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REMEXCA

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Article

Arbuscular mycorrhizae in blackberries under conventional management and propagation with different hosts

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Abstract

Tacámbaro is one of the municipalities of greatest agricultural importance in the state of Michoacán; it stands out for its production of avocado and blackberry; however, the conventional farming methods used have a significant impact on the environment and human health, showing the importance of valuing sustainable agriculture practices that also allow meeting the demand for such products abroad under organic production. An alternative is to use arbuscular mycorrhizal fungi (AMF) obtained from crops established in regions where they will be applied, so that they have greater adaptation to soil and climatic conditions. For this reason and because there is no information on the symbiosis for blackberry crops, the objectives were to evaluate AMF colonization in blackberry plants and identify appropriate hosts for their propagation. Mycorrhizal structures were identified in blackberry roots and the number of spores was quantified in rhizospheric soil samples from two sites in the region. Lettuce, wheat and bean plants were used for propagation, inoculated with rhizosphere and blackberry roots. At the La Magdalena site, it showed greater colonization (100% hyphae, 35% vesicles and 7.6 spores 50 g of rhizosphere). The highest colonization as a trap plant was in lettuce (63.33% hyphae and 48.33% vesicles). Natural colonization in blackberry roots shows that symbiosis is established despite agronomic practices and that this crop is an important source of inoculum that can be propagated in lettuce plants.

Keywords:

Rubus fructicosus var. Tupi., lettuce, native AMF, trap plants

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Introduction

As a result of the response to the demand for production and consumerism, agricultural activity has affected the environment due to the excessive use of agrochemicals, which has led to the need to create alternatives that generate less impact (Cortés-Hernández *et al*., 2023); therefore, most developed and developing countries produce, consume and import organic products preferably and in the case of developing countries, production under organic agriculture is carried out only for export, as is the case of Mexico, with an increasing presence in the production and marketing of organic foods in the world (Kuila and Ghosh, 2022; Ortega *et al*., 2022).

Michoacán contributes 70% of organic production nationwide, with avocado being its main product, followed by blackberry; this production is mainly obtained in the municipality of Tacámbaro (Ortega *et al*., 2022; FIRA, 2024; Ruvalcaba-Cázares *et al*., 2024). Mexico's presence and its maintenance in the world market could enable a transition to sustainable production and increase production systems based on organic or biological products (Reyes-Gómez *et al*., 2023).

Therefore, the use of microorganisms such as arbuscular mycorrhizal fungi (AMF) would be an important biotechnology for application; they allow improving the vigor and yield of plants, increasing the absorption of water, nitrogen, phosphorus and other nutrients that are not very mobile; they increase tolerance to stress due to soil salinity; they protect against pathogens and improve soil quality (Kuila and Ghosh, 2022). Nevertheless, the use of inoculants in any type of soil and crop may not present good results, which could be caused by containing microorganisms that are not adapted to the soil conditions and climate in which they are established, so it is suggested that the inoculum be selected in the environment in which they will be applied (Kuila and Ghosh, 2022; Salomon *et al*., 2022).

For the state of Michoacán, information on AMF species has been reported from some sites in the avocado-growing region (González-Cortés *et al*., 2012; Carreón-Abud *et al*., 2016); however, there is no information for all regions and crops of agricultural importance that could be the basis for obtaining native inocula; therefore, the objectives of this work were to evaluate AMF colonization in blackberry plants and to identify a host species suitable for propagating these fungi in the region of Tacámbaro, Michoacán.

Materials and methods

The work was conducted in Tacámbaro, Michoacán, in a semi-warm subhumid climate with summer rainfall, winter rain <5%, intermediate humidity, and an Acrisol-type soil (INEGI, 2007; Gutiérrez-Contreras *et al*., 2010).

Native AMF in blackberry crops

Rhizosphere and root samples were taken from blackberry (*Rubus fruticosus* var. Tupi) plants at two sites in the region; these were selected considering that the conventional agronomic management, area, and climatic conditions were similar (Table 1).

Table 1. Characteristics of sampling sites.

Site	Coordinates	Area (m2)	pH	EC (μS cm-1)	Nutrient concentration (mg kg-1)		
					N	P	K
La Magdalena	19°13'27.45" N 101°28'49.89" W	1 181	6.1	180	8.3	12.3	28
Instituto	19°13'6.59" N 101°29'10.50" W	1 795	6.4	100	4.5	6.5	15.5

EC= electrical conductivity.

