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Arbuscular Mycorrhizal Fungi Consortia in Six Vegetable Crops in the Tepeaca Valley, Puebla, Mexico
Consorcios de Hongos Micorrízicos Arbusculares en Seis Cultivos de Hortalizas en el Valle de Tepeaca, Puebla, México

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SUMMARY

The objectives of this work were to search for and evaluate arbuscular mycorrhizal fungi (AMF) in six crops of agricultural importance in the horticultural region of Tepeaca valley, Puebla, Mexico, where Cambisols type soil predominates since the communities and consortia of AMF and their functionality in various crops of economic importance in Mexico are still unknown. The search for and identification of AMF spores was carried out in different vegetable crops. Five subsamples were collected from 6 different crop fields with vegetables (cilantro, cabbage, spinach, parsley, lettuce, and pumpkin); each subsample consisted of soil and roots. Roots 5 mm long were selected and stained using the Phillips-Hayman technique. Spore extractions and physicochemical determinations (soil texture, pH, relative humidity (RH), and electrical conductivity (EC)) were performed from soil. The results obtained from staining showed vesicles and spores in roots except in the pumpkin subsamples; of spore extractions from the soil of the six crops, 9 genera and 14 morphotypes of mycorrhizae were found, highlighting *Funneliformis mosseae*, *Claroideoglomus Claroideum,* and *Glomus* sp. as dominant species. By soil physicochemical characterizations were found: sandy texture, pH between 5.8 to 6.8; RH of 9.4 to 13%, and EC of 0.10 to 0.93 mS cm^{-1}. In conclusion, the analyses of similarity and main components suggested that species of vegetables and soil conditions do not affect the spore abundance, but they do affect the richness and composition of AMF species, observing particular mycorrhizal consortia for each crop; therefore, it is suggested to evaluate their effectiveness in each of the crops to promote them as biofertilizer in the agricultural region of Tepeaca valley, Puebla, Mexico.

Index words: *agroecosystems richness, cambisols type soil, glomeromycota, horticultural systems, plant-fungus association.*

RESUMEN

Los objetivos de este trabajo fueron buscar y evaluar hongos micorrízicos arbusculares (HMA) en seis cultivos de importancia agrícola en la región hortícola del valle de Tepeaca, Puebla, México, donde predomina suelo tipo Cambisoles, ya que las comunidades y consorcios de HMA y su funcionalidad en diversos cultivos de importancia económica en México aún se desconocen. La búsqueda e identificación de esporas de HMA fueron realizadas en diferentes cultivos de hortalizas. Se recolectaron cinco submuestras de 6 campos de cultivo diferentes con

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hortalizas (cilantro, repollo, espinaca, perejil, lechuga y calabaza); cada submuestra consistió en suelo y raíces. Se seleccionaron raíces de 5 mm de largo y se tiñeron mediante la técnica de Phillips-Hayman. Se realizaron extracciones de esporas y determinaciones fisicoquímicas (textura del suelo, pH, humedad relativa (HR) y conductividad eléctrica (CE)) del suelo. Los resultados obtenidos de la tinción mostraron vesículas y esporas en raíces excepto en las submuestras de calabaza; de las extracciones de esporas del suelo de los seis cultivos se encontraron 9 géneros y 14 morfotipos de micorrizas, destacando *Funneliformis mosseae, Claroideoglomus Claroideum* y *Glomus* sp. como especie dominante. Mediante caracterizaciones fisicoquímicas del suelo se encontraron: textura arenosa, pH entre 5.8 a 6.8; HR de 9.4 a 13% y CE de 0.10 a 0.93 mS cm^{-1}. En conclusiones, los análisis de similitud y componentes principales sugirieron que las especies de hortalizas y las condiciones del suelo no afectan la abundancia de esporas, pero sí la riqueza y composición de especies de HMA, observándose consorcios micorrícicos particulares para cada cultivo; por lo que se sugiere evaluar su efectividad en cada uno de los cultivos para promoverlos como biofertilizantes en la región agrícola del valle de Tepeaca, Puebla, México.

Palabras clave: *riqueza de agroecosistemas, suelo tipo cambisoles, glomeromycota, sistemas hortícolas, asociación planta-hongo*.

INTRODUCTION

At the national level, the Puebla State stands out as the main producer of vegetables, occupying first place in the production of coriander since it produces 22 thousand tons per year by planting (3499 ha) generating a monetary production value of $70 629 000.00; spinach occupies the second place with (428 ha), in third place is finds lettuce at the national level with a total of 66 501 tons and parsley, with a total production of 814 tons and pumpkin in fifth with a total production of 2791 tons (SIAP, 2022). For all the above, strategies that do not harm and help to improve the environment and improve food quality are needed, such as the use of biofertilizers (Montes de Oca, 2015).

