IDENTIFICATION OF SOIL-CULTURABLE MICROMYCETES IN AVOCADO ORCHARDS IN NORTHEASTERN MEXICO

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Abstract. Mexico is the world's leading producer and exporter of avocado (*Persea americana* Miller). There are few studies on soil microorganisms associated with this crop, which are important to establish the role they can play in the production system and their use in different areas of interest. In this re-search, the biodiversity of cultivable micromycetes in the soil of five avocado orchards located in two municipalities of the state of Nuevo León was determined. The isolates obtained were grouped into morphotypes and identified to genus level or grouped by common morphological characteristics. Shannon-Wiener, Simpson, and Margalef diversity indices were determined for morphotypes, as well as for and genera and groups of micromycetes with common characteristics in each orchard. The Hutcheson's test (p= 0.05) was performed to determine significant differences between Shannon-Wiener diversity index of genera and micromycetes with common characteristics between orchards. A total of 518 isolates were obtained and classified into 291 morphotypes that were grouped into 44 genera and 7 groups with common characteristics. Hutcheson's test showed that the Shannon-Wiener index of genera and groups of a single orchard is different to the others. The sterile mycelium group was the most abundant in four of the five orchards. Genera reported as phytopathogens (*Fusarium*, *Verticillium*), entomopathogens (*Beauveria*, *Paecilomyces*, *Metarhizium*) and mycoparasites (*Trichoderma*, *Gliocladium*) were identified. In addition, 28 new genera associated with avocado orchard soil are reported in this research.
Keywords: *Persea americana, ecological indices, ecological function, community compotition, fungal morphotypes*

Introduction

Mexico is the world's largest avocado (*Persea americana* Miller) producer and is the main exporter of avocado worldwide, bringing important foreign exchange into the country. As in other countries, in Mexico this crop faces several phytosanitary problems among which numerous species of fungi and oomycetes cause root rot, such as *Phytophthora cinnamomi* (Ochoa-Fuentes et al., 2015), *Phytopythium vexans* (Hernández et al., 2019), *Pythium amazonianum* (Ochoa et al., 2018), *Mortierella elongata* (Hernández et al., 2018), *Fusarium oxysporum* and *F. solani* (Olalde-Lira et al., 2020) and *Scytalidium* sp. (Solís-García et al., 2021). Also, several species cause trunk cankers (Ceja-Torres et al., 2020) and fruit damage (Picos-Muñoz et al., 2015; Trinidad-Ángel, et al., 2017; Fuentes-Aragón et al., 2020). In addition to these problems, avocado in Mexico

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faces the risk of fungi of quarantine importance such as *Fusarium ewallaceae*, *Paracremonium* (*Acremonium*) *pembeum* and *Graphium ewallaceae* transmitted by the ambrosial beetle *Euwallacea* nr. *fornicatus* (Lynch et al., 2016), and *Raffaelea lauricola* transmitted by *Xyleborus glabratus* (López-Buenfil et al., 2017).

In avocado orchards, several fungi and oomycetes have been identified from soil (Borneman and Hartin, 2000; Ruano-Rosa et al., 2014; Bonilla et al., 2015; Arjona-Girona and López-Herrera, 2018; Vega-Torres et al., 2019; Aguirre-von-Wobeser et al., 2021), bark (Aguirre-von-Wobeser et al., 2021), in various plant tissues as endophytes (Pérez-Martínez et al., 2018; Andrade-Hoyos et al., 2020) and even suspended in the air of avocado orchards (Valle-Aguirre et al., 2016). Soil shows the greatest diversity of microorganisms with highly complex interactions among them, where they perform diverse functions as organic matter degraders, pathogens of plants and other organisms, and fungal parasites. Although the study of the diversity of soil microorganisms has evolved greatly through the metagenomic studies, the classical methods of their study by culture techniques offer the advantage of isolating strains to investigate their potential in various areas of knowledge. In the avocado rhizosphere, up to 4,500 OTUs have been identified by metagenomic analysis (Solís-García et al., 2021) but only 25 genera of micromycetes have been identified in avocado orchard soil (Borneman and Hartin, 2000; Tofiño et al., 2012; Arjona-Girona and López-Herrera, 2018; Vega-Torres et al., 2019; Aguirre-von-Wobeser et al., 2021) using culture media. Among the latter, biocontrol agents have been identified, such as the entomopathogenic *Paecilomyces* and the mycoparasite *Trichoderma*, as well as the genus *Fusarium*, which contains phytopathogenic species (Tofiño et al., 2012).

