

# Root colonization of tamarind tree (*Tamarindus indica* L.) and occurrence of arbuscular mycorrhizal fungi in soils – Sopetrán, Antioquia

Colonización de raíces de tamarindo (*Tamarindus indica* L.)  
y ocurrencia de hongos micorrízicos arbusculares en los  
suelos – Sopetrán, Antioquia

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## ABSTRACT

### Keywords:

AMF diversity  
Native agroecosystems  
Rhizosphere  
Soil microorganisms  
Symbiotic associations  
Tropical dry forest

The tamarind tree (*Tamarindus indica* L.) is a multipurpose species, primarily used in the food and medical industries, cultivated by traditional growers who do not employ a fertilization process in their crop fields. In Colombia, few studies have related the presence of this plant in low-fertility soils to the colonization of arbuscular mycorrhizal (AM) fungi. This study aims to know the AMF associated with the rhizosphere of tamarind trees in the tropical dry forest from the Western Antioquia region, and to establish the AMF colonization of the roots. For this purpose, samples of the rhizosphere and roots from a production plot were taken, spores were extracted and morphotype identification was made, and after slide mounting, the spores were described under the microscope based on their morphological characteristics and identified using specialized identification keys. In the previously decolorized and dyed roots, the fungi colonization was determined, distinguishing hyphae, arbuscules and vesicles presence. Fifteen species of AMF were described, 53% of them belonging to the Glomeraceae family and 20% to the Acaulosporaceae family. The mycorrhizal colonization was observed in 50% of samples, hyphae were found in 39%, arbuscules in 31%, and vesicles in 14%. This record is higher than other reports on *T. indica*, which may indicate the importance of this symbiosis for the plant in traditional production systems studied.


## RESUMEN

### Palabras clave:

Diversidad de HMA  
Agroecosistemas nativos  
Rizosfera  
Microorganismos del suelo  
Asociaciones simbióticas  
Bosque seco tropical

El tamarindo (*Tamarindus indica* L.) es una especie multipropósito, usada principalmente con fines alimenticios y medicinales, cultivada por productores tradicionales, quienes no realizan fertilización del cultivo. En Colombia hay pocos estudios que relacionen la presencia de estas plantas en suelos de baja fertilidad natural y la colonización por hongos micorrízicos arbusculares (HMA). Este trabajo tuvo como objetivo conocer los HMA asociados a la rizosfera de árboles de *T. indica*, en el bosque seco tropical del occidente cercano de Antioquia y establecer la colonización de HMA de las raíces. Para ello se tomaron muestras de rizosfera y raíces de un lote en producción, se hizo extracción de esporas para su separación por morfotipo, luego del montaje las esporas fueron descritas en sus características morfológicas en microscopio y determinadas con claves especializadas. En las raíces previamente decoloradas y teñidas se determinó la colonización por los hongos, discriminando la presencia de hifas, arbuscúlos y vesículas. Se describieron 15 especies de HMA, un 53% de ellas pertenecientes a Glomeraceae y 20% a Acaulosporaceae. La colonización micorrícica encontrada fue de 50%, en el 39% de las muestras se hallaron hifas, en el 31% arbuscúlos y en el 14% vesículas. Este registro es mayor a otros reportes en *T. indica*, esto puede ser indicativo de la importancia de esta simbiosis para la planta en los sistemas de producción tradicionales estudiados.

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**T***amarindus indica* L. is a species from the monotypic genus *Tamarindus* (subfamily Detarioideae, family Fabaceae) (LPWG 2017), native from tropical Africa (Van der Stege et al. 2011), cultivated in tropical and subtropical regions of the world (Kareem et al. 2023). The tamarind is a long-lived tree that can reach heights of up to 30 meters and produce fruit for up to 200 years (Bahru et al. 2014; Ndiaye et al. 2022). It is considered a multipurpose species, as all its parts are usable. In Western Africa, the tree is attributed to up to 250 different uses, making it an essential resource for the survival of many people (Van der Stege et al. 2011). These uses span a wide array of categories, including—but not limited to—food, medicine, spirituality, ethnoveterinary applications, industrial uses, human and animal welfare (e.g., relief from heat and radiation), and wood utilization, either as an energy source or for cabinetmaking (Muriel et al. 2021; Zeleke 2022). Studies on its chemical composition underscore tamarind's nutritional value due to its content of amino acids, vitamins, proteins, minerals, and bioactive compounds, particularly its high concentration of tartaric acid (Bhadoriya et al. 2011).

