trypan blue 0.05% dissolved in 50% lactic acid. To determine the colonization %, the roots were cut into 2 cm long segments, 25 segments were mounted per slide and the presence of AMF structures (arbuscules, spores, vesicles, hyphae) was observed under a stereomicroscope (Leica EZ4) to quantify the number of colonized roots and record the colonization %.

Colonization (%) = (colonized fields) / (total fields observed) x 100

Ecological and statistical analysis

AMF communities were described by spore abundance, morphospecies richness, and isolation frequency. The Pieolou evenness, Simpson dominance, and Shannon-Wiener diversity indexes were calculated according to Masebo et al. (2023).

Differences in morphospecies richness, spore density, and mycorrhizal colonization were evaluated using analysis of variance (ANOVA) in Minitab ver 21. The experimental design was performed in complete randomized with three replications. One-way ANOVAs were performed to compare the soil properties of the five quelite species. Tukey honest significant difference (α=0.05) was used to evaluate pairwise differences between means. The relationship between soil characteristics, AMF spore density, and species richness was also explored using multivariate analysis (Masebo et al. 2023)

RESULTS AND DISCUSSION

Composition and structure of the AMF community associated with five species of *quelites*

The composition of AMF communities in five quelite species studied was significantly different (Table 1). *Acaulospora alpina*, *Ambispora appendicula*, and *Glomus macrocarpum* were reported exclusively in HP quelite (Table 1). *A. laevis* and *Scutellospora* sp.1 were only recorded in HM, and *Gigaspora gigantea* was only found in the GU quelite. When comparing the composition of AMF community using the Jaccard coefficient, greater similarity was recorded in the composition of AMF morphospecies between the CE and CH *quelites*; the GU and HM *quelites* formed a second group, while the HP quelite formed a third group (Figure 2).

A total of 42 morphospecies of AMF spores were reported, including five families and 12 genera (Table 1). The highest number of morphospecies (42% of the total) was recorded in the Acaulosporaceae family, followed by Glomeraceae and Ambisporaceae families (33% and 9.5% respectively). The species of quelite influences the AMF morphospecies richness; in HM the highest richness was recorded (37), followed by the *quelites* HP and GU with 32 and 31, respectively. In contrast, the *quelites* with the lowest number of AMF morphospecies were CH and CE (Tables 1 and 2). Regardless of the quelite species, 16 of the 42 mosphospecies were recorded associated with five plants (Table 1). Additionally, two morphospecies (*Acaulospora nivalis* and *Entrophospora nevadensis*) were reported for the first time from Mexico, and one morphospecies from Oaxaca (*Sclerocystis taiwanensis*).