The arbuscular mycorrhiza is one of the most interesting and important symbioses in agricultural systems due to its potential to improve the quality, growth, and nutrition of a variety of crops such as corn, beans, chili, papaya, coffee, and various fruit trees; however, there is still a profound lack of knowledge of the communities and consortia of arbuscular mycorrhizal fungi (AMF, Glomeromycota) and their functionalities in various crops of economic importance for Mexico (Alarcón, Hernández, Ferrera-Cerrato, and Franco, 2012). It is recognized that AMF, when associated with plant roots, facilitates the absorption and access to micro and macronutrients of low availability and mobility in the soil, such as phosphorus, nitrogen, copper, and zinc; while plants provide AMF with carbohydrates and lipids for their vital functions to complement their life cycle (Smith and Read, 2008; Keymer *et al*., 2017). The use of biofertilizers or bioinoculants based on beneficial microorganisms worldwide has been seen as an alternative to agrochemicals, which have a negative impact on soil fertility and health (Alarcón *et al*., 2012; Hart, Antunes, Chaudhary, and Abbott, 2017). Given the growing demand for agricultural foods, various strategies have been developed at the national level to improve the productivity of the Mexican countryside, one of them is the use of biofertilizers based on fungi that form the arbuscular mycorrhiza; For example, the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) with the support of the National Program of Biofertilizers of the Federal Government, have observed the efficiency of the inoculation of *Glomus intraradices* in *Sorgum vulgare var. sudanensis* (Aguirre-Medina, 2008). However, it is recognized that a single AMF species is not necessarily the most efficient for a wide diversity of agricultural plants that are cultivated under different cultivation practices or climatic and edaphic conditions throughout the mexican territory (Alarcón *et al*., 2012; Hart *et al.,* 2017). Therefore, the selection of mycorrhizal consortia with native AMF species is recommended for the preparation of inoculants that promote plant growth and health (Alarcón *et al*., 2012; Trejo *et al*., 2011).

In this regard, Trejo *et al*. (2011) evaluated the effectiveness of seven arbuscular mycorrhizal consortia in the coffee zone of Veracruz, and they observed that the most effective AMF consortia in promoting the height and survival of coffee plants under field conditions were those that came from agroecosystems with a medium level of technology since it correlated with a greater richness of AMF species. Likewise, Carballar-Hernández *et al*. (2017)

reported variations in the native communities of AMF associated with chile Poblano crops in the region of Puebla, Mexico; with a significant change in the composition of fungal species depending on agronomic management and soil properties. The foregoing suggests that it is essential to recognize what are the agricultural practices, the intensity, and the input of chemical fertilizers in certain crops, where there is an interest in knowing the AMF communities for the purpose of selecting effective and potential mycorrhizal consortia in agriculture in Mexico (Alarcón *et al*., 2012; Trejo *et al*., 2011). Therefore, the objectives of this research were: i) to evaluate the presence or absence of arbuscular mycorrhizal colonization in six vegetable crops (spinach, cilantro, cabbage, pumpkin, lettuce, and parsley) in the Tepeaca Valley, Puebla, Mexico; ii) determine the arbuscular mycorrhizal consortia associated with each agricultural crop and iii) explore the relationship of AMF communities with soil properties in each crop due to the lack of information in this regard.

MATERIALS AND METHODS

Agricultural Region of Study

This research was carried out in the Tepeaca Valley, located in the state of Puebla. The map of the Mexican Republic indicates that the Tepeaca valley is located at the central part of the state of Puebla. Due to its geographical position, it is located between coordinates 18° 55′ 30″ and 19° 06′ 18″ N of the Tropic of Cancer and between 97° 48′ 18″ and 97° 59′ 18″ longitude west of the Greenwich Meridian. The relief of the Tepeaca Valley is mountainous, with hills and mountains. All this causes the valley to have an altitude of 2080 and 2840 meters. The Tepeaca valley is characterized by having a temperate sub-humid climate, an average temperature of 12 to 18 °C throughout the year, and the dominant type of soil in the region is Cambisols suitable for agricultural activities, it has a territorial extension of 125 976 hectares and consists of 14 municipalities: Acatzingo, Quecholac, Acajete, Los Reyes de Juárez, Tepeaca, Tepeyahualco de Cuauhtémoc, Amozoc, Tecamacalcho, San Salvador Huixcolotla, Tecali de Herrera, Cuautinchan, Tochtepec, Tlanepantla, and General Felipe Angeles.