In Colombia, tamarind is cultivated in the departments of Antioquia and Atlántico (Agronet 2021); however, production remains secondary and is characterized by low levels of technification. The western region of Antioquia, located within the tropical dry forest (T-df) ecosystem, has traditionally been a tamarind-producing area. There the trees can be in silvopastoral systems, like trees scattered through paths, pasturelands, vegetable patches, and backyards in urban houses that are seldom harvested or managed (Álvarez et al. 2015). The fruit is the most used part of the plant in the production of sweets, sauces and juices, which are offered to the region's tourists (Álvarez et al. 2015). This constitutes the main economic support to the individuals dedicated to the manufacture and selling of tamarind sweets. Nonetheless, increasing land demand for other uses has led to the falling of tamarind trees, resulting in a local demand for the fruit that exceeds its supply. This situation highlights the need to promote and expand its cultivation.

The tamarind tree is well adapted to semi-arid tropical regions and is capable of withstanding drought conditions. It can grow in a wide variety of soils; however, it thrives best in deep, well-drained soils that are slightly acidic or saline

(Bhadoriya et al. 2011). Although there are no studies about the appropriate soil features for the tamarind farming, it has been reported to establish relationships with mycorrhizal fungi and nitrifying bacteria (Parrotta 2000; Bourou et al. 2010); therefore, it is expected that soils with a significant presence of organic matter, could offer the right setting to foster the development of plants. Within the important organisms for crop nutrition, the arbuscular mycorrhizal fungi (AMF) can benefit their host, mainly by increasing the absorption of water and soil nutrients, such as phosphate ions which are relatively still, due to the fungus mycelium capacity to grow beyond the phosphorus depletion zone and to develop rapidly around the root (Smith and Read 2008; Rini et al. 2020). Moreover, the AMF protects the plant from soil pathogens, from stress conditions and provides regulation of hydric interactions.

Regarding *Tamarindus indica*, few studies link the role of AMF with the presence of trees in low fertility soils. In one of these studies, Bourou et al. (2010) characterized the diversity of AMF associated with the tamarind rhizosphere in three agroecological zones in Senegal, finding species of the genera *Acaulospora*, *Glomus* and *Scutellospora* and a higher mycorrhization in soils under poor conditions or exposed to drought. AMF studies have been carried out mostly in India and Africa; in Colombia, there is only one study that has been documented about the case of AMF associated with *Tamarindus indica* cultivations (Arcos 2015), in which it is pointed out the presence of 10 morphotypes, from which 40% corresponded to *Acaulospora* and 30% to *Glomus*. Understanding the role of this symbiosis in the establishment of new crop plants is important, as it may facilitate the growth of tamarind seedlings (Waddar and Lakshman 2010; Kareem et al. 2023). In consequence, this study aimed to identify the AMF associated with the tamarind trees' rhizosphere, and to determine their root colonization.

## MATERIALS AND METHODS

### Study site

The study was carried out in a *T. indica* L. productive lot from Los Comunes farm, located in 'El Llano de Montaña' village in the municipality of Sopetrán (Antioquia, Colombia, coordinates 06°29'44.6" N 75°45'55.2" W). The climatic conditions of the location were consistent with tropical dry forest according to Holdridge (1987), with an average temperature of 27.6 – 29 °C, annual precipitation of 1,097

mm and 73.2% humidity (Álvarez et al. 2015). In the study, tamarind trees were about 40 years old, under a traditional silvopastoral cropping system, with periodic irrigation, since to rainless periods take place in the tropical dry forest. Plants were not fertilized, and minimal management activity was carried out. In a physicochemical soil analysis conducted

before starting fieldwork, it was established that the soil had a clay loam texture, with a pH of 6.2%, organic material content of 8.8% and 15 mg kg<sup>-1</sup> of P content (Table 1). In addition, the soil was rich in organic matter, with a high salt content and a medium level of phosphorus, which are conditions for growing tamarind trees.