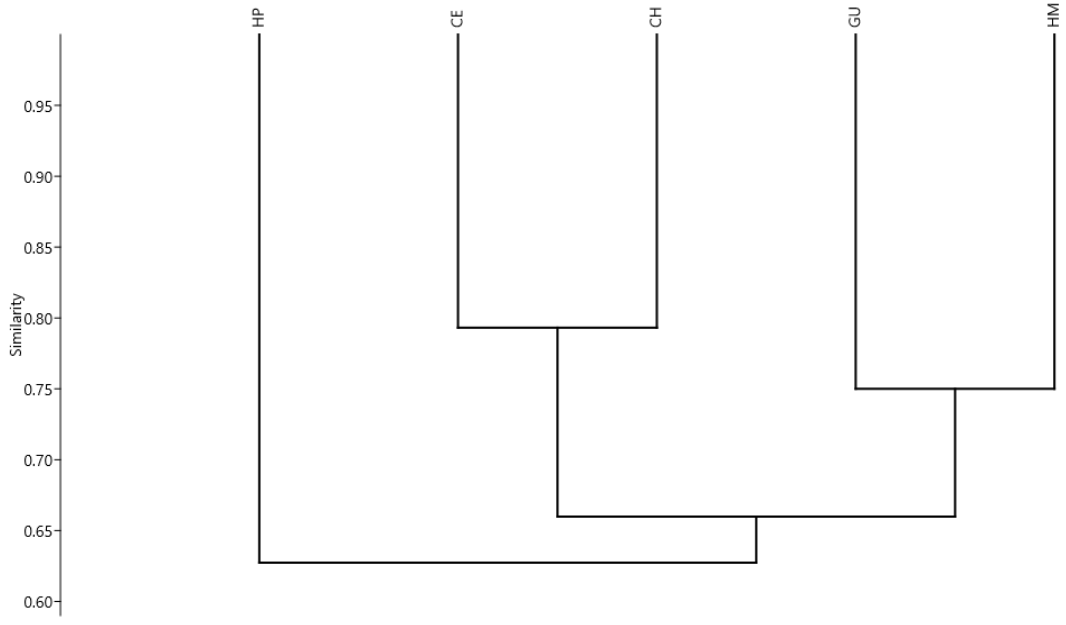

Figure 2. Dendrogram of similarity of the arbuscular mycorrhizal fungi species composition among five *quelites*. Abbreviations: CH: *Crotalaria longirostrata*, GU: *Gonolobus aff. stenanthus*, CE: *Eryngium foetidum*, HM: *Solanum americanum*, HP: *Alloispermum* sp.

The AMF communities of five quelite species were dominated by *Funneliformis geosporum* and *Claroideoglomus claroideum* (29 and 18% respectively of the total spores and 100% of isolation frequency). Morphospecies such as *A. mellea*, *A. scrobiculata*, *F. mosseae*, *G. microcarpum*, and *Claroideoglomus* sp.1 had a high frequency of isolation, but with lower abundance than the first two. On the other hand, several species had low relative abundance and isolation frequency (Table 2). The HMA communities with greater diversity and equity were recorded in the HP and HM *quelites*, while the HMA community with greater dominance and less diversity and equity was recorded in the CH quelite (Table 3). In last quelite, 58% of the spores correspond to *F. geosporum* and *C. claroideum* (Table 2).

Effect of soil properties on the distribution and abundance of AMF

The canonical correspondence analysis explained 24% of the variance and grouped the AMF communities based on the five species of *quelites* and the soil properties. Available-P content and soil pH affect the distribution and abundance of AMF morphospecies. *Acaulospora laevis, Acaulospora aff. mellea, Acaulospora nivalis, Glomus microcarpum,* and *Gigaspora gigantea* were grouped towards the P-available vector, in the first axis. In contrast, most AMF morphospecies distribute inversely to the P-available vector. Furthermore, the species *A. laevis* and *A. aff mellea* present a positive relationship with the soil organic matter vector (Figure 3 and Table 1).

Mycorrhizal colonization and number of spores

Mycorrhizal colonization had significant differences between the quelite species ($p \leq 0.05$). On average, in CE the highest colonization was 21% and the lowest in GU (8.3%) (Figure 4.A). On the other hand, the number of spores ranged from 244 to 344 spores in 50 g of dry soil, although there were no significant differences between the quelite species ($p \leq 0.05$). As for the colonization %, the number of spores was lowest in GU quelite and highest in CE quelite (Figure 4.B).

Soil properties

Results showed that there were no significant ($p < 0.05$) differences in the characteristics of the soils where quelite species grow. The pH was moderately acidic, texture was sandy loam, and the electric conductivity was low in all soil samples. The content of available P and total N varied from medium to high; the highest content of these elements was reported in the rhizospheric soil of CE, while the highest content of organic matter was recorded in the soil where CH was grown, and the lowest in HP (Table 4).

Table 1. Presence of arbuscular mycorrhizal fungi (AMF) morphospecies recorded in the rhizosphere of five species of *quelites*

AMF species / plant species	CH	GU	CE	HM	HP
Acaulosporaceae					
Acaulospora alpina					●
A. denticulata	●	●	●	●	
A. excavata		●	●	●	
A. laevis				●	
A. mellea	●	●	●	●	●
A. morrowiae	●	●	●	●	
A. nivalis		●		●	
A. paulinae				●	●
A. rehmii	●	●	●	●	●
A. scrobiculata	●	●	●	●	●
A. spinosa	●		●	●	●
A. splendida	●		●	●	●
A. aff. mellea	●	●		●	●
A. aff. nivalis		●		●	●
A. aff. reducta		●		●	
Acaulospora sp.1		●		●	
Acaulospora sp.2		●	●		●
Acaulospora sp.3				●	●
Ambisporaceae					
Ambispora gerdemannii	●	●		●	●
A. reticulata				●	●
A. appendicula					●
Ambispora sp.1	●	●	●	●	
Entrophosporaceae					
Entrophospora infrequens	●	●		●	●
E. nevadensis		●	●	●	●
Gigasporaceae					
Dentiscutata sp.1	●		●	●	●
Gigaspora gigantea		●			
Scutellospora dipurpurescens		●		●	●
Scutellospora sp.1				●	
Glomeraceae					
Claroideoglomus claroideum	●	●	●	●	●
Claroideoglomus sp.1	●	●	●	●	●
Funneliformis geosporum	●	●	●	●	●
F. mosseae	●	●	●	●	●
Glomus microcarpum	●	●	●	●	●
G. macrocarpum					●
G. spinuliferum	●	●	●	●	●
Glomus sp.1	●	●	●	●	●
Glomus sp.2	●	●	●	●	●
Glomus sp.3	●	●	●	●	●
Rhizophagus fasciculatus	●	●	●	●	●
Sclerocystis sinuosa	●	●	●	●	●
S. taiwanensis	●	●	●	●	●
Septoglomus constrictum	●	●	●	●	●
Total of AMF species	25	31	25	37	32