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At each site, 10 subsamples were randomly taken following a zigzag methodology to form a composite sample, each of 200 g of rhizosphere and secondary roots (Prendes *et al*., 2006; Sánchez *et al*., 2010; Carreón-Abud *et al*., 2013). In the laboratory, the roots were stained by the method established by Phillips and Hayman (1970) to evidence mycorrhizal structures. The preparations were observed under an optical microscope (Revelation III Lw scientific®) to identify mycelium, vesicles, and arbuscules, and to determine the percentage of colonization by means of the formula established by McGonigle *et al*. (1990):

$$\%of\,colonization = \frac{Number of colonized fields}{total number of observed fields} x100$$

To extract and count spores from the rhizosphere, the techniques of wet sieving and decantation, modified by Gedermann and Nicolson (1963), and sucrose gradient centrifugation, modified by Danielsl and Skipper (1982), were used. The samples were observed under a stereoscopic microscope.

Trap plants for propagating blackberry native AMF

Once the presence of AMF in the crop was identified, the rhizosphere and roots of plants from the two study sites were used to inoculate lettuce (*Lactuca sativa* L.), wheat (*Triticum durum*), and bean (*Phaseolus vulgaris*); these species are from families that are considered suitable for their susceptibility to colonization, short life cycle and root system (Sánchez *et al*., 2010). The plants were established in greenhouse conditions under a completely randomized design with two treatments (inoculum from the two sites), and the experimental unit was one plant with ten replications in each treatment.

Sand was used as a substrate, sterilized in a Felisa® autoclave at 121 °C and 15 lbf in-2. When germinating the trap plant seeds, they were inoculated with 1 kg of rhizosphere and 4 g of blackberry roots. They were kept in a greenhouse for three months, applying Long Aston nutrient solution once a week. At the end of the indicated time, the percentage of colonization in the roots of the host plants and the number of spores in 50 g of the substrate for propagation were determined.

Data analysis

To carry out the analysis of variance (#= 0.05), the data were previously analyzed for the verification of normality (Shapiro-Wilk) and homogeneity of variance (Levene) and since the assumptions were not met, the percentages of colonization of blackberry plants and trap plants, the data were transformed into ranges using the InfoStat software (Di Rienzo *et al*., 2020), except for the number of spores. Finally, a comparison of means was performed (Tukey α= 0.05).

An analysis of variance between sites was performed using the following model:

$$Y_{ijk} = \mu + S_i + P_{j(i)} + e_{ijk}$$

Where: Yijk= is the observation of treatment i in observation; Si= effect of site i; Pj(i)= effect of the plant within site i; eijk= random error associated with observation Yijk;

for the analysis of the variance within sites, the following model was used:

$$Y_{ij} = \mu + P_i + e_{ij}$$

Where: Yij= is the observation of the treatment; Pi= effect of the plant; eij= random error associated with observation Yij.

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Results and discussion

Native AMF in blackberry crops

The presence of AMF in blackberry plants was identified at both sites (Figure 1), with no statistically significant differences between the percentages of colonization (p= 0.91459), both cases with high colonization by hyphae (99% on average) and low colonization by vesicles (38.05% on average) (Figure 2).

Figure 1. Hyphae and vesicles from samples a) La Magdalena; and b) Instituto.

