



## Effect of arbuscular mycorrhizae on sugarcane and on the biocontrol of *Fusarium andiyazi*

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### ABSTRACT

**Background/Objective.** Within the *Fusarium* species complex that causes sugarcane root rot, *F. andiyazi* stands out for its pathogenicity. The aim of this study was to evaluate the effect of native arbuscular mycorrhizal fungi (AMF) from Morelos on sugarcane growth and their potential for the biocontrol of *F. andiyazi*.

**Experimental development.** Soil samples were collected from sugarcane fields in Jojutla, Morelos, where fifteen AMF morphospecies were identified and used as treatments in two bioassays. In the first bioassay, growth parameters and mycorrhizal colonization were evaluated. In the second, the biomass of plants inoculated with *F. andiyazi* and the degree of root necrosis were analyzed as indicators of biocontrol. Data were analyzed using ANOVA and mean separation tests ( $p \leq 0.05$ ).

**Results.** In the first bioassay, the genera *Acaulospora* and *Glomus* showed the highest mycorrhization values and significantly increased plant height, as well as root length and fresh weight. In the second bioassay, mycorrhizal plants inoculated with *F. andiyazi* showed significant increases in root length and dry weight. The morphospecies *G. ambisporum* and *G. mosseae* limited root infection to approximately 10% damage.

**Conclusion.** Native AMF from Morelos demonstrated their ability to stimulate sugarcane growth and reduced the damage caused by *F. andiyazi*.

**Keywords:** Glomeromycota, Mycorrhizal colonization, Mycorrhizae, *Fusarium*, Sugarcane



## INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a crop of high economic value in tropical regions worldwide; its juice provides 80% of the world's sugar and 40% of fuel ethanol (Juntahum *et al.*, 2020). Mexico ranks as the sixth-largest sugarcane producer globally, with more than 800,000 ha cultivated across 267 municipalities in 15 states. In Morelos, production is high, reaching 101 t ha<sup>-1</sup> (SIAP, 2023).

Diseases are among the main factors limiting sugarcane productivity worldwide and are caused by various groups of fungi, bacteria, viruses, and nematodes. Fungal diseases are the most common in Mexico, including smut (*Sporisorium scitamineum*), orange rust (*Puccinia kuehnii*), pokkah boeng or top deformation (*Gibberella fujikuroi*), eye spot (*Bipolaris sacchari*), red rot (*Physalospora tucumanensis* = *Colletotrichum falcatum*), brown spot (*Cercospora longipes*), and pineapple disease (*Ceratocystis paradoxa*) (Salgado-García *et al.*, 2013), as well as root rot (*Fusarium* spp.) (Martínez-Fernández *et al.*, 2015). The latter involves a complex of *Fusarium* species, among which *F. andiyazi* stands out for its pathogenicity (Martínez-Jaimes *et al.*, 2020).

New management practices are currently being developed to increase sugarcane production, including improved tillage systems, fertilizer application, and the management of soil microorganism communities. Among beneficial organisms, arbuscular mycorrhizal fungi (AMF) stand out for their wide distribution. They act as obligate symbionts in plant roots and play a key role in the functioning of agroecosystems (Verbruggen *et al.*, 2010). It has also been documented that AMF are adapted to various environments and form symbiotic relationships with more than 200,000 plant species; however, only 240 AMF species have been described to date, although molecular studies indicate that species diversity is even higher (Lee *et al.*, 2013).

AMF colonize plant roots, where they develop hyphae, arbuscules, and vesicles in cortical cells, as well as form spores and hyphae in the rhizosphere. The formation of a hyphal network by AMF significantly increases root access to a larger soil area, promoting plant growth (Bowles *et al.*, 2016). This symbiosis is particularly important because it enhances water and nutrient uptake by the plant—mainly phosphorus and nitrogen—while AMF obtain carbohydrates in return for their development (Campo *et al.*, 2020). AMF colonization increases resistance to pathogens that cause root necrosis through several mechanisms: by modifying root morphology, regulating the synthesis of secondary metabolites, improving the rhizosphere microenvironment, directly competing with pathogenic microorganisms for invasion sites, and inducing the plant's defense system (Weng *et al.*, 2022). The objectives of this study were to isolate arbuscular mycorrhizal fungi species from sugarcane soils in Morelos and to evaluate their potential to promote plant growth and limit *Fusarium andiyazi* infection in the roots.