Note: CH: *Crotalaria longirostrata*, GU: *Gonolobus aff. stenanthus*, CE: *Eryngium foetidum*, HM: *Solanum americanum*, HP: *Alloispermum* sp.

Table 2. Spore number (SP), relative abundance (RA), and isolation frequency (IF) of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of five species of *quelites*

HMA species	CH			GU			CE			HM			HP		
	SP	**RA**	**IF**	**SP**	**RA**	**IF**	**SP**	**RA**	**IF**	**SP**	**RA**	**IF**	**SP**	**RA**	**IF**
Acaulosporaceae	**161**	**19.33**	**100**	**130**	**12.60**	**100**	**151**	**14.86**	**100**	**232**	**25.95**	**100**	**151**	**20.94**	**100**
Acaulospora alpina	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	67	9.29	100
A. denticulata	10	1.20	66.67	3	0.29	33.33	1	0.10	33.33	17	1.90	100	0	0.00	0.00
A. excavata	0	0.00	0.00	4	0.39	33.33	1	0.10	33.33	1	0.11	33.33	0	0.00	0.00
A. laevis	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	1	0.11	33.33	0	0.00	0.00
A. mellea	88	10.56	100	40	3.88	100	78	7.68	100	112	12.53	100	24	3.33	100
A. morrowiae	23	2.76	66.67	8	0.78	33.33	11	1.08	33.33	18	2.01	66.67	0	0.00	0.00
A. nivalis	0	0.00	0.00	2	0.19	33.33	0	0.00	0.00	2	0.22	33.33	0	0.00	0.00
A. paulinae	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	6	0.67	33.33	1	0.14	33.33
A. rehmii	1	0.12	33.33	4	0.39	33.33	1	0.10	33.33	10	1.12	66.67	1	0.14	33.33
A. scrobiculata	29	3.48	100	37	3.59	100	31	3.05	100	46	5.15	100	29	4.02	66.67
A. spinosa	7	0.84	100	0	0.00	0.00	2	0.20	33.33	1	0.11	33.33	1	0.14	33.33
A. splendida	2	0.24	33.33	0	0.00	0.00	3	0.30	33.33	6	0.67	33.33	11	1.53	66.67
Acaulospora aff. mellea	1	0.12	33.33	4	0.39	33.33	0	0.00	0.00	2	0.22	33.33	7	0.97	33.33
Acaulospora aff. nivalis	0	0.00	0.00	3	0.29	33.33	0	0.00	0.00	3	0.34	33.33	4	0.55	33.33
Acaulospora aff. reducta	0	0.00	0.00	3	0.29	33.33	0	0.00	0.00	4	0.45	66.67	0	0.00	0.00
Acaulospora sp.1	0	0.00	0.00	5	0.48	33.33	0	0.00	0.00	2	0.22	33.33	0	0.00	0.00
Acaulospora sp.2	0	0.00	0.00	17	1.65	66.67	23	2.26	66.67	0	0.00	0.00	5	0.69	33.33
Acaulospora sp.3	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	1	0.11	33.33	1	0.14	33.33
Ambisporaceae	**15**	**1.80**	**100**	**10**	**0.97**	**100**	**3**	**0.30**	**33.33**	**3**	**0.34**	**33.33**	**20**	**2.77**	**66.67**
Ambispora gerdemannii	12	1.44	100	5	0.48	100	0	0.00	0.00	1	0.11	33.33	11	1.53	66.67
A. reticulata	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	1	0.11	33.33	1	0.14	33.33
A. appendicula	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	8	1.11	66.67