The state of Nuevo León, located in northeastern Mexico, represents a small area planted with avocado in the country (677 ha; https://prod.senasica.gob.mx/SIRVEF/ContenidoPublico/Accion%20operativa/PTA/VEF/2019/Nuevo%20Leon.pdf). In this state, which has a great morphological and genetic diversity of avocados (Acosta et al., 2020), there is a lack of knowledge regarding the diversity of cultivable soil-culturable micromycetes fungi that can allow establishing the importance of these on the crop. In this research, the diversity of morphotypes and genera of cultivable micromycetes present in soils of avocado orchards in the state of Nuevo León, Mexico, and the similarity in fungal composition among orchards analyzed were determined, and the possible role of the main micromycetes identified in the avocado production system is discussed.

Materials and Methods

Study orchards

In 2020, soil samples were obtained from five avocado orchards at least 30 years of age, three in the month of August from the municipality of Aramberri (MA-1, MA-2, MA-3) where the predominant soil is Phaeozem, and two in the month of November from the municipality of Bustamante (MB-1, MB-2) where the predominant soil is Luvisol, both municipalities in the state of Nuevo Leon, Mexico. Bustamante is characterized by an average temperature of 21°C while Aramberri has an average temperature of 14°C. The georeferenced location of the orchards and the dates of collection are shown in *Table 1* and a satellite image with the location of the orchards is shown in *Fig. 1*. Sampling was carried out by collecting soil samples under the canopy of trees at a depth of 15 - 30 cm situated at one meter from the trunk, on a diagonal drawn arbitrarily in each

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orchard. The soil collected from each tree consisted of approximately 50 grams and was deposited in a single rubber bag for each orchard. The samples were transported to the Laboratory of Mycology and Phytopathology of the Faculty of Biological Sciences of the Autonomous University of Nuevo Leon.

Table 1. *Information on avocado orchards from which soil samples were obtained for isolation of micromycetes*

Municipality-orchard	Owner	Coordinates	Number of subsamples	Date of collection
Aramberri 1 (MA-1)	María Gordiano	24.019339, -99.788944	5	08/15/2020
Aramberri 2 (MA-2)	Huerta la Cabaña	24.055258, -99.804128	6	08/15/2020
Aramberri 3 (MA-3)	Lilia Villanueva	24.120603, -99.818822	5	08/15/2020
Bustamante 1 (MB-1)	Daniel Santos	26.538965, -100.514231	7	11/28/2020
Bustamante 2 (MB-2)	Daniel Santos	26.533027, -100.498780	5	11/28/2020

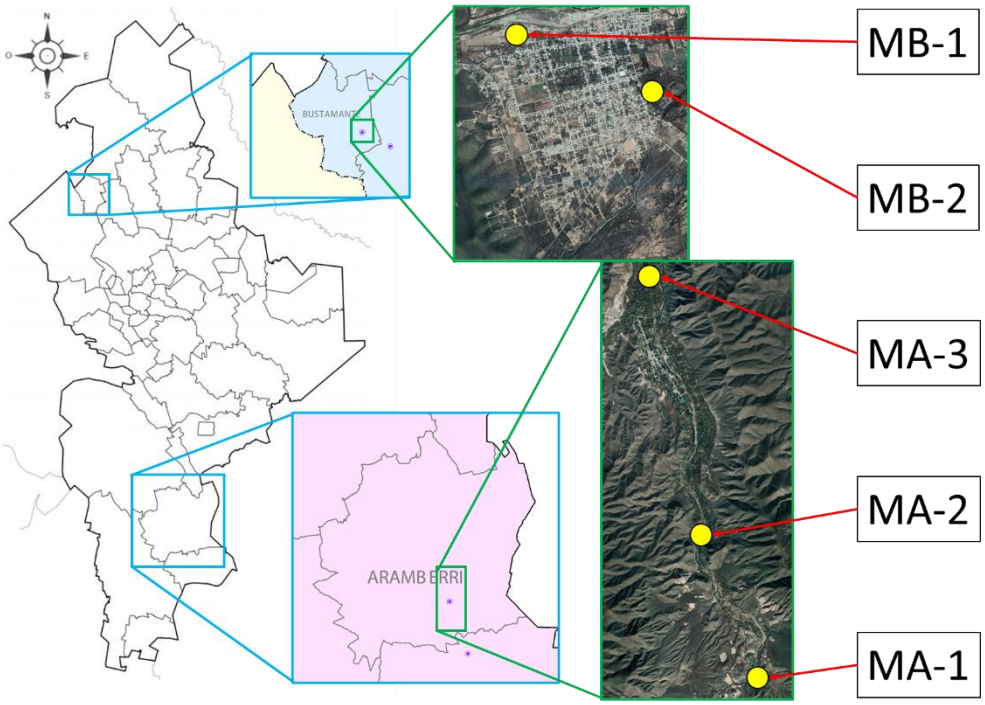

Figure 1. *Location of avocado orchards sampled in the municipalities of Bustamante and Aramberri, Nuevo Leon, Mexico*