## EXPERIMENTAL DEVELOPMENT

This study was conducted in the Biological Control Laboratory of the Biotechnology Research Center (CEIB) and in the Entomology and Phytopathology Laboratory of the Biological Research Center (CIB) at the Autonomous University of the State of Morelos.

**Soil sampling.** Soil samples for AMF isolation were collected from sugarcane crops in the municipality of Jojutla, Morelos. Five sites were selected, and samples were taken from the rhizosphere of asymptomatic sugarcane plants of the CP 72-2086 variety, with five months

of growth. Soil samples were collected 10 cm from the base of the plants and at a depth between 10 and 30 cm (Juntahum *et al.*, 2020). From each collection site, two 1-kg soil samples were obtained and placed in sealed bags.

**Isolation of AMF spores.** AMF spores were recovered from the collected soil samples using the wet sieving and decanting technique (Gerdemann and Nicolson, 1963) followed by centrifugation with 50% sucrose (Brundrett *et al.*, 1996). Spores were quantified under a stereomicroscope, and the number of spores obtained per 100 g of dry soil was determined.

**Identification of AMF spores.** The analyzed soil fractions were placed on watch glasses for observation under a Leica DM50 stereomicroscope to separate spores according to their morphological characteristics, such as shape, size, and color, as described in the INVAM guide (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi). Temporary slides were prepared from the selected spores using a PVLG (polyvinyl-lacto-glycerol) solution. These preparations were then observed under a Nikon Eclipse Ni light microscope at 40× magnification, and AMF spores were identified based on the taxonomic works of Pérez and Schenk (1990), Schüßler and Walker (2010), and Souza (2015), with additional reference to the INVAM database (<http://invam.caf.wvu.edu>)

**Propagation of native AMF from sugarcane in trap plants.** The collected spores were grouped according to their morphological characteristics and subjected to a disinfection process with 0.5 mL of gentamicin for 10 minutes. Each selected spore group, identified as a morphospecies, served as the initial inoculum using sorghum plants as hosts in 2-L pots containing a mixture of peat, perlite, and vermiculite (1:1:1) (Marro *et al.*, 2014). Sorghum plants inoculated with the different identified AMF groups (Table 1) were grown for 45 days and then removed from the pots. Roots were separated along with the adhering substrate and placed in a plastic tray, where they were manually fragmented. These mixtures of root fragments and substrate residues containing AMF spores from each morphospecies were used to establish the two sugarcane bioassays.

**Table 1.** Morphospecies of arbuscular mycorrhizal fungi (AMF) used as treatments in the sugarcane bioassays.

Treatments	Morphospecies AMF
T1	<i>Acaulospora lacunosa/Acaulospora tuberculata</i>
T2	<i>Claroideoglossum lamellosum</i>
T3	<i>Acaulospora lacunosa</i>
T4	<i>Septoglossum deserticola</i>
T5	<i>Glomus ambisporum</i>
T6	<i>Rhizophagus irregularis</i>
T7	<i>Acaulospora tuberculata</i>
T8	<i>Glomus aggregatum</i>
T9	<i>Acaulospora 1/Glomus aggregatum/Sclerocystis 2</i>
T10	<i>Claroideoglossum luteum</i>
T11	<i>Glomus mosseae/ Funneliformis geosporum</i>
T12	<i>Sclerocystis 1</i>
T13	<i>Glomus mosseae</i>
T14	<i>Paraglossum occultum</i>
T15	<i>Funneliformis geosporum</i>
T16	Control