**Table 1.** Los Comunes farm physicochemical soil analysis (El Llano de Montaña village, Sopetrán municipality).

Variable	Units	Value	Interpretation
Sand	%	34	Soil class: Clayey
Clay	%	24	
Silt	%	42	
pH	-	6.2	Slightly acidic
O.M.	%	8.8	High
Al	cmolc kg <sup>-1</sup>	0	-
Ca	cmolc kg <sup>-1</sup>	16.4	High
Mg	cmolc kg <sup>-1</sup>	9.9	High
K	cmolc kg <sup>-1</sup>	2.55	Medium
Na	cmolc kg <sup>-1</sup>	0.14	Low
CECe	cmolc kg <sup>-1</sup>	28.99	High
P	mg kg <sup>-1</sup>	15	Medium
S	mg kg <sup>-1</sup>	1.6	Low
Fe	mg kg <sup>-1</sup>	30	Medium
Mn	mg kg <sup>-1</sup>	5	Medium
Cu	mg kg <sup>-1</sup>	4	High
Zn	mg kg <sup>-1</sup>	3	High
B	mg kg <sup>-1</sup>	0.2	Medium

O.M.: Organic matter; CECe: Effective cation exchange capacity.

### Field sampling

To extract spores and perform morphological identification of arbuscular mycorrhizal fungi (AMF), soil samples were collected from the rhizosphere of seven trees at a depth of 20 cm. To determine the percentage of AMF colonization, the thinnest tertiary roots from five trees were gathered. The sampling was completely random. Then, samples were stored and refrigerated at 4 °C and then taken to the laboratory for post-processing.

### Spore extraction and AMF slide mounting

First, spores were extracted through humid sieving and sucrose gradient centrifugation (Gerdemann and Nicolson 1963). Then, spores were separated using a

light microscope and according to size, shape, color and hyphae accessory characteristics. Next, spores were mounted on microscope slides with PVLG (Polyvinyl-lactoglycerol) mixed with Melzer (1:1 v/v). Finally, slides were stored at 35 °C for 3-4 days for fixation (Stürmer and Siqueira 2011).

### Determination of morphotypes

Spore phenotypes were compared with original studies available in Glomeromycota-PHYLOGENY (<http://www.amf-phylogeny.com/>) and online references of species' descriptions from the International Collection of Arbuscular Mycorrhizal Fungi - INVAM from the West Virginia University, USA (<https://invam.wvu.edu>) and the Department of Plant

Protection of the Agricultural University of Szczecin, Poland (<http://www.zor.zut.edu.pl/Glomeromycota/index.html>). Correct names of the species were verified by Index Fungorum (<http://www.indexfungorum.org/>).

### Analysis of AMF species richness

Species richness was calculated as the number of AMF species identified from field samples and trap culture. The frequency of occurrence (F) was calculated by the equation  $F = (J_i/K) \times 100$ , where F is the frequency of species I,  $J_i$  is the number of samples in which the species was detected, and K is the total number of samples. Species frequency was classified as dominant ( $85\% \leq FO \leq 100\%$ ), very common ( $50\% \leq FO < 85\%$ ), common ( $30\% \leq FO < 50\%$ ) and rare ( $FO < 30\%$ ), according to Zhang et al. (2004).

### Determination of AMF colonization

The root staining and discoloration method described by Koske and Gemma (1989) was used. Since *T. indica* roots are characterized as being woody and reddish, the method was adapted and roots were submerged for 1 hour in 20% of KOH at 90 °C, 20 minutes in 1% hypochlorite, and 5 minutes in 10% of HCL. Lastly, roots were immersed in 0.05% trypan blue to stain internal tissues, as well as AMF vesicles and arbuscules present inside the tree roots. The percentage of colonization was determined in samples

taken from five trees, through the microscope slide intersection method, using one slide per sample (McGonigle et al. 1990). In this method, roots were aligned parallel to the long axis of the slide and observed at 200x magnification. The number of intersections was examined by counting arbuscules, vesicles and hyphae. This procedure was repeated in 12 visual fields of root simples from each tree.