Isolation of micromycetes

From each sample obtained from the orchards, four serial dilutions were made in sterile distilled water from one gram of soil (10^{-1} to 10^{-4}). From the last three dilutions, a volume of 50 µL was added to 20 Petri dishes containing acidified potato dextrose agar medium (200 µL L-1 of 85% lactic acid, added before pouring the medium into Petri dishes), and

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dispersed with a Digralsky loop. The Petri dishes were kept half-open to allow drying on the surface of the culture medium and closed. Petri dishes were opened at 24-48 hr and observed under a compound microscope; fungal microcolonies were extracted with an entomological needle attached to a dissecting needle and transferred to new Petri dishes with the same culture medium. The purity of the colonies was inspected during the following 72 hr.

Morphotypic characterization of micromycetes

From each orchard, the number of isolates was recorded and the initially subcultured microcolonies were visually characterized after seven days based on colony appearance and diameter, surface mycelial color, as well as the appearance and color of the lower part of the colony, color production on the substrate and by the microscopic appearance of hyphae at the edge of the colonies (Silva et al., 2013; Rodriguez-Guerra et al., 2020). Colonies that exhibited a particular set of characteristics were defined as morphotypes. One strain selected from the various morphotypes were identified to genus level using the keys of Barnett and Hunter (1998), and the strains that could not be identified were defined in groups based on common distinguishing characteristics. Morphotypes corresponding to the genus *Fusarium* were identified to species level using the keys of Nelson et al. (1983) and the *Fusarium* species description of Leslie and Summerell (2008). For identification, morphotypes were inoculated onto PDA and SNA (Papa Dextrose Agar; Spezieller Nahrstoffarmer Agar) media and incubated for 10 days. The cultures were observed to determine their macro- and microscopic characteristics. The percentage of species from each sampled orchard was obtained.

Morphotype and genus diversity

Shannon-Wiener (Shannon, 1984) and Simpson's diversity indices, Simpson's dominance and Margalef's richness indices were determined for morphotypes, and for the defined genera and groups, using the formulas:

Shannon-Wiener index: $H' = -\sum (pi \times \ln(pi))$

Simpson's diversity index: $1-\lambda = 1-\sum (p_i^2)$

Simpson's dominance index: $\lambda = \sum (p_i^2)$

Margalef's richness index: $DMg = (S-1) / \ln(N)$

where:

pi= Proportion of isolates of each morphotype, and of genera and groups with common characteristics with respect to the total number of isolates.

ln= Natural logarithm.

S= Total number of morphotypes or genera and groups evaluated.

N= Total number of isolates evaluated.

Also, diversity indices for genera and groups were compared (p= 0.05) among orchards using Hutcheson's t-test (1970), a test that has been used to compare diversity indices of samples from different sampling sites (Pitágoras et al., 2007), and of samples from different collection times (Silva-González et al., 2022). With the genera identified, it was established which ones have been previously reported as entomopathogens or biocontrol agents against phytopathogens. Additionally, the literature was reviewed to establish which identified genera have been reported as phytopathogens on avocado.

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Results

A total of 518 fungal isolates were obtained from the five orchards sampled, varying from 88 (MA-3) to 114 (MA-2) between orchards. Of the isolates obtained, 291 morphotypes were determined, with the number of morphotypes varying from 46 (MB-2) to 71 (MA-2) between orchards; the morphotypes determined from each orchard are unique to each orchard, since the relationship of common morphotypes between orchards was not established. A sample of the morphotype diversity present in orchard MA-1 is presented in *Fig. 2*.

