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Experimental Evidence of Negative Effect of Mycorrhizal Fungi on Pearl Millet Germination in a Cement Polluted Soil

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ABSTRACT

Recently, there has been increased focus on how cement manufacture affects the environment, especially with regards to the toxicity and permanence of heavy metal pollutants. Pear millet (*Pennisetum glaucum*) is a significant multipurpose cereal, particularly in Asia and Africa. However, the effects of arbuscular mycorrhizal fungi (AMF) propagules on seed germination and early seedling development under cement-contaminated soil conditions remain poorly understood. The goal of this study was to evaluate how AMF affects seed germination and early growth stage of two varieties of pearl millet (among local varieties in Jazan region, Saudi Arabia) in soil that have been contaminated by cement. The treatments were as follows: Baladi variety + AMF (Bal-AMF), Baladi variety without AMF (Bal), Hendi variety with AMF (Hen-AMF), and Hendi variety without AMF (Hen). For the germination percentage (GP) and rate (GR), each treatment had 20 replicates. After calculation GR and GP, three healthy seedlings were transplanted into pots, and grown for 45 days in a growth chamber under controlled conditions. The results showed that AMF negatively impacted germination rates and percentages of pearl millet. AMF did not significantly affect the early growth of the Bal variety, but negatively impacted the Hen variety, indicating a possible parasitic effect. The result of this study supports recent findings of possible negative effect of AMF on seed germination. It also suggests potentially varying interactions between AMF and pearl millet varieties in the early growth stage under controlled conditions, indicating the need for field trials with more replicates under different biotic and abiotic conditions to fully understand how crop varieties possibly differ in their responsiveness to AMF.

Keywords: arbuscular mycorrhizal fungi, cement-contaminated soil, pearl millet, seed germination, germination percentage, germination rate, early seedling growth.

INTRODUCTION

One of the main human-caused sources of particulate matter (PM), greenhouse gas emissions, and the discharge of heavy metals into the environment is the cement industry. Arsenic (As), lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), chromium (Cr), mercury (Hg), and cadmium (Cd) make up most of the heavy metals found in cement dust (Addo et al., 2012; Dong et al., 2015). Because heavy metals are persistent in the environment and bioaccumulate in the food chain, they pose a serious threat to human health and food security on a worldwide scale (Peralta-Videa et al., 2009; Wuana and Okieimen, 2011). Thus, the effects of cement manufacturing on the environment, particularly the toxicity and persistence of heavy metal emissions, have drawn more attention recently (Li et al., 2019; Olatunde et al., 2020).

Pearl millet (*Pennisetum glaucum*) is an important multipurpose cereal grown for grain, fodder, and forage (Arora et al., 2003) especially across Africa and Asia (Nambiar et al., 2011). The crop is highly tolerant to drought and elevated temperatures, making it well suited to arid and semi-arid regions where other cereals such as wheat and maize often fail to perform. Globally, pearl millet is cultivated on more than 29 million hectares, with production largely concentrated in Africa (about 15 million hectares) and Asia (around 11 million hectares) (Rathore et al., 2016). Over 95% of total production occurs in developing countries, and India ranks as the leading producer (Basavaraj et al., 2010), accounting for approximately 9.8 million hectares of the global cultivated area (Rathore et al., 2016). In Saudi Arabia, pearl millet is cultivated in many provinces such as Madinah, Aseer and Jazan where favorable

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climatic conditions exist in these regions. Applying plant-beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF), has been shown to enhance plant performance under environmental stress conditions (Shrivastava and Kumar, 2015; Kumar et al., 2015; Racic et al., 2025).

Approximately 80% of all terrestrial plants, including many agricultural crops, form symbiotic associations with AMF (Smith and Read, 2010). This type of interactions between the AMF and plants is considered mutualistic since the AMF absorbs and distribute soil nutrients (mainly phosphorus) to the plant in return for the complete reliance of the heterotrophic AMF on the host plant for carbon acquisition (Smith and Read, 2010). In addition, AMF also improves soil structure and plant water uptake by the extensive hyphal networks and production of glomalin (Köhler et al., 2010).