Working Farm Field

In the agricultural zone of Acatzingo, Puebla, two samplings were carried out on April 26 and August 17 2017, the important agricultural season of the state. On each date, 5 subsamples of roots and soil near the rhizosphere were randomly collected from spinach (*Spinacia oleracea* L.), coriander (*Coriandrum sativum* L.), cabbage (*Brassica oleracea* L.), pumpkin (*Cucurbita pepo* L.), lettuce (*Lactuca sativa var. capitata* L.) and parsley (*Petroselinum sativum* Hoffm. ex Gaudin). The plants with their roots and soil were placed in polyethylene bags and labelled for transport to the laboratory, where they were kept under refrigeration (4 °C) for conservation until processing.

Root Staining

To evaluate the presence of arbuscular mycorrhizal colonization in the plants of the six crops, a root staining process was carried out to highlight the AMF structures, using the Phillips-Hayman technique (Phillips and Hayman, 1970), of spinach, cilantro, cabbage, pumpkin, lettuce, and parsley. For this, secondary roots were cut from each of the collected plants into 2.0 cm long fragments and placed in vials with a 10% potassium hydroxide (KOH) solution until the roots were covered and placed in an autoclave for 10 min at 15 Lb of pressure and 120 °C to eliminate the cellular content and the radical pigments; at the end of the time, the KOH was removed and washed with sufficient water, then 1% hydrochloric acid (HCl) was added until the roots were covered and it was allowed to acidify for 5 min. Finally, they were stained with acid fuchsin and trypan blue, leaving them to act for 24 hours.

Presence of Arbuscular Mycorrhizal Colonization

At the end of the staining time, the fragments of the roots of each plant were placed on slides of 4 to 5 pieces depending on the thickness of the root, a drop of glycerol was added and placed on a coverslip, and the observation was made of the structures in an optical microscope. The observation consisted of recording the structures of the AMF for the evaluation of the percentage of arbuscular colonization of the samples (Hernández-Cuevas and García-Sánchez, 2008).

Extraction of Spores From AMF

Using the wet sieving and decantation method (Gerdeman and Nicolson, 1963) followed by centrifugation with a sucrose solution (Hernández-Cuevas and García-Sánchez, 2008), the extraction of AMF spores from each of the soil samples that were previously sieved and air-dried. The procedure consisted of weighing 50 g of soil that was placed in a glass with approximately 400 mL of water and mechanically stirred for 5 min; it was allowed to stand for 10 s to allow the sedimentation of particles such as sand, and later the suspension was decanted in a series of sieves with a mesh opening of 44 105 and 1000 μm. The fractions obtained in the 105 and 44 μm sieves were added to tubes to be centrifuged with water at 2000 rpm for 5 min; subsequently, the supernatant of each tube (dead spores and traces of organic matter) was removed. The sample retained in the tube was resuspended in a 60% sucrose solution and centrifuged at 1000 rpm for 5 min to remove the mineral component, in this way, the sucrose solution was decanted on a 44 μm sieve, was washed with water, and recovered in a Petri dish with distilled water. With the help of a dissection microscope, the extraction, counting, and selection of AMF spores were carried out, which were mounted in permanent preparations in polyvinyl alcohol in lactoglycerol (PVLG) and in a mixture of PVLG and Melzer's reagent (1:1) for subsequent taxonomic determination (Hernández-Cuevas and García-Sánchez, 2008).

Taxonomic Determination of AMF

The permanent slides with the isolated, grouped, and selected AMF spores from each individual and culture species were reviewed under an optical microscope with Nomarski illumination, which allowed a record of the morphological characteristics of the AMF spores such as colour, size, the presence/absence of hyphae, the types of hyphae, the number of layers of the spore wall, the presence/absence of ornamentations, the reaction or not to the Melzer reagent of the layers of the spore wall and the presence of specific structures such as germination shields. Together, these morphological characteristics allowed a comparison with the AMF species described worldwide (Invam, 2023; Amf-phylogeny, 2023; Zor zut Edu, 2023).