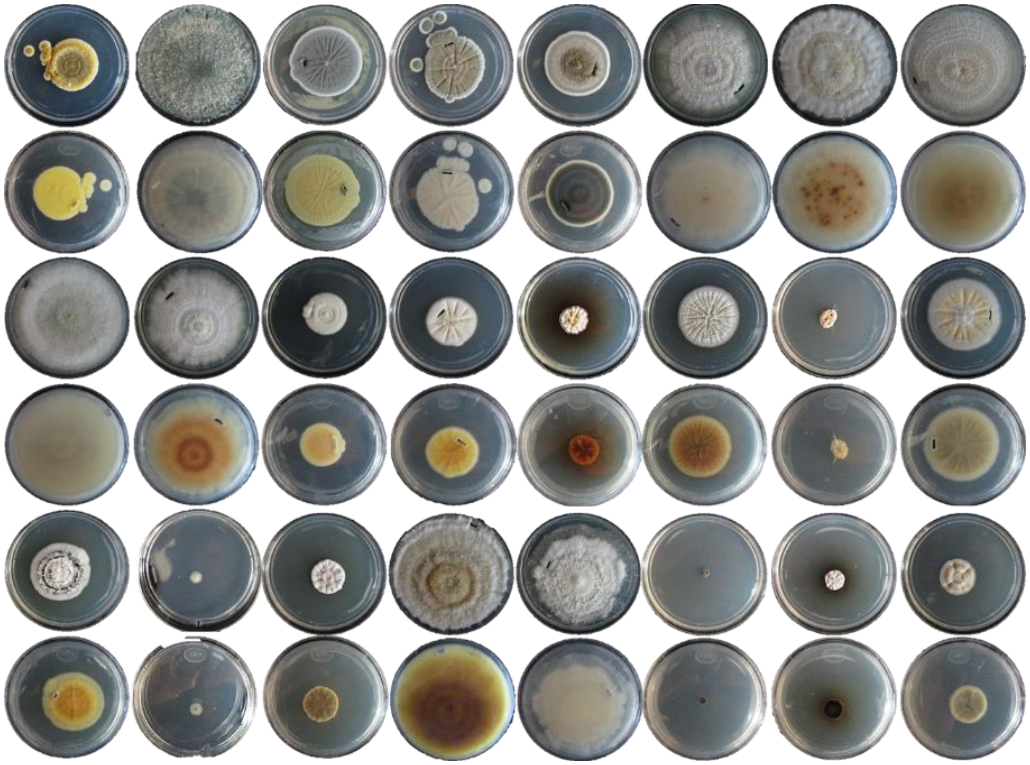

Figure 2. *Sample of morphotypes isolated from sample one from Aramberri (MA-1). Frontal view (rows 1, 3 and 5) and reverse view (rows 2, 4 and 6) of the colonies*

The diversity found in the morphotypes was greater than when genera and defined groups were studied, because in the latter case the isolates were grouped in larger clusters (*Table 2*). The Shannon-Wiener and Simpson diversity indices and the Margalef richness index with respect to the morphotypes found ranged from 3.77 to 4.18, 0.976 to 0.984 and from 9.57 to 14.78 in orchards MB-2 and MA-2, respectively. For genera and groups, the same diversity indices ranged from 2.46 to 2.93 and 0.881 to 0.928 in MA-3 and MA-2 orchards, respectively, while Margalef richness ranged from 3.62 to 5.70 in MB-2 and MA-2 orchards.

Considering the total number of genera and groups of fungi present in each municipality, in orchard soils of the municipality of Aramberri there is the highest richness (6.95) with 41 genera and groups with respect to the municipality of Bustamante (5.08) with 28 genera and groups (*Table 3*). In addition, ten genera and groups occur exclusively in Bustamante, while 23 occur in Aramberri; and only 18 are shared between both municipalities (*Table 4*).

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Table 2. *Distribution of morphotypes and genus and groups of micromycetes isolated from avocado orchard soils and indices obtained in each orchard analyzed*

	MA-1	**MA-2**	**MA-3**	**MB-1**	**MB-2**
Number of isolates	113	114	88	93	110
Number of morphotypes	62	71	63	49	46
Number of genera and groups	24	28	18	20	18
Indices for morphotypes					
Shannon-Wiener Diversity Index	4.04	4.18	4.08	3.83	3.77
Simpson's dominance	0.019	0.016	0.018	0.023	0.024
Simpson's diversity index	0.981	0.984	0.982	0.977	0.976
Margalef's richness index	12.90	14.78	13.85	10.59	9.57
Indices for genera and groups with common characteristics					
Shannon-Wiener Diversity Index	2.67	2.93	2.46	2.63	2.51
Simpson's dominance	0.105	0.072	0.119	0.103	0.101
Simpson's Diversity Index	0.895	0.928	0.881	0.897	0.899
Margalef's richness index	4.86	5.70	3.80	4.19	3.62