Even though the role of AMF in improving plant growth is extensively studied, most of these studies examine the effects of AMF colonization on seedlings after the seed germination stage, and how this enhances nutrient uptake and plant growth (Adesemoye et al., 2008; Meddad, 2017; Dieu-Béni et al., 2022). However, only few studies have investigated the effects of AMF on seed germination (Rueda-Puente et al., 2010; Barber et al., 2013; Wu et al., 2014). Seed germination is a crucial stage within the life cycle of plants as it marks the shift from dormancy to active development. The effects of the presence of AMF propagules on seed germination in soil remain largely absent and unexplored (Varga, 2015; Eldridge et al., 2021). Therefore, the present study aimed to evaluate the effects of AMF inoculation on seed germination and early seedling growth of two locally cultivated pearl millet varieties, Baladi and Hendi, under cement-contaminated soil conditions. Specifically, the study assessed germination percentage and germination rate and evaluated early seedling growth under controlled environmental conditions. The results are expected to provide preliminary information on variety-specific responses to AMF under cement-associated soil stress and to support the development of further field-based studies.

MATERIAL AND METHODS

Soil samples

The soil samples used in this study were collected from an area adjacent to a cement factory (Figure 1) located in Jazan region, Saudi Arabia (16°43'36.3 "N latitude and 43°02'29.7 "E longitude). Five soil samples were collected using a zigzag pattern from an area of approximately 500 m^2, then a composite soil sample was made by mixing all samples into a single melded sample.

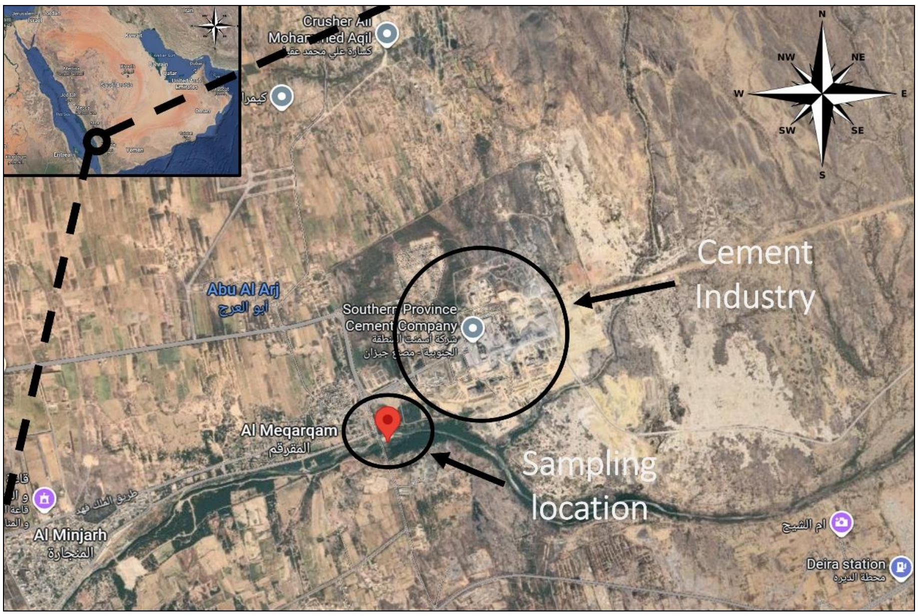

Figure 1. Soil sampling location near the cement factory in Jazan, Saudi Arabia

Soil physical and chemical properties

Soil texture was determined using the hydrometer method (Bouyoucos, 1962). The percentage of soil organic matter (SOM %) was determined using the wet oxidation method (Walkley and Black, 1934). Using a saturated paste extract, soil pH and electrical conductivity (EC) were measured with a pH meter, bicarbonate (HCO_3^-) and chloride (Cl^-) were measured using titration methods (Richards, 1954; APHA, 2005).

Soluble soil nitrate (NO_3^-) was measured using the Vanadium (III) reduction method (Doane and Horwath, 2003). Total soil phosphorus was analyzed using sulfuric acid digestion procedure and the APHA 4500-P E method (APHA, 2005).

In addition, concentration of some heavy metals in the soil was determined using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), according to the standard US-EPA 6010B method (EPA, 1996).

Pearl millet seeds and AMF inoculum

Seeds of two pearl millet varieties (Baladi and Hendi) were obtained from the local market in Jazan city, Saudi Arabia. These two varieties are the most grown by the local farmers. A mixture of five AMF strains was used as an inoculum (Plantonix LLC, Ashland, OR, USA): *Rhizophagus irregularis, Rhizophagus aggregatus, Rhizophagus prolifeerum, Rhizophagus clarus, Claroideoglomus etunicatum*. Each gram (powder) contains approximately 1000 propagules.