**Bioassay 1. Influence of AMF on sugarcane plant development.** To evaluate the effect of AMF on sugarcane plant development, a completely randomized design with 16 treatments was established. The morphospecies were applied individually or in consortia, along with an absolute control, with three replicates per treatment. Each plant represented

an experimental unit. For each treatment, nursery pots were filled halfway with the substrate prepared as described above, and 50 g of the mixtures containing AMF spores were added (Djocgoue *et al.*, 2019). Healthy sugarcane plants one month old (variety CP 72-2086), obtained from in vitro culture, were then placed and covered up to the stem base with another layer of substrate. Control plants were not inoculated with AMF. After 60 days, the following variables were evaluated: height (cm), stem diameter (cm), root length (cm), root fresh weight (g), and root dry weight (g). Plants were fertilized twice a week with Hoagland's solution without P and watered with tap water as needed (Ravnskov *et al.*, 2020).

**Evaluation of AMF colonization in sugarcane roots.** Plants were analyzed 30 days after inoculation with the treatments indicated in Table 1. From each treatment, fine root fragments (1–2 cm) were collected, cleared with 10% KOH, and stained with 0.05% trypan blue in lactoglycerol following the method of Phillips and Hayman (1970). Preparations were made with 50% glycerol and observed under a Nikon Ni light microscope (40×). The percentage of mycorrhizal colonization was determined using the magnified intersection method, considering the presence of hyphae, arbuscules, or vesicles (McGonigle *et al.*, 1990).

**Bioassay 2. Effect of AMF application on the biocontrol of *Fusarium andiyazi*.** A strain of *F. andiyazi* from the collection of phytopathogenic fungi of the Entomology and Phytopathology Laboratory at the CIB was used, whose pathogenicity had been previously verified (Martínez-Jaimes *et al.*, 2020).

Thirty days after applying the different AMF treatments to the sugarcane plants, *F. andiyazi* inoculation was carried out. A 50 mL suspension containing  $10^6$  conidia  $\text{mL}^{-1}$  was applied directly to the base of the stems (Yadav and Naik, 2014). Control plants without AMF were inoculated with 50 mL of the *F. andiyazi* conidial suspension. After 30 days, the following variables were evaluated: height (cm), number of stems, stem diameter (cm), root length (cm), root fresh weight (g), and root dry weight (g). At the end of the experiment, disease severity in sugarcane plants was assessed according to the following scale (Asran and Buchenauer, 2003): 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% root necrosis, 5 = dead and fragmented root system, 6 = entire plant dead.

**Statistical analysis.** Data from the variables analyzed in bioassays 1 and 2 were subjected to analysis of variance (ANOVA), and Tukey's multiple mean comparison tests were performed at a 95% significance level to compare differences among treatments. The percentages of root colonization by AMF that did not meet the assumptions of normal distribution were transformed using the arcsine function to homogenize variances. ANOVA and Tukey tests ( $p \leq 0.05$ ) were conducted using the SAS v9.0 statistical package.

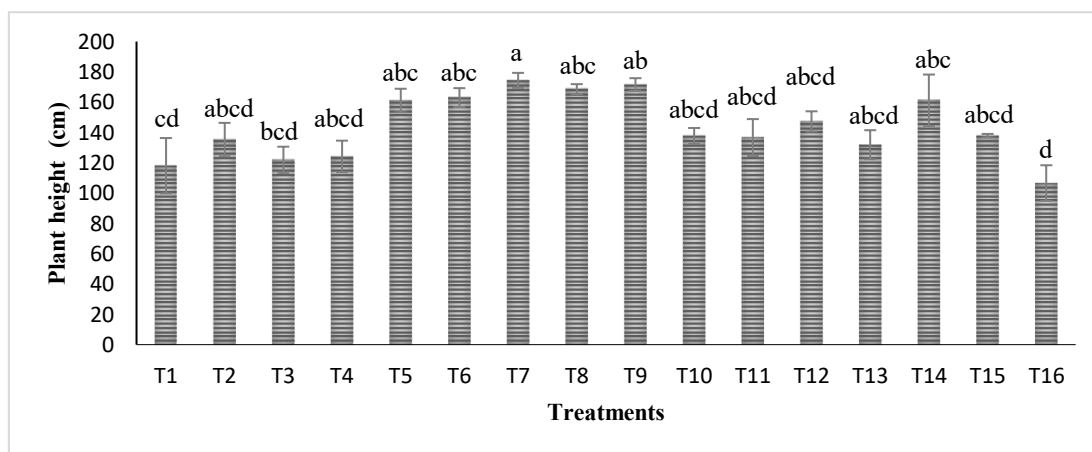
In the soil samples analyzed from the five sugarcane fields in Jojutla, Morelos, native AMF propagules were found. AMF population variability ranged from 280 to 863, with an average of 497 spores per 100 g of soil. These results are consistent with reports from fields in Jalisco, which showed an average of  $248 \pm 37$  spores per 100 g of soil (Sánchez-Lizarraga *et al.*, 2017). These values are higher than those reported by Juntahum *et al.* (2020), who found 11–168 spores per 100 g of soil in northeastern Thailand. However, they are lower than those reported by Salgado-García *et al.* (2013), who mentioned an average of 943 spores per 100 g of soil. It has been documented that variation in the number of