Table 3. *Distribution of morphotypes and genus and groups of micromycetes isolated from avocado orchard soils and indices obtained in each orchard analyzed*

	Aramberri	**Bustamante**
Number of isolates	315	203
Genera and groups	41	28
Shannon-Wiener diversity index	2.99	2.76
Simpson's dominance	0.080	0.092
Simpson's diversity index	0.920	0.908
Margalef's richness index	6.95	5.08

Of the 291 characterized morphotypes, 225 (77.3%) were identified to genus level, while 66 (22.7%) were distributed in seven defined groups of fungi. The total number of genera identified in the orchards of both municipalities was 44, and the morphotypes that could not be identified to genus level were included in 7 groups defined based on common characteristics in each (*Table 4*). Of the latter, in the groups of fungi with aleuriospora, arthrospora, sporodochia, and the glomeromycete group, the characteristics of their structures did not agree with any genus included in the keys used. In addition, in the sterile mycelium group, no hyphal fibulae were observed to suggest that any morphotype corresponded to basidiomycetes, or characteristics that could suggest their identity to genera such as *Rhizoctonia*, *Macrophomina* and *Sclerotinia*. In addition, of the 43 morphotypes present among the 68 isolates obtained from the genus *Fusarium*, 32 were identified. Sixty-nine percent of the morphotypes were identified as *F. solani*, 25% as *F. oxysporum* and 3% as *F. equiseti* and *F. semitectum*.

The distribution of identified genera and defined groups of fungi in orchards of each municipality is presented in *Fig. 3*. In the municipality of Aramberri, 41 genera and groups were determined, of which 21.9% (9) were distributed in the three sampled orchards and 12.2% (5), 26.8% (11) and 12.2% (5) were exclusive to MA-1, MA-2 and MA-3 respectively, and the rest were shared between pairs of orchards. While in Bustamante 28 genera and groups occurred, of which 35.7% (10) occurred in both orchards and 35.7% (10) and 28.6% (8) were exclusive to MB-1 and MB-2.

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Table 4. *Number of isolates/morphotypes of fungal genera and groups with common characteristics in avocado orchards in the state of Nuevo León*

Orchard	**MA-1**	**MA-2**	**MA-3**	**MB-1**	**MB-2**	**Orchard**	**MA-1**	**MA-2**	**MA-3**	**MB-1**	**MB-2**
Genus						Genus					
Aposphaeria					1/1	*Paecilomyces*	1/1	3/2	3/2	2/1	2/1
Aspergillus	7/3	11/5	5/3	8/4	11/5	*Penicillium*	4/3	5/2	9/5	7/3	13/5
Aureobasidium				2/1		*Phialophora*			2/1		
Beauveria	1/1	1/1		2/1		*Periconia*		2/1			
Bipolaris		5/2				*Pestalotia*	5/2	2/2			
Botryotrichum					1/1	*Pseudotorula*	2/1				
Cephalosporium	14/6	14/9	5/3	7/4	5/2	*Pyrenochaeta*	2/1				
Chaetomium		1/1	3/2			*Robillarda*		2/1		2/1	
Cladosporium	4/2	3/2		9/4	15/5	*Sporothrix*		1/1			
Colletotrichum			1/1			*Stachybotrys*	2/1	6/4			
Cylindrocarpon		2/1				*Synnematium*					3/1
Cylindrocladium	2/1	1/1				*Trichocladium*	3/1		1/1		7/3
Curvularia				3/1		*Trichoderma*	3/1	2/2	5/4	3/2	1/1
Diplosporium			2/1			*Tritirachium*				3/1	
Doratomyces		4/2				*Verticillium*	1/1	1/1		2/1	3/1
Epicoccum					3/1	*Wallemia*		1/1			
Fusarium	18/11	10/7	22/17	3/2	15/6	Total genera	21	24	14	16	17
Gliocladium	5/3	8/4		3/2	6/3						
Gliomastix		2/1									
Graphium					3/1	Group					
Heterosporium	2/1					Aleuriospores	2/1		3/3	5/3	
Humicola	2/2	3/3	13/9	1/1		Arthrospores		2/1		1/1	
Hymenella		1/1				Ascomycete			1/1		
Illosporium	2/2			2/1		Sporodochia		2/2			
Metarhizium	3/1		3/2			Yeast				5/3	
Microsporum			1/1			Sterile mycelium	25/13	17/10	8/6	23/12	18/7
Monocillium	2/2				2/1	Glomeromiceto	1/1	2/1	1/1		
Nodulosporium					1/1	Total groups	3	4	4	4	1