Physicochemical Analyses of Soil

All the physicochemical analyses of soil were carried out following the Norma Oficial Mexicana NOM-021-RECNAT-2000 (NOM-021-SEMARNAT-2000, 2002), which establishes the specifications for the study, sampling, and analysis of fertility, salinity, and soil classification (NOM-021-SEMARNAT-2000, 2002). To determine the texture of the soil, it was carried out using the Bouyoucos technique which consisted of weighing 60 g of soil with 40 mL of hydrogen peroxide (H_2O_2), the sample was left to dry for 24 h at 80 °C and subsequently 50 g were weighed, at which 5 mL of sodium oxalate ($Na_2C_2O_4$) and 5 mL of sodium metasilicate (Na_2SiO_3) were added and allowed to stand for 15 min. The sample was mixed for 5 min and placed in a 1000 mL test tube, which was calibrated to the specified volume with distilled water. After this, the first reading was made at 40 s and a second reading at 2 h with a Hanna brand hydrometer. The correction of the readings was made based on the temperature of the experiment (Navarro-García and Navarro, 2013). The following Equation was used for the determination of soil texture.

$$\%\, silt + clay = \frac{Corrected\ first\ reading}{sample\ weight} \times 100 \tag{1}$$

$$\%\ \text{sand} = 100 - \%\ \text{silt} + \text{clay} \tag{2}$$

$$\%\ \text{silt} = 100 - \%\ \text{sand} + \text{clay} \tag{3}$$

The determination of the Relative Humidity (RH) of the soil was carried out by means of the gravimetric procedure; for which 10 g of fresh soil was weighed and dried in an oven at 80 °C for 24 h. After this time, the sample was weighed again, and this procedure was repeated until constant weights were obtained in the samples. For the determination of the RH, the following formula was used:

$$\text{Humidity (H)} = (\text{wet soil weight}) - (\text{dry soil weight}) \tag{4}$$

$$\%RH = \frac{H \times 100}{10} \tag{5}$$

Determination of the Water Retention Capacity (WRC)

10 g of oven-dried soil was weighed and placed in a crucible with a screened base, then the sample was hydrated until the hydrated surface was observed, and the excess water was drained by the effect of gravity and weighed. The WRC was expressed as a percentage.

Retained water = Weight of crucible with soil saturated with water) - (Weight of crucible with unsaturated soil)

$$\%WRC = \frac{Retained\ water \times 100}{10} \tag{6}$$

The determination of soil pH (1:2) was carried out by weighing 10 g of soil in a beaker and 20 mL of distilled water were added and mixed for 1 min, left to stand for 30 min and after the time, the pH reading with a previously calibrated potentiometer.

The determination of Electrical Conductivity (EC) was made by weighing 20 g of dry soil, each soil sample was placed in a plastic beaker with 40 mL of distilled water, mixed for 30 s, and allowed to stand for one hour. EC readings were made with previously calibrated HI 76304 plastic cover electrodes.

Data Analysis

The variable abundance of arbuscular mycorrhizal fungi spores in the six vegetable crops was first analysed with a Shapiro-Wilk test to evaluate the normality of data and with a Levene test to evaluate the equality of variances; Since the data did not comply with normality, a non-parametric analysis of variance was applied with a single-factor Kruskal-Wallis test. The analyses were performed with the NCSS 2007 software (NCSS, 2007). To compare the similarity in the composition of AMF species in the six vegetable crops, a multivariate cluster analysis was applied to generate a similarity dendrogram with the Jaccard index and the presence/absence data of AMF species. Likewise, to determine if the generated dendrogram and the differences in the species composition in each crop were significantly different, a multivariate test of similarity analysis (ANOSIM) was applied by means of permutation (n = 9999) and a $P \leq 0.05$. This ANOSIM was carried out with the PAST software version 3.14 (Hammer, Harper, and Ryan, 2001).

In order to explore the relationship between the composition of AMF species and soil properties in the six agricultural crops evaluated, a main component analysis was applied; the analysis was performed with the MVSP version 3.22 software (MVSP, 2013).

RESULTS AND DISCUSSION

Presence of Arbuscular Mycorrhizal Colonization

The presence of AMF structures was observed in most of the studied vegetables, except for pumpkin and parsley, both crops belonging to the Santa María Aticpan area. On the other hand, in all the cultures collected in the Acatzingo zone, as well as in the El Cristo locality, only mycelium, vesicles inside the roots, and spores near the surface of the roots were observed (Table 1).

Abundance of AMF Spores

All the crops analysed in the Tepeaca Valley, Puebla presented AMF spores associated with the rhizosphere soil of the vegetables Cilantro, Parsley, Pumpkin, Spinach, Lettuce, and Cabbage. The abundance of AMF spores varied from an average of 92.5 spores in 100 g of dry soil for spinach up to a maximum of 150.7 AMF spores in the coriander cultivar. The non-parametric analysis of variance (ANOVA) did not detect significant differences in spore abundance among the six cultures analysed (Figure 1).