spores at sampling sites depends on several factors, including cultivation practices, environmental conditions, soil types, and host plant species (Kadian *et al.*, 2018).

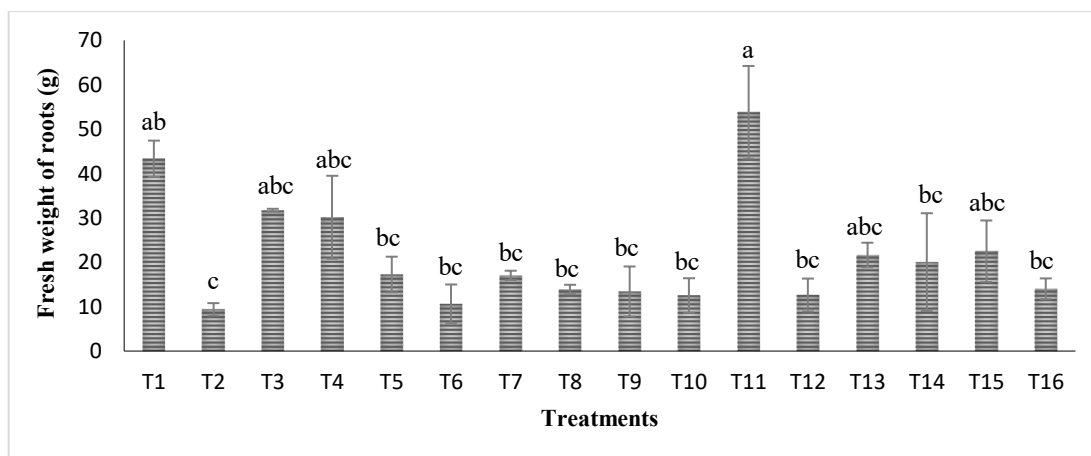
From the sugarcane soil samples, 15 AMF morphospecies were isolated—12 identified at the species level and three at the genus level. These morphospecies belong to the families Acaulosporaceae, Claroideoglomeraceae, Glomeraceae, and Paraglomeraceae, within the orders Diversisporales, Glomerales, and Paraglomerales.

**Bioassay 1. Influence of AMF on sugarcane plant development.** Sixty days after treatment application, some differences were observed among the evaluated variables. According to the statistical analysis, only plant height, root fresh weight, and root length showed statistically significant differences ( $p \leq 0.05$ ). Regarding height, the highest value was obtained with treatment T7 (*A. tuberculata*), reaching 174.37 cm, compared with 106.63 cm in T16 (control) (Figure 1). The positive effect of mycorrhizal inocula applied to sugarcane agrees with Wilches-Ortiz *et al.* (2019), who reported significant differences in plant height and root diameter when applying native Colombian AMF from the genera *Glomus*, *Acaulospora*, and *Funneliformis* compared with commercial mycorrhizae and chemical fertilization. Similarly, Ventura *et al.* (2018) observed greater height development in sugarcane varieties IAC SP 955094 and CTC 9004M cultivated in Brazil when treated with native AMF (*Acaulospora*, *Claroideoglosum*, *Diversispora*, *Glomus*, and *Gigaspora*).