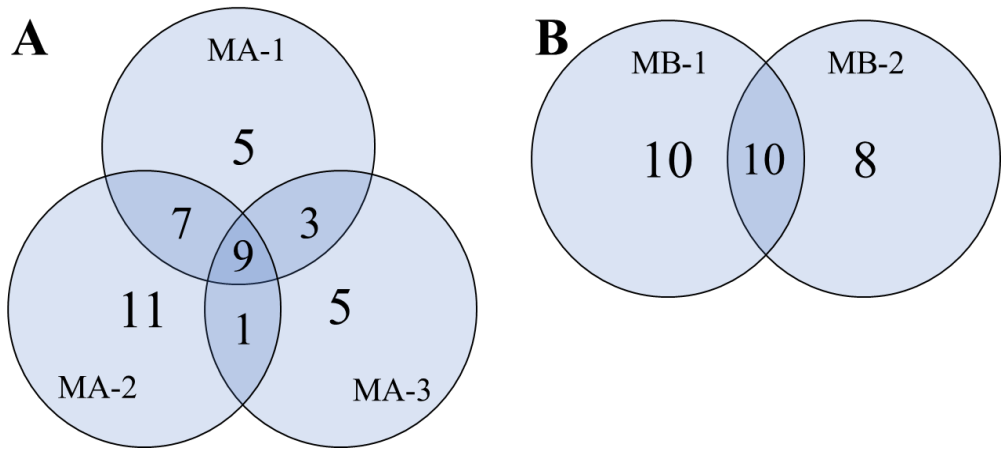

Figure 3. *Distribution of genera and groups in sampled orchards in the municipalities of Aramberri (A) and Bustamante (B)*

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The Shannon-Wiener diversity index for genera and groups ranged from 2.46 (MA-3) to 2.93 (MA-2), while for morphotypes, it ranged from 3.77 (MB-2) to 4.18 (MA-2) and although Simpson's dominance index shows that the orchards lack dominant genera or groups (*Table 2*), those genera and groups that presented an amount of isolates equal to or greater than 10% of the total present in each orchard, or of the total in the five orchards, were considered the most predominant. In the MA-1 orchard, sterile mycelium, *Fusarium* and *Cephalosporium* predominated, in the MA-2 orchard sterile mycelium and *Cephalosporium*, in MA-3 *Fusarium*, *Humicola* and *Penicillium*, in MB-1 sterile mycelium and in MB-2 sterile mycelium, *Fusarium*, *Cladosporium*, *Penicillium* and *Aspergillus*. The predominant genera and groups considering the five orchards were sterile mycelium (91 isolates) and *Fusarium* (68 isolates); other genera that were abundant were *Cephalosporium* (45 isolates), *Aspergillus* (42 isolates) and *Penicillium* (38 isolates). Six fungal genera (*Aspergillus*, *Cephalosporium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Trichoderma*) and the sterile mycelium group occurred commonly in the five orchards of both municipalities. The group determined as glomeromycete is only represented in the three orchards of Aramberri; no other genus or group is represented exclusively in the three orchards of Aramberri and absent in both samples of Bustamante, and vice versa.

Of the genera identified, three of them (*Beauveria*, *Metharrizium* and *Paecillomyces*) are recognized to contain entomopathogenic species; while *Trichoderma* and *Gliocladium* contain species used as biocontrol agents against phytopathogenic fungi.

According to Hutcheson's test (p= 0.05), it was found that the Shannon-Wiener diversity index of orchard MA-2 is the only one that presents significant differences compared to the indices of the other orchards of Aramberri and Bustamante (*Table 5*).