Table 1. Structures of arbuscular mycorrhizal fungi in the soil associated with six species of vegetables in the Tepeaca Valley, Puebla, Mexico.

Host	Location	Structures	Spores
Spinach (*Spinacia oleracea* L.) Chenopodiaceae	Acatzingo	V	+
Cabbage (*Brassica oleracea* L.) Brassicaceae	Acatzingo	V	+
Coriander (*Coriandrum sativum* L.) Apiaceae	Acatzingo	V	+
Pumpkin (*Cucurbita pepo* L.) Cucurbitaceae	Santa María Aticpan	S/E	-
Lettuce (*Lactuca sativa var. capitata* L.) Asteraceae	El Cristo	V	+
Parsley (*Petroselinum sativum Hoffm*. ex Gaudin) Apiaceae	Santa María Aticpan	S/E	+

V = vesicles; (+) = present; (-) = absent; S/E = no structures.

AMF Species Richness and Composition

The richness of AMF species recorded in the six vegetable crops consisted of 21 morphospecies, distributed in the genera *Acaulospora* (5 spp, 23.8%), *Claroideoglomus* (2 spp, 9.5%), *Diversipora* (2 spp, 9.5%), *Entrophospora* (1 sp, 4.75%), *Funneliformis* (2 spp, 9.5%), *Gigaspora* (1 sp, 4.75%), *Glomus* (6 morphospecies, 28.6%), *Scutellospora* (1 sp, 4.75%) and *Septoglomus* (1 sp, 4.75 %) (Table 2). The composition of AMF species associated with the six vegetable crops varied significantly according to the parametric analysis of similarity (ANOSIM, $P = 0.0001$). According to ANOSIM, the similarity in the composition of species among the six vegetable species was low (12% similarity) between coriander versus lettuce, and in the case of parsley versus spinach they were the ones that presented the highest values (40%) of similarity in the composition of AMF species (Figure 2).

Soil Properties

The relative humidity values in the soil associated with the six vegetables were generally low; the values varied from 9.4% for lettuce to a maximum value of 13% for the soil associated with the spinach crop (Table 3). The values of the soil water retention capacity varied from 41.3% in the parsley crop to 50.9% in the cabbage crop. The soil pH varied from slightly acidic in the parsley crop (pH = 5.87) to neutral in the lettuce crop (pH = 6.87) as can be seen in Table 3. The electrical conductivity (EC) of the soil in the six vegetable crops was less than 1 mS cm^{-1}; crops such as pumpkin and lettuce presented soils with low EC (0.1 mS cm^{-1}) compared to coriander (EC = 0.93 mS cm^{-1}) and spinach (EC = 0.44 mS cm^{-1}) crops that presented the highest values (Table 3). Regarding the textural classification of the soil, three crops with a sandy loam texture were observed, two with a loamy sandy texture, and finally only one crop with a sandy textured soil, corresponding to the Cambisols type (Table 3).

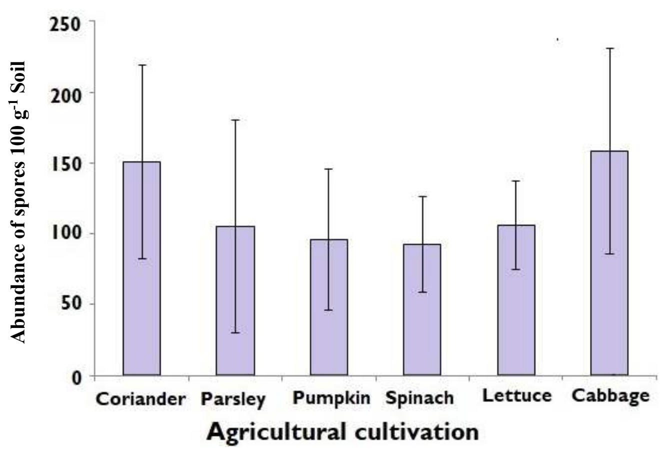

Figure 1. Abundance of arbuscular mycorrhizal fungi spores in the soil associated with six species of vegetables in the Tepeaca Valley, Puebla, Mexico. Kruskal-Wallis non-parametric one-way ANOVA (vegetable species): H = 6.14; P = 0.292; not significant.

Table 2. Consortia of arbuscular mycorrhizal fungal species in the soil associated with six species of vegetables in the Tepeaca Valley, Puebla, Mexico.