Ambispora sp.1	3	0.36	33.33	5	0.48	33.33	3	0.30	33.33	1	0.11	33.33	0	0.00	0.00
Entrophosporaceae	**5**	**0.60**	**100**	**8**	**0.78**	**66.67**	**1**	**0.10**	**33.33**	**10**	**1.12**	**66.67**	**19**	**2.64**	**66.67**
Entrophospora infrequens	5	0.60	100	1	0.10	33.33	0	0.00	0.00	7	0.78	66.67	16	2.22	66.67
E. nevadensis	0	0.00	0.00	7	0.68	66.67	1	0.10	33.33	3	0.34	33.33	3	0.42	66.67
Gigasporaceae	**1**	**0.12**	**33.33**	**4**	**0.00**	**33.33**	**1**	**0.10**	**33.33**	**16**	**1.79**	**100**	**7**	**0.28**	**33.33**
Dentiscutata sp.1	1	0.12	33.33	0	0.00	0.00	1	0.10	33.33	8	0.89	100	2	0.28	33.33
Gigaspora gigantea	0	0.00	0.00	2	0.19	33.33	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Scutellospora dipurpurescens	0	0.00	0.00	2	0.19	33.33	0	0.00	0.00	3	0.34	33.33	5	0.69	33.33
Scutellospora sp.1	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	5	0.56	66.67	0	0.00	0.00
Glomeraceae	**651**	**28.45**	**100**	**880**	**15.60**	**100**	**860**	**18.41**	**100**	**633**	**70.81**	**100**	**524**	**15.12**	**100**
Claroideoglomus claroideum	223	26.77	100	150	14.53	100	173	17.03	100	149	16.67	100	98	13.59	100
Claroideoglomus sp.1	14	1.68	100	11	1.07	100	14	1.38	100	36	4.03	100	11	1.53	100
Funneliformis geosporum	264	31.69	100	306	29.65	100	252	24.80	100	282	31.54	100	195	27.05	100
F. mosseae	26	3.12	100	8	0.78	66.67	15	1.48	100	11	1.23	66.67	10	1.39	100
Glomus microcarpum	17	2.04	100	154	14.92	100	17	1.67	100	20	2.24	66.67	11	1.53	100
G. macrocarpum	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	1	0.11	33.33	1	0.14	33.33
G. spinuliferum	8	0.96	66.67	3	0.29	33.33	1	0.10	33.33	2	0.22	66.67	9	1.25	33.33
Glomus sp.1	14	1.68	33.33	27	2.62	100	52	5.12	100	31	3.47	100	23	3.19	66.67
Glomus sp.2	3	0.36	66.67	4	0.39	66.67	6	0.59	66.67	16	1.79	66.67	1	0.14	33.33
Glomus sp.3	19	2.28	66.67	41	3.97	66.67	137	13.48	100	9	1.01	100.00	28	3.88	66.67
Rhizophagus fasciculatus	9	1.08	66.67	77	7.46	100	29	2.85	100	41	4.59	100.00	16	2.22	100
Sclerocystis sinuosa	28	3.36	66.67	20	1.94	100	30	2.95	100	15	1.68	33.33	36	4.99	66.67
S. taiwanensis	18	2.16	33.33	10	0.97	33.33	1	0.10	33.33	3	0.34	33.33	63	8.74	66.67
Septoglomus constrictum	8	0.96	66.67	69	6.69	66.67	133	13.09	100	17	1.90	100.00	22	3.05	33.33
Total	833	100		1032	100		1016	100		893	100		721	100	

Note: CH: *Crotalaria longirostrata*, GU: *Gonolobus aff. stenanthus*, CE: *Eryngium foetidum*, HM: *Solanum americanum*, HP: *Alloispermum* sp.