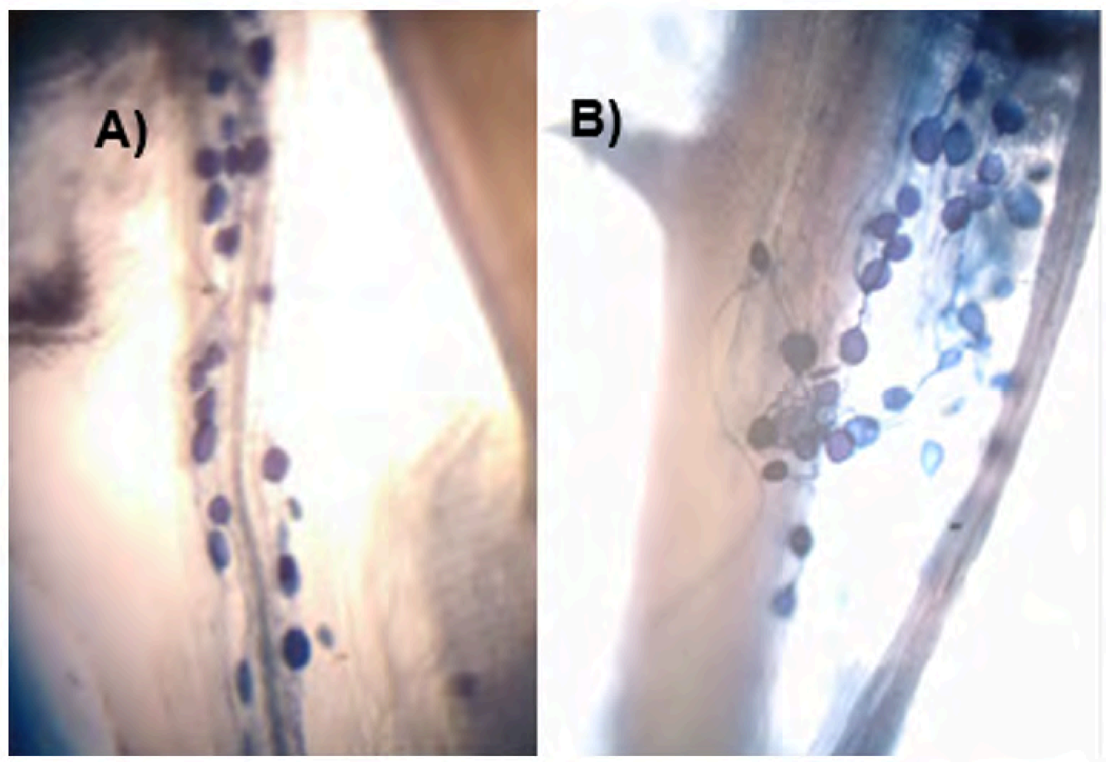

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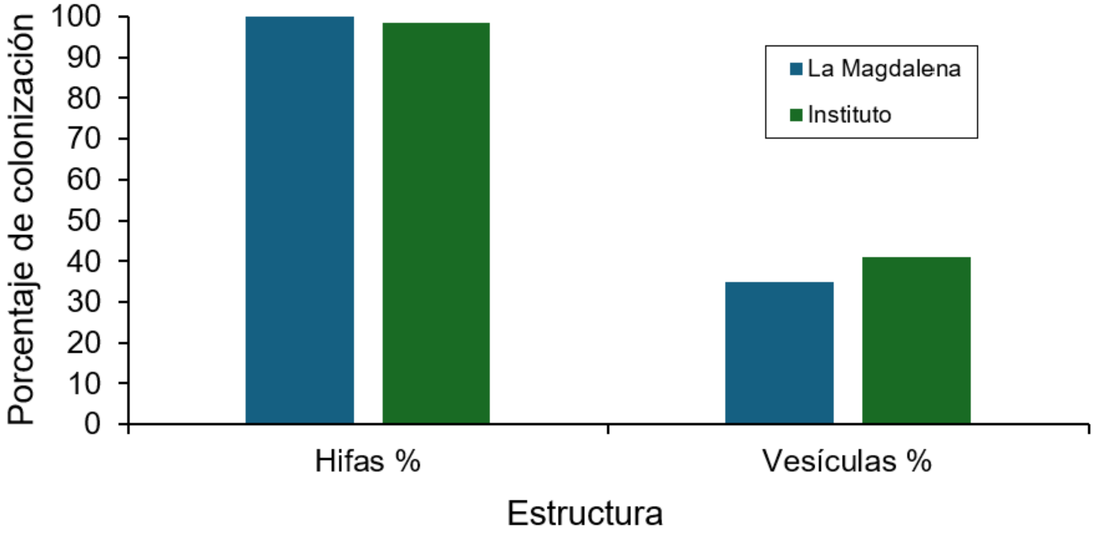
Figure 2. Percentages of AMF colonization in blackberry plants.



The percentages of colonization by hyphae and vesicles were higher than those found by Rodríguez *et al*. (2022) in three species of *Rubus* (*R. alpinus*, *R. urticifolius*, and *R. floribundus*), obtaining an average of 13.4% and 9.7%, respectively and 6.5% colonization by arbuscules. The data are higher than those found in other Rosaceae species; Albornoz *et al*. (2014) reported percentages of hyphal colonization of 67.63, 66.35 and 34.69 for *Duchesnea indica*, *Fragaria vesca*, and *Potentilla tucumanensis*, respectively; in contrast, in strawberry *Fragaria ananassa* var. Camino Real, Lizarraga *et al*. (2015) found a colonization by vesicles of 1.2% and by hyphae of 20.5%.

