Colegio de Postgraduados

# Infectivity and effectiveness of an arbuscular mycorrhizal fungi native inoculum on the growth and absorption of macroelements in maize (*Zea mays* L.) plants

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## ABSTRACT

**Objective**: To evaluate the impact of an arbuscular mycorrhizal fungi (AMF) native inoculum on the growth and absorption of macroelements in maize (*Zea mays*) under seedbed conditions.

**Design/Methodology/Approach**: The experiment consisted of a completely randomized experimental design with four treatments (three inocula of arbuscular mycorrhizal fungi and an uninoculated control) and 30 repetitions, resulting in 120 experimental units. Two consortia of commercial AMF were used: AMF1, AMF2 and, one native AMF3 treatment. The experiment included a control (T) without inoculation. The variables evaluated were: total dry weight and mycorrhizal colonization in plants and nutritional content of nitrogen (N), phosphorus (P), and potassium (K) in plant tissue.

**Results**: The application of the native inoculum (AMF3) had a significantly greater impact on total dry weight, as well as on P and K content in plant tissue, than the rest of the treatments (particularly the control). AMF3 showed 18% more mycorrhizal colonization than the rest of the treatments.

**Study Limitations/Implications**: The experiment was carried out under seedbed conditions and did not include the production stage; therefore, the impact of the treatments on maize production is unknown.

**Findings/Conclusions**: Maize (*Zea mays*) plants had a positive response to inoculation with arbuscular mycorrhiza-forming fungi. The bio-technological potential of AMF3 (*Claroideoglomus claroideum*), a mycorrhizal consortium native to the rhizosphere, can be used to reinforce the development of maize plants, increasing the absorption of macroelements and inducing greater growth and root development.

**Keywords**: Root growth, mycorrhizal colonization, phosphorus, nitrogen, potassium.

## INTRODUCTION

Maize (*Zea mays*) originated in the Americas, specifically central-eastern Mexico (Reyes *et al.*, 2022). The cultivation of maize has significant economic importance worldwide. Mexico ranks eighth in global production with 27,228,242 tons produced in 2019, behind

the United States, China, Brazil, Argentina, Ukraine, Indonesia, and India (FAOSTAT, 2022). Within Mexico, Sinaloa ranks first with 6,440,204 tons, surpassing states such as Jalisco, Estado de México, and Guanajuato (FAOSTAT, 2022). The plant development of this species is positively influenced by certain groups of naturally-occurring soil microorganisms, including arbuscular mycorrhizal fungi (AMF), which establish a symbiotic association with the roots of most vascular plants found in natural ecosystems and crops with economic and agricultural importance (Villavicencio and Garces, 2023).

However, several factors limit the production of maize (*Zea mays*), particularly the lack of nutrients and water, as well as pests and diseases caused by soilborne phytopathogens. These challenges have led to a dependence on the intensive use of synthetic products in maize cultivation, whose residues contribute to the pollution of soil-water-atmosphere ecosystems (Alvarado *et al.*, 2021).

Therefore, Palacios-Chávez (2023) and Torán-Figueroa (2023) suggest that the use of biofertilizers based on native arbuscular mycorrhizal fungi (AMF) and the application of vermicompost are viable alternatives that can reduce the use of chemical products, providing benefits to the plant and mitigating environmental problems. AMF are soil-borne fungi from the phylum *Glomeromycota* that establish symbiotic associations with more than 90% of terrestrial plants (Delgado and Gutiérrez, 2022; Schüßler *et al.*, 2001). Plants provide carbohydrates as a food source for the fungi, which in exchange offer various benefits to the plants. AMF form a mycelial network that allows greater soil exploration, enhancing the plants' capacity for water and nutrient absorption (Zhang *et al.*, 2020). They also provide resistance against biotic (pathogens, herbivores) and abiotic (drought, salinity, heavy metals) factors (Ravnskov *et al.*, 2020).

Meanwhile, consortia of native arbuscular mycorrhizal fungi are usually more effective than those composed of exotic species or a single species (Bashan *et al.*, 2000; Ortas and Ustuner, 2014), as a result of the adaptation of fungi to specific natural conditions. Their introduction to different environments, can lead to maladaptations to the new conditions (Rillig and Mummey, 2006).

Therefore, this study evaluated the impact of a native arbuscular mycorrhizal fungi inoculum on the growth and macronutrient absorption of maize (*Zea mays*) under seedling conditions.

## MATERIALS AND METHODS

### Study location

The research was conducted at seedbed-level under shade house conditions at the Faculty of Agronomy of the Universidad Autónoma de Sinaloa, in Culiacán, Sinaloa, Mexico, at 24° 37' 29" N, 107° 24' 30" W, and 38 m.a.s.l. From February 27 to May 8, 2020, the temperature ranged from 35 °C (maximum) to 18 °C (minimum).