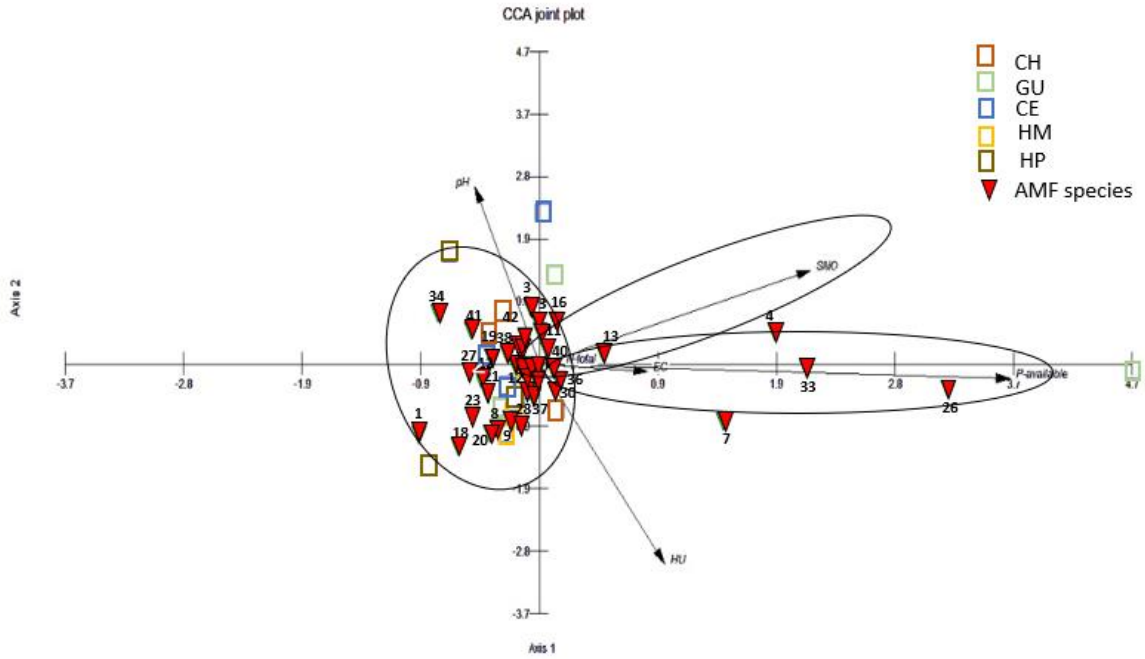

Figure 3. Ordination diagram of the effect of soil properties on the distribution of arbuscular mycorrhizal fungi species in five *quelites*. Abbreviations: CH: *Crotalaria longirostrata,* GU: *Gonolobus aff. Stenanthus,* CE: *Eryngium foetidum,* HM: *Solanum americanum,* HP: *Alloispermum* sp., SOM: Soil organic matter. AMF species represented by numbers: 1=*A. alpina* 2=*A. denticustata* 3=*A. excavata* 4=*A. laevis* 5=*A. mellea* 6=*A. morrowiae* 7=*A. nivalis* 8=*A. paulinae* 9=*A. rehmii* 10=*A. scrobiculata* 11=*A. spinosa* 12=*A. splendida* 13=*A. aff. mellea* 14=*A. aff.nivalis* 15=*A. aff. reducta* 16=*A.* sp.1 17=*A.* sp.2 18=*A.* sp.3 19=*A. gerdemannii* 20=*A. reticulata* 21=*A. appendicula* 22=*A.* sp.1 23=*E. infrequens* 24=*E. nevadensis* 25=*D.* sp.1 26=*G gigantea* 27=*S. dipurpurescens* 28=*S.* sp.1 29=*C. claroideum* 30=*C.* sp.1 31=*F. geosporum* 32=*F. mosseae* 33=*G. microcarpum* 34=*G. macrocarpum* 35=*G. spinuliferum* 36=*G.* sp.1 37=*G.* sp.2 38=*G.* sp.3 39=*R. fasciculatus* 40=*S. sinuosa* 41=*S. taiwanensis* 42=*S. constrictum*