## RESULTS AND DISCUSSION

A total of 15 AMF species associated with tamarind (*Tamarindus indica*) crop soils were identified and are listed with their abundances in Table 2. Of the species found, 53% belonged to the Glomeraceae family, 20% to the Acaulosporaceae, and 13% to the Diversisporaceae. In terms of genus-level diversity, *Glomus* was the most abundant, representing 33% of the identified species, followed by *Acaulospora*, *Diversispora*, and *Rhizophagus*, each accounting for 13% of the total species (Figure 1). The average percentage of colonized trees was of 50%, and regarding the observed structures in the microscope's visual field, 39% were found with hyphae, 31% with arbuscules and 14% with vesicles (Figure 2). On the other hand, the present investigation found that in the roots of *T. indica* evaluated, there were other structures corresponding to dark septate endophytes, which are also part of the rhizosphere.

**Table 2.** Occurrence frequency and global frequency (F%) of arbuscular mycorrhizal fungus species in soil around of trees of *Tamarindus indica* trees in the municipality of Sopetrán.

ID	Family	Species	Size / diameter spore (µm)	Characteristics & INVAM spore color	Frequency of occurrence	F (%)
a	Acaulosporaceae	<i>Acaulospora aff. delicata</i>	100	Globose spore, color (0-10/40-0 melon color)	R	14.29
b	Acaulosporaceae	<i>Acaulospora aff. denticulata cf.</i>	58 to 60	Globose spore, color (0-20/80-0 RGB orange color)	C	42.86
c	Acaulosporaceae	<i>Entrophospora aff. infrequens</i>	120 to 130	Globose spore to subglobose, color (0-10/40-0 melon color)	R	14.29
d	Claroideoglomeraceae	<i>Claroideoglomus aff. etunicatum</i>	110 to 140	Globose spore to ovoid, color (0-40/100-10 orange color)	MC	71.43
e	Diversisporaceae	<i>Diversispora sp1 cf.</i>	90	Globose spore to subglobose, color (0-0/20-0 light yellow color)	R	14.29
f	Diversisporaceae	<i>Diversispora sp2 cf.</i>	70	Globose spore to subglobose, color (0-0/50-0 pale orange color)	R	14.29

Table 2

ID	Family	Species	Size / diameter spore (µm)	Characteristics & INVAM spore color	Frequency of occurrence	F (%)
g	Glomeraceae	<i>Funneliformis geosporum</i>	130 to 140	Globose spore to ovoid, color (0-10/20-10 yellow color)	R	14.29
h	Glomeraceae	<i>Glomus</i> aff. <i>macrocarpum</i>	120	Globose spore to subglobose, color (0-60/70-10 mid orange color)	R	28.57
i	Glomeraceae	<i>Glomus glomerulatum</i>	56	Globose spore to subglobose, color (20-20/40-0 persian orange color)	MC	57.14
j	Glomeraceae	<i>Glomus rubiforme</i>	40 to 50	Ovoid spore to subglobose, color (0-30/70-10 mandarin color)	C	42.86
k	Glomeraceae	<i>Glomus</i> sp1	150	Globose spore, (20/100-0 yellow color)	D	85.71
l	Glomeraceae	<i>Glomus</i> sp2 aff. <i>badium</i>	80	Globose spore to ovoid, color (0-40/50-10 ochre color)	R	28.57
m	Glomeraceae	<i>Rhizophagus</i> aff. <i>clarus</i>	50 to 71	Globose spore to ovoid, color (0-10/30-10 melon color)	MC	57.14
n	Glomeraceae	<i>Rhizophagus</i> sp1.	70	Globose spore to subglobose, color (0-30/70-10 mandarin color)	R	14.29
o	Paraglomeraceae	<i>Paraglomus</i> aff. <i>occultum</i>	70	Globose spore to avoid, color (0-0/10-0 light yellow color)	R	14.29