The absence of arbuscules at both sites is similar to that found in *Alliaria petiolata* (Trombley *et al*., 2025) and to that reported by Acoltzi-Conde *et al*. (2024) in crops of squash, parsley, spinach, cabbage, coriander and lettuce, in which it was not possible to detect such a structure, proposing that the application of fertilizers to the crop may cause the absence of arbuscules as nutrient exchange zones between plants and AMF. In the case of the number of spores, there were differences between sites (p= 0.0132), with the La Magdalena site being where the highest number was found (Figure 3 and Figure 4); in this site, the concentration of phosphorus is higher (Table 1) and according to Dube *et al*. (2025), the increase in spore numbers may be a survival strategy in response to increased phosphorus levels.

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Figure 3. Number of spores 50 g of rhizosphere in different sites. Different letters indicate significant differences (α= 0.05).

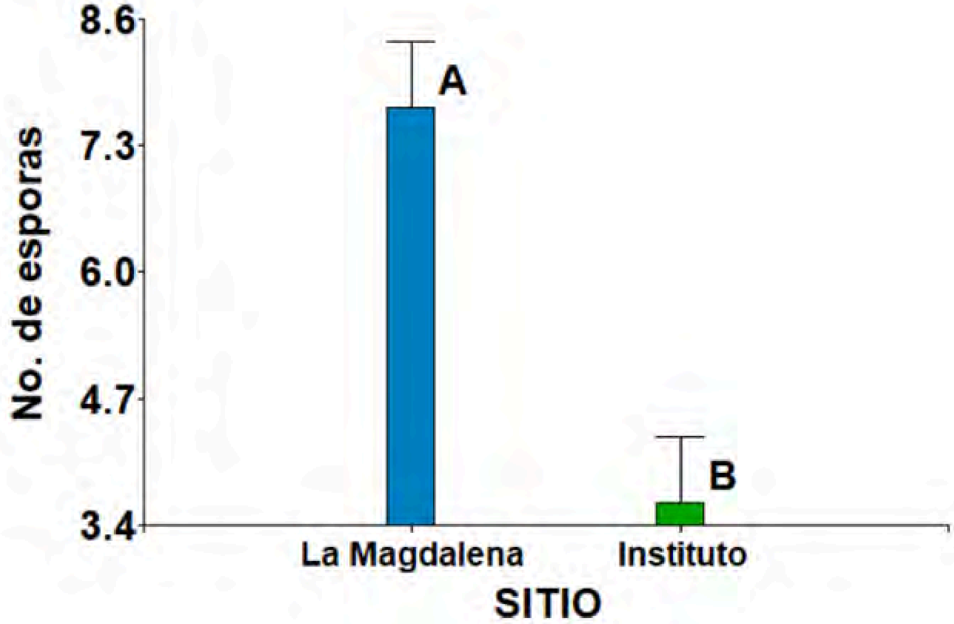
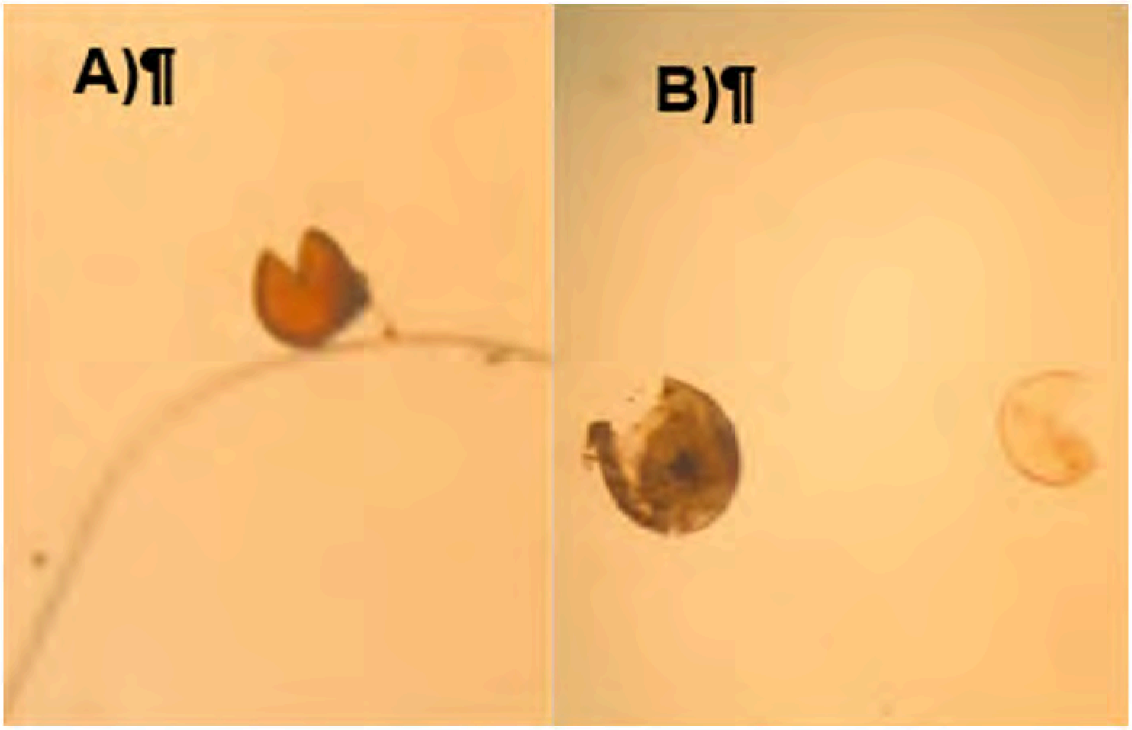