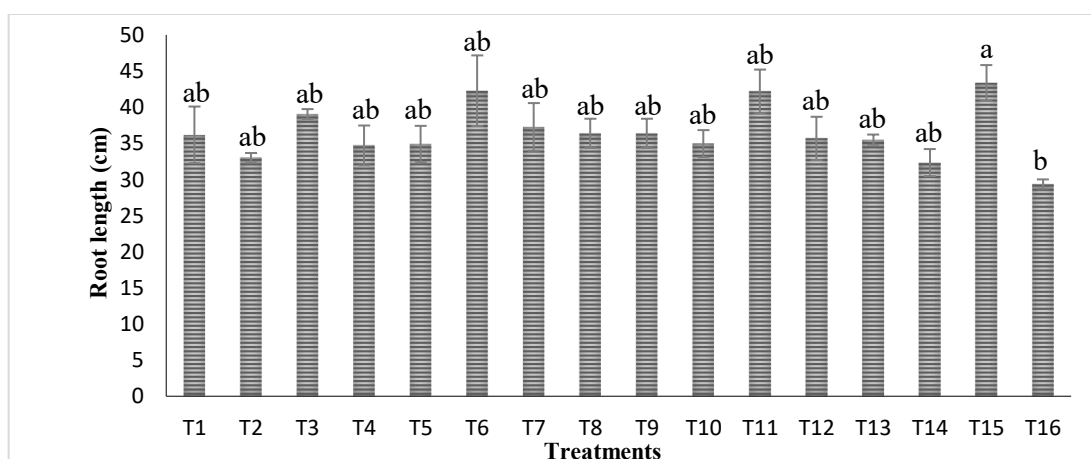
Significant differences were observed in root fresh weight. The analysis of variance showed that the best treatments were T11 (*G. mosseae*/*F. geosporum*) with 53.8 g and T1 (*A. lacunosa*/*A. tuberculata*) with 43.33 g. In contrast, the lowest values were recorded for T2 (*C. lamellosum*) with 9.4 g and T6 (*R. irregularis*) with 10.6 g (Figure 2). Regarding root length, the highest value was obtained with T15 (*F. geosporum*) at 43.37 cm, while the lowest value corresponded to T16 (control) at 29.37 cm (Figure 3). Other similar studies have also highlighted the positive effect of these morphospecies. Li *et al.* (2012) found significant increases in plant height, biomass, and grain yield when inoculating rice with *G. mosseae*. Juntahum *et al.* (2020) reported increased sugarcane productivity when incorporating the arbuscular mycorrhiza *F. mosseae* into the soil as a complement to native mycorrhizae in a field in Thailand. Similarly, Rojas-Martínez (2014) found that applying *Acaulospora lacunosa* to chili pepper (*Capsicum annuum*) plants increased root length and dry weight.



**Figure 1.** Effect of AMF inoculation on sugarcane plant height in Bioassay 1 (mean  $\pm$  standard error). Different letters indicate significant differences among treatment means according to Tukey's test ( $p \leq 0.05$ ), C.V. = 11.81.



**Figure 2.** Effect of AMF inoculation on sugarcane root fresh weight in Bioassay 1 (mean  $\pm$  standard error). Different letters indicate significant differences among treatment means according to Tukey's test ( $p \leq 0.05$ ), C.V. = 17.67.