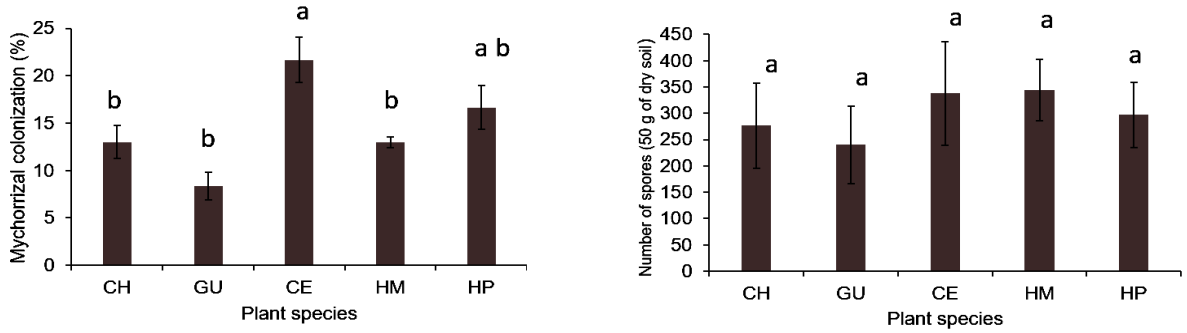

Figure 4. Mycorrhizal colonization (A) and number of spores (B) in roots of five quelite species. Note: CH: *Crotalaria longirostrata,* GU: *Gonolobus aff. stenanthus,* CE: *Eryngium foetidum,* HM: *Solanum americanum,* HP: *Alloispermum* sp. Identical letters above bars are not significantly different (P <0.05)

Discussion

This research contributes to the knowledge of AMF communities that are associated with wild species of *quelites* that grow in the pine-oak forest of the Sierra Sur region of Oaxaca, Mexico. The presence of spores of the morphospecies *Acaulospora alpina*, *Ambispora appendicula*, and *Glomus macrocarpum* associated only with the quelite HP, allowed us to consider these AMF as specialist symbionts, since they were found only under certain ecological conditions. In general, the mycorrhizal relationship was not considered specific, since any fungus can colonize any plant; however, under certain edaphoclimatic conditions, some fungi can benefit a particular host (Pérez and Vertel 2020; Tedersoo et al. 2020).

The high similarity in AMF morphospecies composition among quelite species (68%) indicates low species turnover or low beta diversity (Carballar-Hernández et al. 2017), because many species have a wide geographic distribution; for example, *Funneliformis geosporum, Acaulospora mellea, Claroideoglomus claroideum, A. scrobiculata, F. mosseae*, and *G. microcarpum*, which has already been reported in other studies (Oehl et al. 2010; Stürmer et al. 2013; Chávez-Hernández et al. 2021).

Table 3. Diversity measurements of arbuscular mycorrhizal fungi associated with five species of *quelites*

Ecological parameters	CH	GU	CE	HM	HP
Richness	25	31	25	37	32
Simpson dominance-diversity index	0.1901	0.1476	0.1383	0.1539	0.1192
Shannon-Wiener diversity index	2.181	2.389	2.294	2.455	2.627
Pieolou evenness index	0.6776	0.6957	0.7126	0.6798	0.7581

Note: Means with the same letter in the same column are not significantly different (*t*-student, α =0.05). CH: *Crotalaria longirostrata,* GU: *Gonolobus aff. stenanthus,* CE: *Eryngium foetidum,* HM: *Solanum americanum,* HP: *Alloispermum* sp.

Table 4. Physical and chemical properties of rhizospheric soil samples collected from five quelite species

Samples	pH	EC	SOM	Moisture	P-available	N-total	Soil texture
CH	5.06±0.17a	0.08±0.021 a	13.38±7.35 a	2.38±0.97 a	16.61±7.10 a	42.58±4.98 a	Sandy loam
GU	5.17±0.25 a	0.09±0.00 a	9.86±4.00 a	2.03±0.39 a	53.88±45.58 a	37.92±1.16 a	Sandy loam
CE	5.78±0.14 a	0.18±0.07 a	11.12±5.16 a	2.07±0.24 a	118.24±60.84 a	43.75±0.00 a	Sandy loam
HM	5.35±0.23 a	0.10±0.02 a	12.06±7.25 a	2.72±0.21 a	62.72±47.65 a	34.83±8.22 a	Sandy loam
HP	5.11±0.085 a	0.06±0.00 a	5.80±0.82 a	2.10±0.55 a	29.71±15.78 a	24.20±1.01 a	Sandy loam

Note: Mean with the same letter in the same column are not significantly different (Tukey, α =0.05). CH: *Crotalaria longirostrata,* GU: *Gonolobus aff. stenanthus,* CE: *Eryngium foetidum,* HM: *Solanum americanum,* HP: *Alloispermum* sp.