Figure 4. Sample spore a) La Magdalena and b) Instituto.



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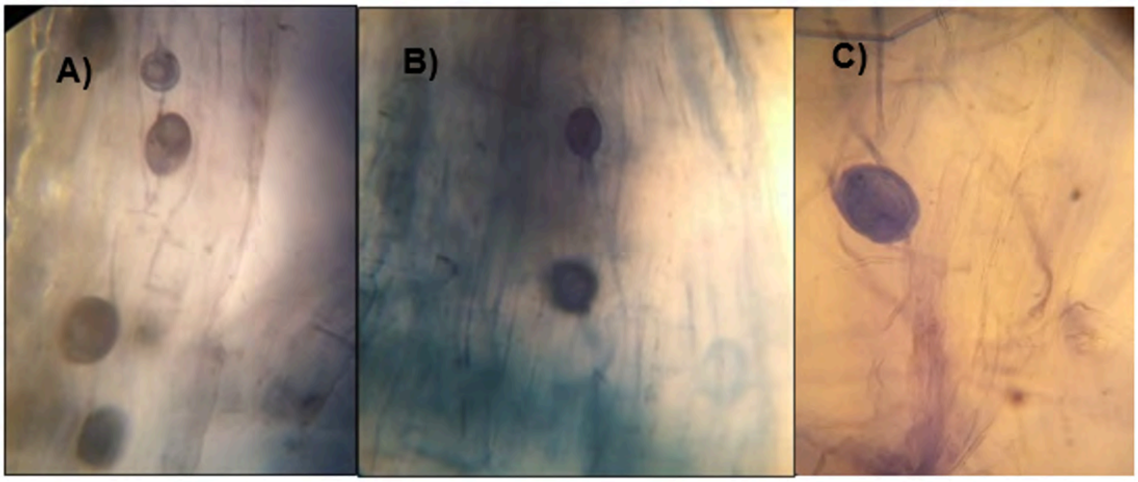
Trap plants for the development of native AMF

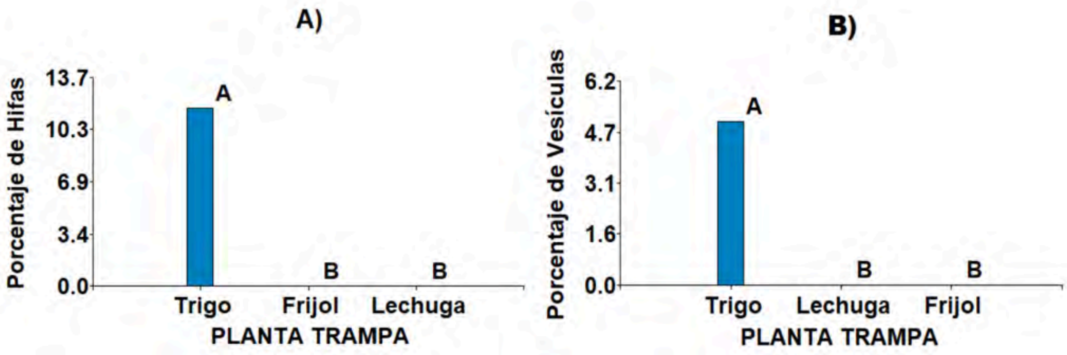
No statistically significant differences were found between the percentages of hyphae and vesicle colonization obtained in the trap plants compared between the inoculums from each site (p= 0.1057), which is similar to what was reported by Arias *et al*. (2024), where inoculum sources did not cause differences in colonization percentages in inoculated trap plants. The analysis carried out within sites between the trap plants (Figures 5a, 5b and 5c) did show statistically significant differences for both variables (p < 0.0001), which differs from what was found by Arias *et al*. (2024), who report the absence of significant statistical differences and a higher percentage of colonization in the trap plants used.