Crop	Morphospecies of arbuscular mycorrhizal fungi
Coriander	*Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker &, A. Schlüβler; *Claroideoglomus drumondii* (Blaszk. & Renker) C. Walker & A. Schlüβler; *Acaulospora spinosa* C.Walker & Trappe; Glomus sp1.
Parsley	*F. mosseae, C. claroideum* N.C. Schenk & G.S,. Sm. C. Walker & A. Schlüβler; *Glomus* sp2.
Pumpkin	*F. mosseae, C. claroideum, Glomus sp3, F. halonatum* Rose & Trappe; *Scutellospora* sp., *Septoglomus* sp., *Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid; *Acaulospora* sp1.
Spinach	*F. mosseae, C. claroideum, Glomus* sp4, F. *halonatum*
Lettuce	*F. mosseae, Glomus* sp5, *Diversispora trimurales* (Koske & Halvorson) C. Walker &, A. Schlüβler C, *Gigaspora gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe, *A. scrobiculata* Trappe, *A. gedanensis* Blaszk.
Cabbage	*F. mosseae, C. claroideum*, *Glomus* sp6, *F. halonatum*, *Diversispora* aff. *globifera*, A. *excavata* Ingleby & C, Walter, *A. gedanensis* Blaszk

Relationship Between the Composition of AMF Species and Soil Properties

Main component analysis (MCA) explained 57.3% of the variance; the first component explained 30% of the variance and the second component 27.3% of the remaining variance. The MCA ordered and separated the six vegetable crops based on the composition of AMF species and the properties of the analysed soil (Figure 3). The soil variables with the highest load in the ordering were relative humidity for the first component, while electrical conductivity, pH, and soil texture had the highest load for the second component.

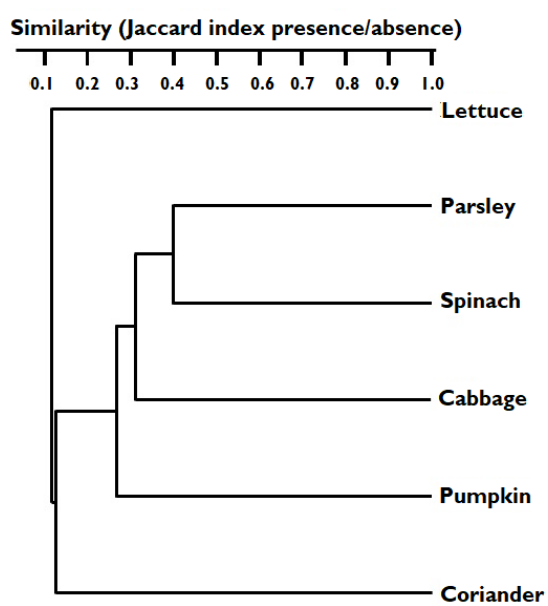

Figure 2. Similarity analysis (presence/absence Jaccard index) in the composition of arbuscular mycorrhizal fungi species in the soil associated with six species of vegetables in the Tepeaca Valley, Puebla, Mexico. ANOSIM (Permutations = 9999; R=1; P = 0.0001).

Table 3. Physical and chemical properties of the soil associated with six species of vegetables in the Tepeaca Valley, Puebla, Mexico.

Soil	Relative Humidity	Water holding capacity	Ph	Electric conductivity	Texture
	- - - - - - - - - - - %	- - - - - - - - - - -		mS cm^{-1}	Bouyoucos
Coriander	12.7	45.1	6.12	0.93	Sandy loam
Parsley	9.9	41.3	5.87	0.33	Loam sand
Pumpkin	12.8	48.4	6.60	0.11	Loam sand
Spinach	13.0	50.2	6.30	0.44	Sandy loam
Lettuce	9.4	45.8	6.87	0.10	Sandy
Cabbage	12.5	50.9	6.24	0.25	Sandy loam