The spore abundance values, greater than 200 spores per 50 g of dry soil, contrast with the low abundance reported in other pine-oak ecosystems (Palta et al. 2018; Chávez-Hernández et al. 2021). In Mexico, 160 AMF species have been reported in different wild and cultivated plant species (Polo-Marcial et al. 2021); in this study, 42 morphospecies of AMF associated with five species of *quelites* are reported, which represents 26% of the morphospecies registered for Mexico and 12.9% of those registered worldwide. In addition, morphospecies are reported that could represent new species. The richness of morphospecies found in present study was greater than that reported for studies carried out in the pine-oak forest in other regions of Mexico (Chávez-Hernández et al. 2021), as well as for previous studies for Oaxaca, in corn crops (Matias et al. 2021), in *Agave potatorum* (Carballar-Hernández et al. 2013), and *Solanum lycopersicum* (de la Cruz-Ortiz et al. 2020). The richness reported in this study is similar to the richness value reported by Reyes-Jaramillo et al. (2019) in *Agave angustifolia* and *A. karwinskii*. The abundance of spores and the richness of AMF species associated with these five species of *quelites* constitute a reservoir of potentially useful resources for the regeneration of this type of disturbed biomes.

Sclerocystis taiwanensis has been reported in agroecosystems and grasslands in the states of Chiapas and Veracruz (Mexico) (Posada et al. 2018). *Acaulospora nivalis* has been reported in the Swiss Alps (Oehl et al. 2012) and Brazil (Teixeira et al. 2017). It is characterized by being found in soil at high altitudes between 2200-3000 meters above sea level (Oehl et al. 2012), however, in this study it is reported at altitudes of 978-1980 meters above sea level. *Entrophospora nevadensis* has been identified in Spain (Palenzuela et al. 2010); it is found in soil at high altitudes, with acidic pH, high moisture and organic carbon content.

The highest richness of AMF was found in the HM quelite with 37 morphospecies, while in the lowest number, 25 morphospecies was recorded in the CH and CE *quelites*, respectively. These results are higher than those reported by Dandan and Zhiwei (2007) in the quelite *Chenopodium ambrosioides* (eight morphospecies) and by Johnson et al. (2013) in *Vigna unguiculata* in tropical environments of Africa (15 morphospecies). Only 18% of the AMF morphospecies found in these two species of *quelites* are common to those recorded in present study. The predominance of morphospecies from the Glomeraceae, Acaulosporaceae, and Gigasporaceae families has been reported by other authors for different ecosystems and agrosystems (Álvarez-Lopeztello et al. 2023), which coincides with what has been reported in current work. The low diversity of AMF in CH and CE *quelites* could be associated with the use of soil in which they grow; a greater number of AMF morphospecies has been reported in soils with less disturbance than in disturbed ones, such as agroecosystems (Xu et al. 2017). The low presence of morphospecies of the *Ambispora* genus in the present study (1%), with soils from conserved areas, coincides with reports that indicate the Ambisporaceae family as an indicator of altered areas, with an abundance of available P (Marinho et al. 2019; Phillips et al. 2019; Devia-Grimaldo et al. 2021).

In our study the AMF communities associated with the quelite species were dominated by *Funneliformis geosporum* and *Claroideoglomus claroideum*, which had the highest abundance and were isolated in all samples. These species have been reported as dominant in the *quelites C. ambrosioides* and *V. unguiculata* (Johnson et al. 2013). Likewise, they have been recorded in other forest studies (Gaonkar and Rodrigues 2021). The Glomeraceae and Claroideoglomeraceae families have been the most abundant and frequent due to the fact that they produce a large number of small spores in short periods of time, with high germination rates and constant renewal of hyphae, which gives them great adaptive capacity to tolerate diverse environmental conditions (Boddington and Dodd 2000;

Martínez-García et al. 2015; Trejo et al. 2016; Stürmer et al. 2018), and survive and dominate AMF communities in different parts of the world (Pande and Tarafdar 2004).

The genera *Gigaspora* and *Scutellospora* appear as plant diversity increases, due to greater sporulation (Mukhongo et al. 2023). In this study, these genera were commonly associated with the HM species, as the sites where this species was located are little disturbed, in contrast to the CH and CE species, which were in the milpa system or on the side of the roads.

According to the Shannon-Wiener index, the alpha diversity of AMF varies from 2.18 to 2.62, values higher than those reported in maize agroecosystems (Hao et al. 2021) and in quelite *C. ambrosioides* (Dandan and Zhiwei 2007). The quelite species with the greatest diversity and equity were HP and HM, while in CH the HMA community with greater dominance and lower diversity and equity. The CH quelite recorded the lowest index, possibly because it is regularly associated with the milpa system and the use of mineral fertilizers (Oehl et al. 2010).