### Microbiological material

Two commercial consortia of arbuscular mycorrhizal fungi (AMF) were used, along with a native consortium and a control without AMF application. The commercial AMF consortia included AMF1 (*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*,

*Acaulospora scrobiculata*, and *Gigaspora margarita*; from the MycorrazineVA brand, produced in Mexico) and AMF2 (formulated with spores from selected strains of vesicular-arbuscular mycorrhizal fungi, from the Glumix brand, distributed in Celaya, Guanajuato). Additionally, an AMF3 treatment with a native arbuscular mycorrhizal fungi inoculum (*Claroideoglomus claroideum*) was used. This inoculum was previously extracted from the rhizosphere of the huamuchil (*Pithecellobium dulce*) tree located in Ciudad de los Niños, Navolato, Sinaloa, with a radial biodiversity of watergrass (*Echinochloa crus-galli*) and Bermuda grass (*Cynodon dactylon*), as well as a control (T) without inoculation.

**Seed sowing and inoculation**

Seeds were sown on March 10, 2020, in 60-cell polystyrene trays, which were then cut into five sections of six cells each. One seed was planted per cell in a mixture of soil (characteristics specified in Table 1) and Kekkila® peat (1:1 v/v), which had been previously sterilized for two consecutive days (121 °C for 3 hours).

Inoculation with arbuscular mycorrhizal fungi was performed at the time of sowing, applying 0.5 g (AMF1), 0.5 g (AMF2), and 21.6 g (AMF3) of inoculum, to ensure that each plant received 20 spores. The plants were watered once every day with purified drinking water until the root ball was completely moistened. Additionally, the plants were fertilized every 30 days with a 25% Steiner solution without P (42 mg $L^{-1}$ of N, 68 mg L−1 of K, 45 mg $L^{-1}$ of Ca, and 12 mg $L^{-1}$ of Mg), adjusting the pH to 6.5 using sulfuric acid.

**Evaluated variables**

The plants were evaluated and harvested 70 days post-sowing (dps) and total dry weight, mycorrhizal colonization, and macronutrient content were measured.

Dry biomass was obtained drying the samples in a Felisa™ FE-292 dehydration oven (70 °C for 72 hours) and separately weighing the shoots and roots using an Aczet CZ 30 analytical balance (Conquer Scientific).

The percentage of mycorrhizal colonization was assessed using the clearing and staining technique (Phillips and Hayman, 1970).

A sample of the shoot of the plants was taken from each treatment to determine the nutrient contents of N, P, and K in plant tissue. These samples were placed in a forced-air oven at 70 °C until a constant weight was achieved; subsequently, they were ground for laboratory analysis. The evaluation of N, P, and K content was performed using the methodologies described below. N was determined using the semi-micro Kjeldahl method (Etchevers, 1987). P was measured by colorimetry of molybdophosphate complexes reduced with ascorbic acid (AOAC, 1980). K was determined by the flame photometry methodology proposed by Rodríguez and Rodríguez (2015). Extracts obtained from dry

**Table 1**. Chemical and physical characteristics of the soil.

| E.C. (mS $cm^{-1}$) | pH | CEC ($Cmol_{(+)}kg^{-1}$) | Texture | N (mg $kg^{-1}$) | P (mg $kg^{-1}$) | K (mg $kg^{-1}$) | Ca (mg $kg^{-1}$) | Mg (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 0.85 | 7.11 | 44.46 | Arcillosa | 15.60 | 13.72 | 584.80 | 6687.50 | 973.30 |

E.C.: electrical conductivity; pH: hydrogen potential; CEC: cation exchange capacity.

digestion were used for K and P. To estimate the total contents, the concentrations of each element in the plant tissue were considered, along with the dry biomass weights of the shoot.

**Experimental design**

The experiment consisted of a completely randomized design with four treatments (three arbuscular mycorrhizal fungi inoculant and an uninoculated control) and 30 repetitions, resulting in 120 experimental units. An analysis of variance (ANOVA) and a means comparison test (Tukey, $\alpha=0.05$) were applied to analyze the data, using SAS for Windows (SAS Institute Inc., 2002).

## RESULTS AND DISCUSSION

The inoculation of maize plants with AMF3 had greater impact on total dry weight, as well as the P and K content in plant tissue than the other treatments, particularly control. This phenomenon indicates the potential of this AMF consortium as a promoter of plant growth, particularly in maize.