Discussion of the Experimental Results

This research contributes to the knowledge of AMF communities and reports the isolation and taxonomic identification of a total of 21 AMF morphospecies in the six vegetable crops in the producing region of Tepeaca Valley, Puebla, México. This richness of AMF species was distributed in the genera *Acaulospora, Claroideoglomus, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Scutellospora,* and *Septoglomus*. Of the determined species, *F. mosseae* was present in all the crops, an AMF species that has shown positive effects on the growth and health of the plants and that is commonly recorded in agroecosystems in Mexico (Alarcón *et al*., 2012); followed by C. *Claroideum* and *Glomus sp*, probably because of, as suggested by (Guadarrama-Chávez, Camargo, Hernández, and Castillo, 2007) AMF of the *Glomus genus* have a higher incidence in agricultural areas with intensive use. On the contrary, some AMF species such as *Diversispora trimurales*, *D. globifera*, among others, have only been reported from natural ecosystems so their functionality in agroecosystems (Chimal-Sánchez, Montaño, Camargo, García, and Hernández, 2015; Alarcón *et al*., 2012). The presented results show that, apart from the pumpkin and parsley crops, the spinach, cabbage, cilantro, and lettuce crops evaluated in the Tepeaca Valley, Puebla, presented arbuscular mycorrhizal colonization, with the presence of mycelium and vesicles; while it was not possible to detect arbuscules in the roots of the six crops. It is recognized that the arbuscular mycorrhizal activity and its functionality can be reduced by the effects of the high availability of phosphorus in the soil (Smith and Read, 2008). Likewise, the amendments of organic fertilizers with high availability of macro and micronutrients (Guerra-Sierra, 2008) or the use of chemical fertilizers and pesticides that have also been reported could reduce mycorrhizal colonization (Martínez-Torrijos, 2013[1]); which may explain the absence of arbuscules as nutrient exchange structures between plants and AMF in the agricultural systems evaluated. Sánchez-Colín (2003) reported 95% arbuscular mycorrhizal colonization in coriander crops in sodic-saline soils amended with compost and inoculated with AMF of the genera *Glomus, Acaulospora,* and *Scutellospora* from an Andisol; which improved the characteristics of the plants and their yield. In contrast, Harris-Valle, Esqueda, Valenzuela, and Castellanos (2011) when inoculating Pumpkin plants with a consortium of seven species of native AMF isolated from an arid region of Sonora, recorded a better response of plants to high salt stress compared to plants inoculated with an isolated AMF (*C. clarioideum*). from a temperate region, a species of AMF that was also recorded in the squash crop in the Tepeaca Valley. This suggests that knowledge of the native arbuscular mycorrhizal consortia in each of the crops evaluated in the Tepeaca Valley would have greater potential to be studied and used as biofertilizers compared to exotic AMF species, as observed by Castillo *et al*. (2009) in the cultivation of chili (*Capsicum annuum* L.) inoculated with *C. Claroideum* in comparison with an exotic AMF (*Rhizophagus intraradices*). The mycorrhiza-plant benefits in the crops under study (cilantro, spinach, lettuce, parsley, cabbage, pumpkin) are to improve and increase the assimilation of nutrients and water. As well as increased branching and root growth, elongating the cells and greater efficiency of the same, the extended hyphae reach more food sources and especially water, where the roots do not reach, which translates into greater efficiency to locate and absorb water, and this implies a highly beneficial effect against hydric and saline stress. Mycorrhizae in the soil mobilize a large amount of nutrients that were previously not available to plants, increasing their fertility, improving the productive capacity of soils affected by desertification, salinization, water, and wind erosion. The abundance of AMF spores did not vary among the six crops, but there were changes in the richness and composition of AMF species. It is clear that the abundance of spores is an indicator of the potential mycorrhizal inoculum available in the soil (Smith and Read, 2008).

[1] Martínez-Torrijos, E. (2013). *Inoculación de calabacita verdura con cepas de hongos micorrízicos arbusculares.* Tesis de maestría para obtener el grado de Maestro en Ciencias en Horticultura. Universidad Autónoma de Chapingo. Chapingo, México. Available at https://repositorio.chapingo.edu.mx/handle/123456789/1497