Table 5. *Hutcheson's t-test (p= 0.05) between Shannon-Wiener diversity indices for genera and defined groups of the sampled orchards*

		MA-1	**MA-2**	**MA-3**	**MB-1**	**MB-2**
Orchard	Shannon-Wiener Diversity Index	2.67	2.93	2.46	2.63	2.51
MA-1	2.67					
MA-2	2.93	*				
MA-3	2.46	ns	*			
MB-1	2.63	ns	*	ns		
MB-2	2.51	ns	*	ns	ns	

Discussion

The study of fungal diversity is useful to establish relationships of their composition in natural ecosystems and those altered by human activity, as well as to know their distribution, abundance, and role in agroecosystems. In this study, the largest number of cultivable fungal isolates were acquired from avocado orchards soil in the state of Nuevo León. The diversity of morphotypes and genera of the micromycetes in Nuevo León were obtained. Of the 518 isolates obtained, 291 morphotypes were defined, although it was not possible to establish the relationship of the morphotypes among the different orchards because it was required to have all the isolates growing simultaneously, and to be evaluated at the same time to establish common morphotypes among them. However, the variation in the number of culturable isolates obtained from each orchard was because it

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was considered that the greatest diversity of microcolonies in each orchard had been obtained in the time established for their isolation. It is not discarded that some cultivable micromycetes could have the capacity to germinate and form microcolonies after 48 hr or have special requirements for their growth, as has been considered by Filion et al. (2003). In Mexico, there is little research aimed at studying the diversity of micromycetes in avocado orchards. Solís-García et al. (2021) conducted a study in an orchard in the state of Veracruz in which they determined the effect of symptomatic and asymptomatic trees by root rot due to *Phytophthora* on the microbiome of the avocado rhizosphere, through a metagenomic analysis of the soil; while Valle-Aguirre et al. (2016) morphologically identified fungi present in the air of orchards in the state of Morelos. Likewise, the search for biocontrol agents in avocado orchard soil in the state of Nayarit has also been directed (Vega-Torres et al., 2019).

The diversity indices observed in this research are lower for defined genera and groups with common characteristics than for isolated fungal morphotypes, the above demonstrates that great variation occurs in terms of morphological characteristics of isolates belonging to the same genus or group (*Table 3*). This is commonly observed when performing morphotypic characterizations of fungi, as has been observed by Solís-García et al. (2021) who from 46 fungal isolates obtained from avocado roots determined 10 morphotypes that corresponded to only four genera. However, it has also been reported that isolates of different morphotypes can be taxonomically identical using restriction patterns of internal transcribed rDNA spacers (Watrud et al., 2006).

The Shannon-Wiener diversity indices obtained for genera and groups in the five orchards under study are lower than those obtained by Solís-García et al. (2021), who by analysis of fungal operational taxonomic units, determined by analysis of internal transcribed spacer sequences (ITS) of soil rDNA from avocado trees symptomatic and asymptomatic for root rot, found indices of 4.56 and 4.81, respectively, in the state of Veracruz. This shows that metagenomic analysis allows defining the occurrence of greater diversity of major fungal taxa in soil with respect to the traditional procedure carried out here; however, in this study a bank of culturable micromycetes was developed with 291 isolates representing each morphotype and with which it is possible to carry out diverse studies that allow their use in the avocado production system.

The greater diversity (Shannon-Wiener: 2.99; Simpson: 0.92) and richness (6.95) of genera and groups, as well as the greater number of genera and exclusive groups (23) in the municipality of Aramberri with respect to the municipality of Bustamante, may be influenced by the different weather conditions present. This is supported since the greatest diversity of fungi in soil occurs in temperate or tropical zones with respect to arid or very arid zones (Aguirre-Acosta et al., 2014; Maestre et al., 2015); in that regard, Aramberri has a temperate climate while in Bustamante it is mainly arid.

Of the 44 genera identified in this study, 16 (*Aspergillus*, *Beauveria*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Colletotrichum*, *Epicoccum*, *Fusarium*, *Humicola*, *Paecilomyces*, *Penicillium*, *Pestalotia*, *Trichocladium*, *Trichoderma*, *Tritirachium*, *Verticillium*) have been previously recorded from metagenomic analysis or by isolation from soil samples (Borneman and Hartin, 2000; Violi et al., 2007; Tofiño et al., 2012; Arjona-Girona and López-Herrera, 2018; Aguirre-von-Wobeser et al., 2021), roots (López-Herrera and Melero-Vara, 1991; Solís-García et al., 2021), leaves in soil (Borneman and Hartin, 2000), air (Valle-Aguirre et al., 2016), bark (Aguirre-von-Wobeser et al., 2021) and as endophytes in various plant tissues (Pérez-Martínez et al., 2018) in avocado orchards. The results of this research allow the inclusion of 28 new

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genera of cultivable micromycetes associated with avocado orchard soil to those previously reported by previous authors. However, it is possible that among the 66 morphotypes in the seven defined groups of fungi, new genera may be found. Among these groups, the most abundant and distributed in the orchards of both municipalities is that of sterile mycelium with 91 isolates and 48 morphotypes, and none of these was recognized as *Rhizoctonia* (characterized by right angles in the ramifications of its hyphae), the only genus of sterile mycelium that has been reported only in roots of avocado plants with wilt symptoms (López-Herrera and Melero-Vara, 1991). This suggests that the analysis of DNA sequences of fungi that do not produce spores as a means of reproduction in culture media may increase the diversity of cultivable mycelia associated with the avocado agroecosystem.