Disturbances in ecosystems are determined according to the dominant species or groups of species (Grime 1998). In terms of dominance, it is possible to observe that the five quelite species generally present a low dominance, with values between 0.12 to 0.19, little or no disturbance in the soil, allowing the coexistence of a greater number of morphospecies and greater diversity of AMF compared to disturbed or stressed soils (Berza et al. 2021).

In the present study results showed that edaphic conditions affect the structure of the AMF community in the rhizosphere of five quelite species. The canonical correspondence analysis indicates that available P was variable that best explains the abundance and distribution of AMF species. Previous studies indicate that increased P availability has negative effects on the composition, distribution, and diversity of AMF species (Bernaola et al. 2018; Javadi et al. 2020). It was observed that AMF species were associated with the *quelites* CE, HM, HP, and CH. The soils in which they grow had moderate to high levels of available P and moderately acidic pH. This behavior was notable in the morphospecies *Acaulospora laevis*, *A. aff. mellea*, *A. nivalis*, *Glomus microcarpum*, and *Gigaspora gigantea*, which were grouped in the vector in which the highest concentrations of available P were found. In contrast, most AMF morphospecies distribute inversely to the P-available vector.

All *quelites* presented mycorrhizal colonization, the highest in CE (22%) and the lowest in GU (8.3%), the first plant species belongs to the Apiaceae family and the second to Apocynaceae. Both families have been reported to be highly mycorrhizal (Santhoshkumar et al. 2019; Walid et al. 2021). This range of values coincides with what was reported by Chávez-Hernández et al. (2021) for "blow grass" (15.5%), a species of the Apiaceae family. The low % of colonization in the five species of *quelites* could be due to factors such as AMF species that colonize them, soil pH, and the presence of nutrients such as Nitrogen and Phosphorus (Shi et al. 2017; Pinargote et al. 2020). The abundance of available P reduces the mycorrhizal dependence of plants, which translates into less colonization (Bernaola et al. 2018).

The presence of AMF structures in the roots of the five quelite species suggests that mycorrhiza is necessary to complement their nutritional requirements under the conditions in which they grow in the study area. It has been recognized that the plant species and the composition of AMF species associated with the roots are components that affect the sporulation response by fungi, affecting spore abundance and species richness in the soil (Scheublin et al. 2004; Chávez-Hernández et al. 2021).

The results showed that spore density was associated with soil pH ($p < 0.05$). This result agrees that reported by Davison et al. (2021), who conducted an analysis of approximately 300 studies, concluding that soil pH is the most important variable to determine the abundance of AMF spores at the local level. Furthermore, it is known that pH works as a filter for AMF (Xu et al. 2017), as other chemical properties change with pH, solubility, and availability of other elements (iron, magnesium, copper, zinc) (Alloish et al. 2000). The formation of AMF requires soils with a pH of 4.5 to 5.5 (Pérez and Vertel 2020). However, previous studies suggest that soil properties such as organic matter, nitrogen, and phosphorus affect soil diversity (Carrenho et al. 2001; Oliveira and Oliveira 2010; Melo et al. 2019) in addition to pH. Thus, the positive relationship between morphospecies richness, spore abundance, and soil properties, suggests that soil acidity could promote turnover between AMF species in the five quelite species. These results show that the relationship between soil properties and spore density cannot be generalized.

In conclusion, the quelite species *Gonolobus aff. stenanthus*, *Solanum americanum*, *Eryngium foetidum*, *Crotalaria longirostrata*, and *Alloispermum* sp. growing in soils of pine-oak forests in Sierra Sur region of Oaxaca (Mexico) were associated with many species of arbuscular mycorrhizal fungi. A total of 42 morphospecies of AMF, with 31, 37, 25, 25, and 32 species respectively in rhizospheric soil of each plant. Acaulosporaceae family recorded 42% of total, followed by Glomeraceae and Ambisporaceae with 33 and 9.5 % respectively. *Funneliformis geosporum* and *Claroideoglomus claroideum* (29 and 18%, respectively, of the total of recovery spores) were the dominant AMF species. Mycorrhizal colonization was present in all plants, ranged from 21 to 8.3%, and number of spores ranged from 244 to 344 in 50 g of dry soil. The results of this study revealed that soil available-P content and soil pH affected the distribution and abundance of AMF morphospecies.

ACKNOWLEDGEMENTS

The authors thanks to the Secretaria de Investigacion y Posgrado of the Instituto Politecnico Nacional for funding de research project SIP20221574. First author is thankful of CONAHCyT for the PhD scholarship (502017).

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