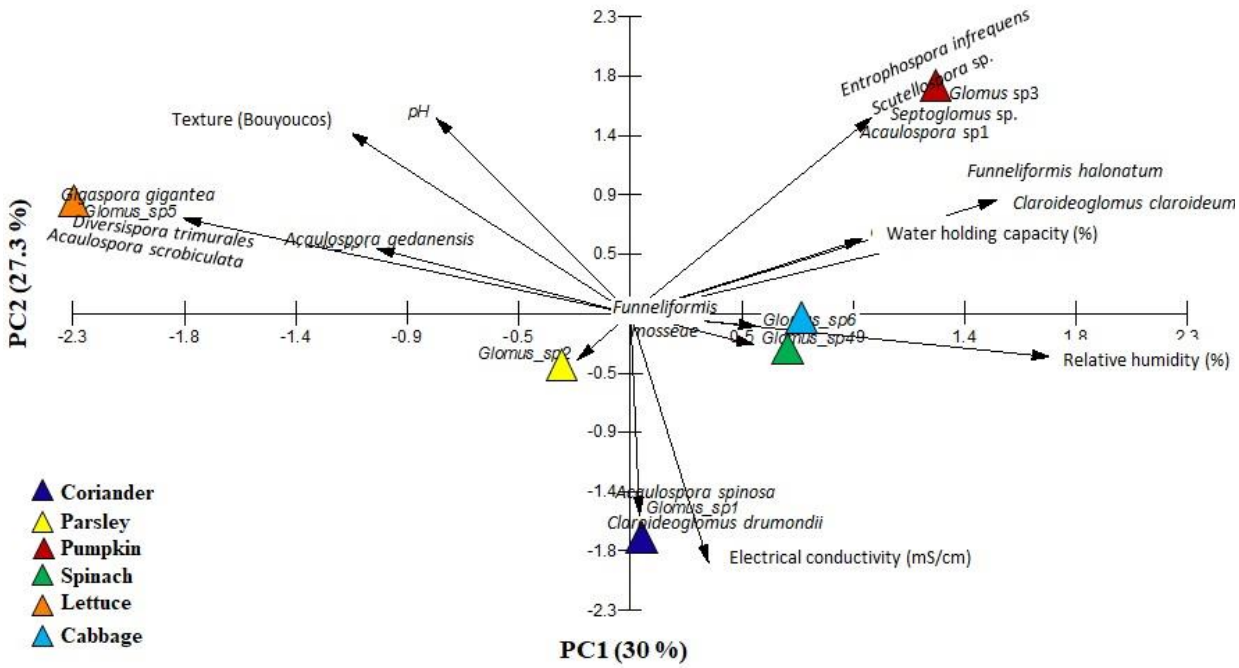

Figure 3. Main component analysis that explore the relationship between the composition of arbuscular mycorrhizal fungi species and the properties of the soil associated with the six species of vegetables in the Tepeaca Valley, Puebla, Mexico (Tolerance of the eingenanalysis = 0.007).

Based on the above, the presented results indirectly suggest that the six crops could have the same inoculum potential in the soil; however, similarity and main component analyses suggested significant changes in the species richness and composition of the six crops evaluated as a function of plant species and soil conditions. Thus, forming arbuscular mycorrhizal consortia, perhaps specific for each cultivar. The experimental results coincide with what is reported in the literature, where soil conditions affect AMF communities, for example, in Chile Poblano (Carballar-Hernández *et al*., 2017) and coffee (Trejo *et al*., 2011).

Regarding the diversity of AMF, Harris-Valle *et al*. (2011) observed that pumpkin crops are colonized by species of the genera *Glomus*, *Claroideoglomus*, *Rhizophagus,* and *Pacispora*. Mbogne *et al*. (2015) reported the genera *Acaulospora*, *Gigaspora,* and *Scutellospora* in this crop; while Ley-Rivas, Ricardo, Sánchez, and Furrazola (2016) reported the association of said vegetable with the genera *Rhizophagus*, *Glomus*, *Scutellospora*, *Acaulospora,* and *Entrophospora*; which some genera coincide with what was recorded in the Tepeaca Valley, Puebla; the first record for this area and crops.

CONCLUSIONS

The research reveals the structure of AMF communities in six vegetable crops in a producing region of importance for the state of Puebla. The analyses of similarity and main components suggested that the vegetable species and the soil conditions did not affect the abundance of spores but affected the richness and composition of AMF species, observing particular mycorrhizal consortia for each crop. The results indicated the presence of mycelium and vesicles of the AMF spinach, coriander, cabbage, and lettuce while in pumpkin and parsley there was no colonization. AMF spore abundance ranged from 92.5 spores 100 g^{-1} of dry soil for spinach to 150.7 spores 100 g^{-1} in coriander without significant differences between crops. The AMF species richness in the six vegetable crops consisted of 21 morphospecies, distributed in the genera *Acaulospora, Claroideoglomus, Diversipora, Entrophospora, Funneliformis, Gigaspora, Glomus, Scutellospora* and *Septoglomus*. Parametric analyses of similarity and main component analysis indicated that plant identity and soil conditions modify the richness and composition of AMF species. Therefore, it is advisable to evaluate its effectiveness in each of the crops to promote its use as a biofertilizer in the agricultural region of the Tepeaca Valley in Puebla, Mexico.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

Analysis research and writing, original draft preparation: M.C.A.C. Extraction and Taxonomy: E.C.S. Collaborator of the sample management project: A.T.S. Writing, review and editing and supervision: J.D.R.

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