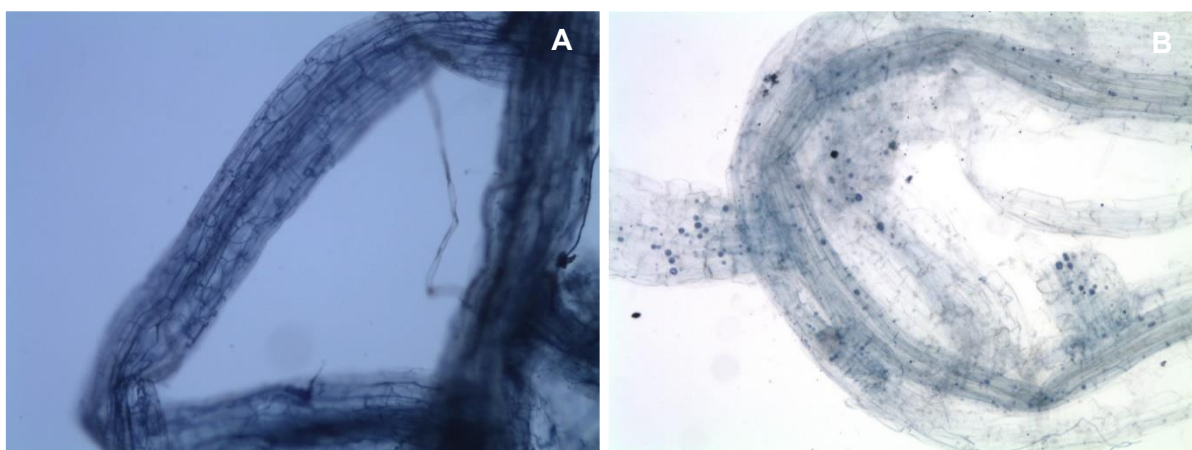
**Figure 3.** Effect of AMF inoculation on sugarcane root length in Bioassay 1 (mean  $\pm$  standard error). Different letters indicate significant differences among treatment means according to Tukey's test ( $p \leq 0.05$ ), C.V. = 12.19.

**AMF colonization in sugarcane roots.** AMF applied individually or in consortia to sugarcane plants of the CP 72-2086 variety colonized the roots to varying degrees, ranging from 13.3% to 93.3%. Microscopic analysis of the roots revealed typical AMF structures such as spores, hyphae, vesicles, and arbuscules. The results showed significant differences among the evaluated treatments ( $p \leq 0.05$ ), with T8 (*G. aggregatum*) exhibiting the highest colonization at 93.3%, followed by T1 (*A. lacunosa/A. tuberculata*) with 73.3%. In contrast, T5 (*G. ambisporum*) showed the lowest mycorrhizal colonization at 13.3% (Table 2). The degree of colonization developed in the roots of plants inoculated with the different treatments is similar to that observed in sugarcane grown in Mexico—for example, an average of 88.9% in Chontalpa, Tabasco (Salgado-García *et al.*, 2013), and 60% in a field in El Arenal, Jalisco (Sánchez-Lizarraga *et al.*, 2017). Similarly, in a sugarcane crop from northeastern Thailand, 10–22% mycorrhizal colonization was reported (Juntahum *et al.*, 2020). In those reports, mycorrhizal colonization in sugarcane was analyzed without specifying the participation of individual morphospecies. The species *G. aggregatum* showed the most efficient root colonization in sugarcane (Figure 4), as has been documented in other plants, such as faba bean, with 30–89% colonization (Abd-Alla *et al.*, 2015); licorice (*Glycyrrhiza glabra*), 86.5% (Selvaraj and Sumithra, 2011); and *Acacia seyal*, 31.57% (Manga *et al.*, 2017).