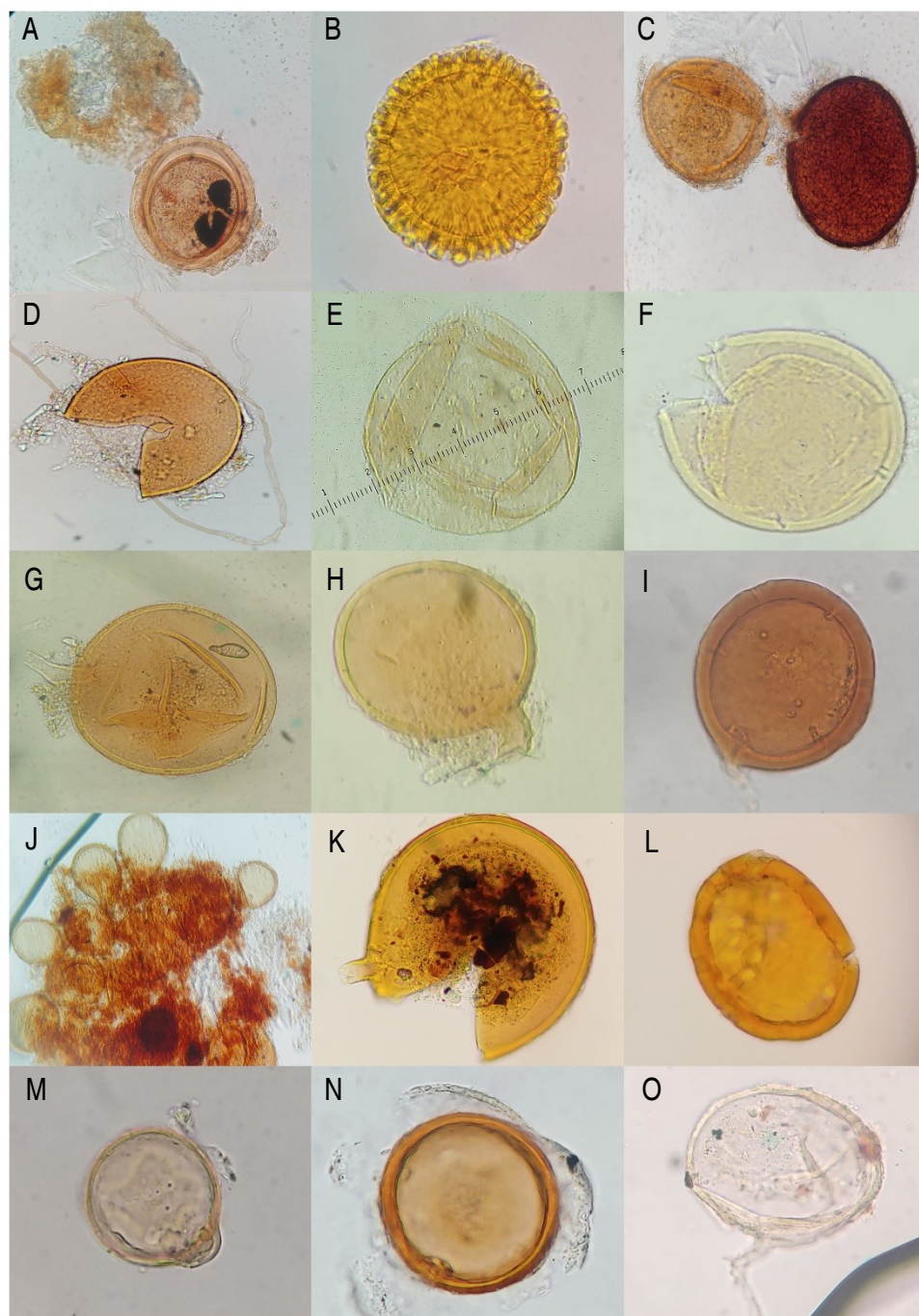
D = dominant ( $85\% \leq FO \leq 100\%$ ), VC = very common ( $50\% \leq FO < 85\%$ ), C = common ( $30\% \leq FO < 50\%$ ), R = rare ( $FO < 30\%$ ) (Zhang et al. 2004).

Bourrou et al. (2010) documented to AMF colonization ranging from 3.9 to 11% of the roots of *T. indica* trees, with higher colonization rates in trees established on soils under poor conditions and subjected to drought. In the present study, the colonization percentage found was significantly greater (50%) than that reported by Arcos (2015), who also assessed trees from the same region as those evaluated in this study and found 12% of colonization. Some authors report that differences in the colonization percentage may be influenced by a series of environmental and functional factors such as host, regional location, temperatures, rain and the availability of nutrients to soils (Smith and Read 2008). It is possible that the tamarind crops particular conditions in this case, such as medium phosphorous content, limited availability of water, high competence with unwanted plants and the absence of other farming practices, set out an environment in which some AMF are adapted to tolerate stress conditions, in the sense suggested by Chagnon