Figure 5. Hyphae and vesicles in a) lettuce; b) beans and c) wheat.



Of the plants with inoculum from La Magdalena, only wheat showed colonization (Figures 6a and 6b), a situation similar to that reported by Covacevich *et al*. (2012) when evaluating the mycotrophic capacity of agricultural and natural soils; not all soils were appropriate sources of inoculum to colonize the trap plants tested, reporting low mycorrhization percentages (12.73%).

Figure 6. Percentage of colonization of a) hyphae and b) vesicles in trap plants. Different letters indicate significant differences (α= 0.05).



In the case of the trap plants with inoculum from Instituto, all showed colonization, with lettuce having a higher percentage of both hyphae and vesicles, whereas wheat plants showed a lower percentage of both structures (Figures 7a and 7b).

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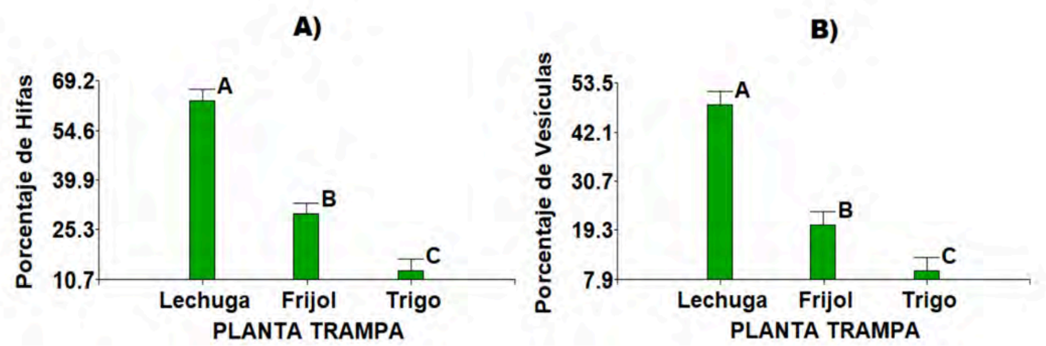
Figure 7. Percentage of colonization of a) hyphae and b) vesicles in trap plants. Different letters indicate significant differences (α= 0.05).



The percentage of colonization found in wheat plants with both inocula is close to that reported for the same species by Covacevich *et al*. (2008), who, in a study with different soil phosphorus contents, found an average colonization of 8.86%. Bean plants showed less colonization by hyphae and higher colonization by vesicles than found in a study conducted by Herrera-Corrales *et al*. (2014) (5< 50% by hyphae; 5< 7% by vesicles) in the same crop. As for the percentages of colonization found in lettuce plants, these were lower than those obtained in a study with the same species that evaluated the infectivity of AMF from five sites with saline soils; the values found were between 68% and 83% (Tapia-Goné *et al*., 2010).

The null or relatively low colonization found in the trap plants may be due to the compaction observed in the substrate used, as the mixture of medium particle sand of between 0.25 and 0.5 mm (Casanova, 2005) was used in combination with the rhizosphere of each site; the low porosity caused in the environment could exert a restrictive and negative influence on the colonization and sporulation of the fungi, as reported by Jiménez-Martínez *et al*. (2014), who evaluated the effect of different particle sizes and found that the lowest percentage of colonization was found in the granulometry <0.6 mm.

Although soil compaction affects plant water and nutrient uptake, it would also be expected to affect symbiosis with AMF; however, in a study conducted with different wheat varieties, they found that colonization in compaction treatments depended on the variety, also suggesting that in the field, the diversity of AMF communities may have different abilities to decrease the effect of compaction (Torppa *et al*., 2023).

Conclusions

The study showed no differences between sites; nevertheless, high colonization was observed in blackberry crops, which is why it can be an important source of native inoculum, adapted to the soil and climatic conditions of the region, useful in production systems under conditions similar to the sites from which it was obtained. Lettuce plants can be used as a trap to reproduce and propagate AMF, which allowed us to develop local inoculants, and based on the results of other research for other crops in the Tacámbaro region, provide a biofertilizer alternative to improve agricultural production in the region, both in conventional management systems, as well as in organic management systems.

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