In cases where crops are sown directly in the field and do not undergo a seedbed stage, the seed should be inoculated at the time of sowing to reduce costs and minimize competition with the inoculated microorganism. This measure also ensures a successful symbiosis during seed germination, allowing the plant root to recognize the presence of the inoculum, before native opportunistic microorganisms displace the inoculated microorganism (Zuluenta *et al*., 2021). The dry weight of the maize plant shoots was significantly higher ($P\leq0.05$, 10.91% difference) in AMF2 than in AMF1 (Figure 1). Meanwhile, the dry weight of the root, was significantly higher ($P\leq0.05$) with AMF3 (Figure 1). Compared to control, it had a 21.73% greater margin. Similarly, plants inoculated with AMF2 and AMF3 recorded 13.0% and 12.7% more total dry weight than the uninoculated control, respectively, indicating a significantly higher ($P\leq0.05$) difference than both the AMF1 and the uninoculated control (Figure 1).

For its part, N content of maize plants was statistically higher with AMF3 than AMF2, recording a 3.42% difference (Figure 3). However, both the control and AMF1 had lower values than AMF3, but no significant difference was observed.

P content was significantly ($P\leq0.05$) higher with AMF3 than with the other treatments, with a difference of 8.10% (*versus* AMF2) and 24.86% (*versus* AMF1 and the control (T)) (Figure 4). The AMF2 treatment was statistically higher (18.23%) than AMF1 and the control (T).

Meanwhile, K content variable was significantly higher ($P\leq0.05$) with the AMF3 treatment than the control (11.20% difference) and statistically similar to AMF1 and AMF2 (Figure 5).

P plays a role in reactions that require energy within the cell, as an integral part of energy storage molecules (*e.g.*, adenosine triphosphate (ATP)). These molecules are formed as a result of photosynthesis and participate in plant respiration. Consequently, P is vital for the generation of new cells, for example, during the production of roots at the beginning of vegetative cycles. An increased P and K content was also observed in the plant tissue of

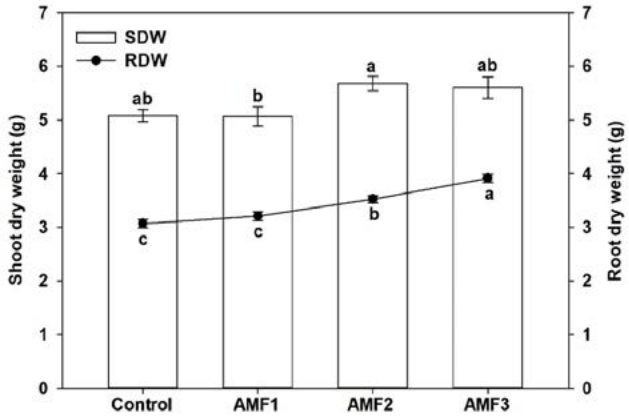


**Figure 1**. Root weight and shoot dry weight in maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). SDW=shoot dry weight, RDW=root dry weight. AMF1 *Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

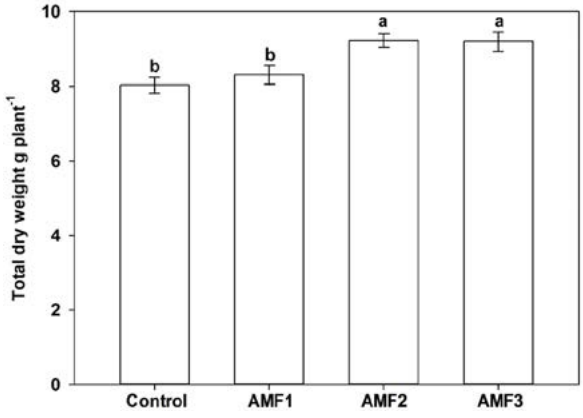


**Figure 2**. Total dry weight of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

maize inoculated with AMF, specifically with AMF3. This phenomenon can be attributed to the ability of AMF to absorb higher amounts of P. Faggioli *et al.* (2020) mention that AMF, through their hyphae and the secretion of extracellular phosphatases, are capable of capturing, transporting, and solubilizing the soil's scarce nutrient elements. Consequently, the plant's increased P uptake may have contributed to greater root development in maize plants. According to Paredes *et al.* (2021), phosphate strengthens the root system by promoting root extension and lateral branching. Additionally, the total dry biomass increased in plants inoculated with AMF, specifically with AMF3. A more developed root system allowed the plant to absorb more nutrients, which in turn resulted in higher dry biomass production, as noted by Ojeda *et al.* (2023).