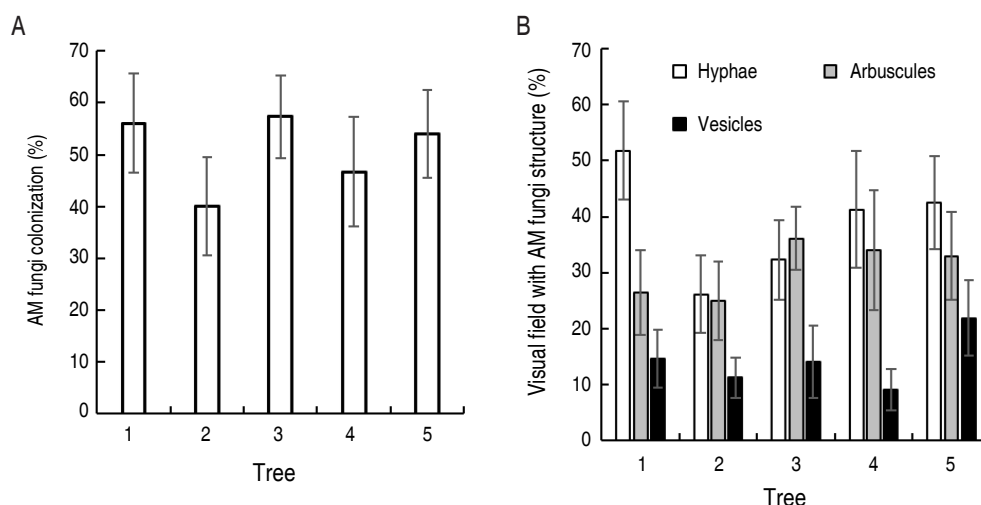
et al. (2013). Different mechanisms have been proposed to explain physiological, molecular and morphological changes in symbiotic plants when overcoming the effects of water stress. These mechanisms explain how the plants survive and maintain vigorous growth under the aforementioned conditions (Abdelmalik et al. 2020; Cheng et al. 2021). Jiménez (2019) demonstrated that tamarind seedlings inoculated with *Glomus* commercial had a greater amount of dry matter in roots and aerial parts than non-inoculated seedlings, as well as a faster recovery when seedlings were attacked by leaf-cutting ants.

In this study, a higher percentage of the genus *Glomus* associated with tamarind was found, while Arcos (2015) found a higher presence of *Acaulospora*. These two genera are abundant in tropical soil (Peña-Venegas et al. 2007; Cofré et al. 2019). Additionally, they contain species that have been reported to be associated with plants exposed to episodes of drought (Lenoir et al. 2016; Bahadur et al. 2019).





**Figure 1.** *Tamarindus indica* spore diversity in Sopetrán municipality, Antioquia - Colombia. **A.** *Acaulospora* aff. *delicata*, **B.** *Acaulospora* aff. *denticulata* cf., **C.** *Entrophospora* aff. *Infrequens*, **D.** *Claroideoglomus* aff. *etunicatum* cf., **E.** *Diversispora* sp1 cf., **F.** *Diversispora* sp2 cf., **G.** *Funneliformis* geosporum, **H.** *Glomus* aff. *macrocarpum*, **I.** *Glomus* *glomerulatum*, **J.** *Glomus* *rubiforme*, **K.** *Glomus* sp1, **L.** *Glomus* sp2 aff. *badium*, **M.** *Rhizophagus* aff. *clarus*, **N.** *Rhizophagus* sp1 and **O.** *Paraglomus* aff. *occultum*.



**Figure 2.** Tamarind roots colonized by AM fungi. **A.** Colonization and **B.** Microscope visual field with AMF presence.

## CONCLUSION

*Tamarindus indica* is a species that establishes symbiotic connections with a great variety of AM fungi, specifically those from the genera *Glomus* and *Acaulospora* in the tropical dry forest of Western Antioquia. The percentage of AMF colonization highlights its importance to tamarind plants in an environment prone to recurring drought. Future research could focus on defining mycorrhizal dependence, exploring its interaction with nitrifying bacteria and confirming the species identity through molecular techniques.

## CONFLICT OF INTEREST

The authors express that they do not have any conflict of interest.

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