Molecular identification of fungal species is currently based on the sequencing of several *loci*, which vary depending on the genus to which they belong. In this study, we limited ourselves to morphological characterization and identification due to the considerable number of isolates obtained (518) which would have to be subjected to identification based on the analysis of numerous DNA loci from 44 genera and 7 groups.

Six genera (*Fusarium*, *Cephalosporium*, *Aspergillus*, *Penicillium*, *Paecilomyces*, *Trichoderma*) occurred commonly in the orchards of both municipalities, and the most abundant among the five orchards under study were *Fusarium* (68 isolates), *Cephalosporium* (45 isolates), *Aspergillus* (42 isolates) and *Penicillium* (38 isolates). Twenty-four genera and three groups with common characteristics were represented by less than five isolates in any of the five orchards under study. The abundance of *Fusarium*, *Aspergillus* and *Penicillium* has also been observed in orchard soils in the state of Michoacán (Aguirre-von-Wobeser et al., 2021), suggesting that they may be performing some function in the avocado agroecosystem. Of these genera, *Fusarium* is recognized for having economically important species that cause root rot in numerous crops, including avocado (Olalde-Lira et al., 2020); and *Verticillium* is reported as a root pathogen of avocado (Pérez-Jiménez, 2008). The high frequency of *Fusarium* isolates observed in soil, and the presence of *F. solani* and *F. oxysporum* species, could suggest their association as avocado root pathogens in the orchards analyzed, as reported by Olalde-Lira et al. (2020). In addition, strains of *F. solani*, *F. equiseti* and *Fusarium* spp. isolated from avocado roots (Solís-García et al., 2021) and *F. oxysporum* isolated from stems (Ceja-Torres et al., 2000) can cause stem necrosis when artificially inoculated.

The observed abundance of the genera *Aspergillus* and *Penicillium* agrees with research conducted in various soils (Gomez et al., 2007; Zhou et al., 2014), although in others both genera have been reported to be widely distributed, but in low frequency (Zhong et al., 2022); furthermore, species of these genera are considered to play a role in phosphate solubilization in soil (Elias et al., 2016). The genus *Cephalosporium* (currently accepted *Acremonium*) is considered as a saprobe in soil and plant debris (Park et al., 2017) and some species cause diseases in humans and plants (Ortiz-Bustos et al., 2015; Summerbell et al., 2018); however, like *Fusarium*, *Aspergillus* and *Penicillium*, their identification to species level or relationship to genera, families and even orders has changed based on phylogenetic analyses of sequences from diverse *loci* (Summerbell et al., 2011; Gräfenhan et al., 2011; Houbraken et al., 2020; Crous et al., 2021). Other genera such as *Beauveria*, *Gliocladium* and *Metarhizium*, as well as *Paecilomyces* and *Trichoderma* present in the five orchards, are considered among the main biocontrol agents against pests and phytopathogenic fungi (Baron et al., 2019; Thambugala et al., 2020). These strains may be potentially used in avocado production systems for the

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control of phytosanitary problems they face. Although a great diversity of fungal genera was found in this study and some of these are common in several study sites, it is possible that each of them is performing a particular and different ecological function in each of them.

The results obtained demonstrate the existence of great diversity of cultivable fungal genera present in avocado orchard soils in small production areas in the state of Nuevo León, Mexico. It is important to implement these studies in other regions of the country and other countries where avocado is produced, in order to have more information on the diversity of mycromycetes present and to have strains of potential use and natural resources to improve their production systems.

Conclusions

In this research, 44 genera were identified, of which 28 are new genera of cultivable micromycetes associated with avocado orchard soil, and 7 groups of fungi, from 291 morphotypes determined. Among the fungal genera identified, 16 are common in other avocado orchards, showing a close relationship with this crop, of which some may contain phytopathogenic, entomopathogenic and mycoparasitic species, most of which are distributed in the orchards under study in both regions. It is considered that this study represents the most important contribution to the diversity at the genus level of cultivable micromycetes present in avocado orchard soils in Mexico.

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