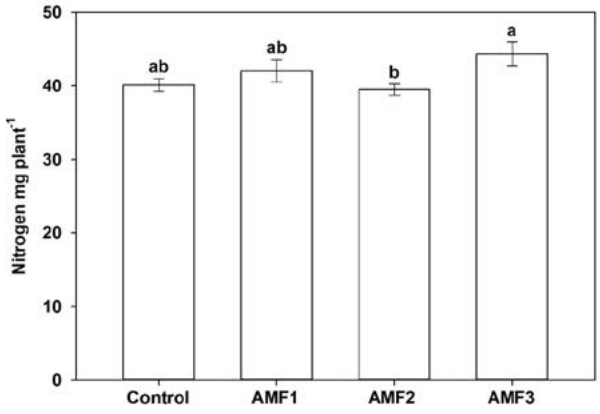


**Figure 3**. Nitrogen content of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

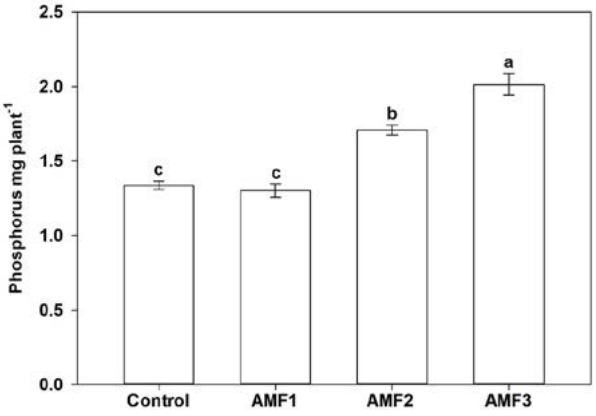


**Figure 4**. Phosphorus content of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

Total mycorrhizal colonization showed significant differences ($P \leq 0.05$) between AMF3 and AMF2, with a 78.53% difference. Specifically, AMF3 was significantly higher than AMF2 regarding vesicular colonization, with a 76.18% difference between these treatments. AMF3 had a statistically greater hyphal presence than AMF2 (80.31% difference). No mycorrhizal colonization was observed in AMF1 or control (T) (Table 2).

Mycorrhizal colonization was low in the commercial AMF treatments and absent in the uninoculated control; however, the response of the plants to the AMF2 and AMF3 consortium was favorable for most variables. Although the total mycorrhizal colonization percentage was low in AMF2, it did not limit the benefits of AMF for the plants. Therefore, the degree of mycorrhizal colonization is not always a clear indicator of its potential benefits for the host plant (Herrera, 2022).

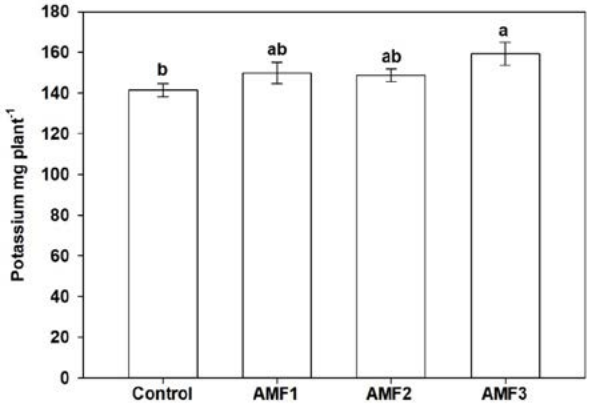


**Figure 5**. Potassium content of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

**Table 2**. Colonization of arbuscular mycorrhizal fungi in corn plants (*Zea mays*).

| HMA | Total colonization (%) | Vesicles (%) | Hyphae (%) |
|---|---|---|---|
| Control | 0.00 c | 0 | 0 |
| AMF1 | 0.00 c | 0 | 0 |
| AMF2 | 5.32 b | 2.92 | 2.4 |
| AMF3 | 24.79 a | 12.26 | 12.19 |

AMF1=*Glomus fasciculatumi*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobicurata*, and *Gigaspora margarita*. AMF2=arbuscular mycorrhizal fungi consortium (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control. The same letters after the means indicate no significant difference (Tukey, $\alpha=0.05$).

Therefore, the AMF-plant relationship is not considered specific, because any AMF species can colonize or form symbiosis with any plant (Delavaux *et al.*, 2019), as a result of their presence in virtually all types of soils (Guachanamá-Sánchez *et al.*, 2021). However, under certain edaphoclimatic conditions, some fungi may improve or provide a more significant benefit to a particular host (Zazueta *et al.*, 2021). In conclusion, the non-native commercial arbuscular mycorrhizal fungi (AMF1 and AMF2) exhibited very low colonization percentages in the root.

## CONCLUSIONS

Maize plants responded positively to inoculation with arbuscular mycorrhizal fungi. The AMF3 consortium —sourced from the rhizosphere of the huamuchil (*Pithecellobium dulce*) tree in Sinaloa— had a significant effect on P and K absorption in maize, unlike commercial mycorrhizal consortia (AMF1 and AMF2) and an uninoculated control. Furthermore, the infectivity of the arbuscular mycorrhizal fungi consortium (AMF3) in maize plants was positive.

The biotechnological potential of AMF3 can be harnessed to enhance the initial development of maize plants, increasing the absorption of macronutrients, and promoting greater root growth and development.

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