**Table 2.** Arbuscular mycorrhizal fungi (AMF) colonization in sugarcane roots.

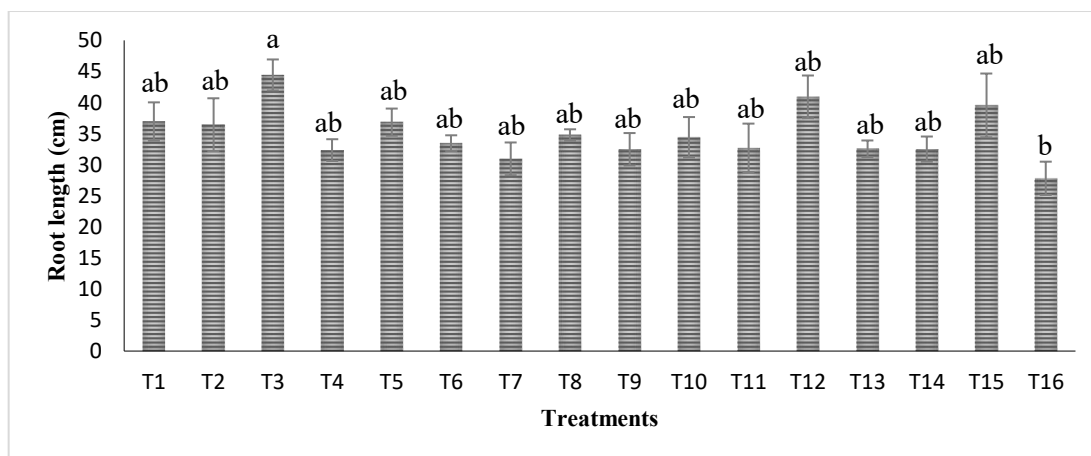
Treatments	Morphospecies AMF	Colonization % $\pm$ SD *
T1	<i>Acaulospora lacunosa/Acaulospora tuberculata</i>	73.3 $\pm$ 3.8 ab
T2	<i>Claroideoglossum lamellosum</i>	23.3 $\pm$ 15.3 bc
T3	<i>Acaulospora lacunosa</i>	16.6 $\pm$ 4.7 bc
T4	<i>Septoglossum deserticola</i>	50.0 $\pm$ 26.6 abc
T5	<i>Glomus ambisporum</i>	13.3 $\pm$ 4.7 c
T6	<i>Rhizophagus irregularis</i>	46.7 $\pm$ 12.3 abc
T7	<i>Acaulospora tuberculata</i>	33.3 $\pm$ 9.3 bc
T8	<i>Glomus aggregatum</i>	93.3 $\pm$ 10.6 a
T9	<i>Acaulospora 1/Glomus aggregatum/Sclerocystis 2</i>	66.6 $\pm$ 16.2 abc
T10	<i>Claroideoglossum luteum</i>	26.7 $\pm$ 10.7 bc
T11	<i>Glomus mosseae/ Funneliformis geosporum</i>	50.0 $\pm$ 18.4 abc
T12	<i>Sclerocystis 1</i>	36.6 $\pm$ 9.4 abc
T13	<i>Glomus mosseae</i>	20.0 $\pm$ 7.4 bc
T14	<i>Paraglossum occultum</i>	70.0 $\pm$ 13.3 abc
T15	<i>Funneliformis geosporum</i>	70.0 $\pm$ 10.6 abc

SD = Standard deviation. \*Means with different letters are statistically significant (Tukey,  $p \leq 0.05$ ). C.V. = 18.4%.

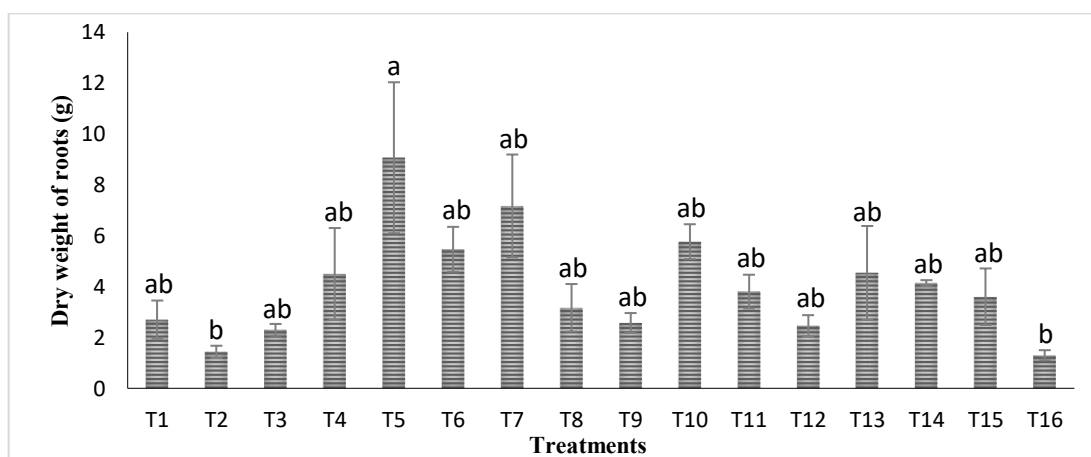


**Figure 4.** Mycorrhizal colonization of AMF in sugarcane roots stained with trypan blue (40 $\times$ ). (A) Hyphal development of *Glomus aggregatum* (T8). (B) Hyphal and spore development of *Claroideoglossum luteum*.

**Bioassay 2. Effect of AMF application on the biocontrol of *Fusarium andiyazi*.** Plants in the control treatment (without AMF application) showed characteristic wilt symptoms, such as leaf flaccidity and slight chlorosis, 30 days after inoculation with *F. andiyazi*, similar to the findings of Martínez-Jaimes *et al.* (2020). In contrast, treatments with plants previously inoculated with AMF and the pathogen developed only slight foliar chlorosis. Additionally, no differences were observed in plant height, number of stems, stem diameter, or root fresh weight. However, statistically significant differences ( $p < 0.05$ ) were observed in root length. The mean analysis showed that plants from T3 (*A. lacunosa*) developed the greatest root length (42.58 cm), whereas plants from T16 (control) had the lowest value (28.13 cm) (Figure 5). Significant differences were also observed in root dry weight, with T5 (*Glomus ambisporum*) showing 7.20 g compared to 1.56 g in T16 (control) (Figure 6).



**Figure 5.** Effect of AMF inoculation on sugarcane root length in Bioassay 2 (mean  $\pm$  standard error). Different letters indicate significant differences among treatment means according to Tukey's test ( $p \leq 0.05$ ), C.V. = 14.39.



**Figure 6.** Effect of AMF inoculation on sugarcane root dry weight in Bioassay 2 (mean  $\pm$  standard error). Different letters indicate significant differences among treatment means according to Tukey's test ( $p \leq 0.05$ ), C.V. = 27.42.

Sugarcane plants without mycorrhizal treatment showed a severity level of 2, corresponding to 25–50% root infection caused by *Fusarium andiyazi*. In contrast, plants inoculated with mycorrhizal treatments showed a severity level of 1, corresponding to 0–25% root infection. In plants from T5 (*G. ambisporum*) and T13 (*G. mosseae*), the infection rate was 10%. In this regard, it has been documented that *G. mosseae* applied to tomato plants reduces the disease index caused by *Rhizoctonia solani* by 60% (Kareen and Hassan, 2014).

This beneficial effect of AMF in reducing disease severity in plants involves a compatible interaction between AMF species and their host. This relationship enhances nutrient uptake efficiency, thereby strengthening the plant against potential pathogen attacks (Olumayoga *et al.*, 2018; Ravnkov *et al.*, 2020). It has also been documented that resistance in mycorrhizal plants is due to the activation of certain defense mechanisms, including the production of secondary metabolites such as phenolic compounds (Corrales-Sánchez *et al.*, 2021), which have antimicrobial properties (Mandal *et al.*, 2010). These compounds accumulate in root exudates, suppressing pathogen development in the rhizosphere (Ren *et al.*, 2015).

## CONCLUSIONS

From sugarcane soil samples collected in Jojutla, Morelos, 15 AMF morphospecies were isolated—12 identified at the species level and three at the genus level. The most abundant AMF genera were *Acaulospora*, *Claroideoglosum*, and *Glomus*.

The application of AMF to sugarcane significantly influenced plant height, root fresh weight, and root length. AMF colonized sugarcane roots to varying degrees, with *G. aggregatum* showing the highest colonization at 93.3%. Sugarcane plants inoculated with *Glomus ambisporum* and *Glomus mosseae* showed reduced disease severity induced by *F. andiyazi*. Finally, mycorrhizal sugarcane plants inoculated with *F. andiyazi* exhibited significant increases in root length and dry weight.

## Limitations

In this study, molecular identification of the arbuscular mycorrhizal fungi was not performed.

## Conflicts of interest

None declared.

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