Experimental design and growth condition

Prior to conducting the experiment, seed viability was tested using a simple germination test for 12 days in a moist filter paper in the dark. The results showed that more than 95% of seeds were viable. Similar size seeds were then used to conduct the experiment. Plant seeds were surface sterilized with 10 % NaOCl for 10 min, then rinsed five times with sterilized DI water. Only the sinked seeds were collected during the water rinsing step as an indication of viability. The germination experiment was conducted using a small seedling tray containing 160 cells, with 15 mL volume per cell. The treatments were as follows: Baladi variety + AMF (Bal-AMF), Baladi variety without AMF (Bal), Hendi variety with AMF (Hen-AMF), and Hendi variety without AMF (Hen). In the control treatments (without AMF), we used a sterilized AMF inoculum. The sterilization step of AMF (autoclaving three times in three consecutive days) was done to rule out any possible effect resulting from the inoculum carrier media. Each treatment had 20 replicates. Seeds from each variety were then sown in cells with AMF or with sterilized AMF inoculum. Each cell was filled with approximately 8 grams of soil, and 2 grams of inoculum at the top. Seeds were then placed approximately 0.5 cm below the surface, in direct contact with the inoculum. The seedling tray was then placed in a growth chamber at 32°C day/22°C night, 16 h/8 h light/dark, 60% humidity, and 1000 µmol/m2/s light intensity. Cells were watered daily using DI water for 14 days.

Germination rate and percentage

Starting from the first day after sowing, the number of seeds that were germinated was recorded daily to calculate the germination rate (GR). The germination percentage (GP) was also calculated at the end of the experiment. The germination rate and percentage were calculated using the following equations (Illori et al., 2012):

$$GR = \frac{X_1}{Y_1} + \frac{X_1 - X_2}{Y_2} + \cdots \frac{X_n - X_n - 1}{Y_n}$$

Where X_n is the number of germinated seeds at the *n*th day and

Y_n is the number of days from sowing until the *n*th sowing time.

$$GP = \frac{Number\ of\ germinated\ seeds}{total\ number\ of\ seeds\ planted} \times 100$$

Seedling transplant and plant growth parameters

After calculating GR and GP, three healthy and similar sized seedlings from each treatment were transplanted into a bigger pot for each, each pot containing approximately

450 grams of soil. In addition, 2 grams of inoculum (either sterilized or unsterilized) were added around the seedling at the time of transport to further stimulate the symbiotic relationship. Plants were grown for 45 days in the same growth chamber under the earlier mentioned conditions. Pots were watered as needed using DI water to approximately 65% of water holding capacity of the soil. After 45 days, plants were removed from pots and shoots were separated from roots. Leaf number, plant height, root and shoot dry weight (oven drying at 60°C for 72 hrs.) were then measured. The whole root dry weight was calculated after recording the entire root moist weight, followed by removing a portion of the root system and weighed prior and after oven drying. The remaining moist roots were then used for root staining procedure.

Root Staining and AMF Colonization

Roots were immersed in preboiled 10% KOH for 10 min (to clear cytoplasmic content), washed 5 times with tap water, then immersed in 2% HCl for 20 min, and then washed 5 times with tap water. After the acidifying step, roots were immersed in preboiled 0.05% trypan blue solution (contains water, glycerin, lactic acid in 1:1:1 volume) for 5 min. Following the staining step, the roots were then washed 5 times with tap water and stored at 4°C for 5 days submerged in DI water to remove excess stain. Percentage of AMF colonization was then determined using the gridline intersect method (Giovannetti and Mosse, 1980).

Statistical analysis

Differences between treatments were tested using one-way ANOVA, followed by pairwise comparisons using Tukey's HSD, with significance level of 0.05 (α). All statistical analysis was performed using XLSTAT (v2024.4.1).

RESULTS AND DISCUSSION

Soil physical and chemical properties

The properties of soil used in this study are listed in Table 1. Based on percentages of clay, silt and sand, the soil has a loam texture, with a low percentage of organic matter. The pH and EC indicated that the soil is considered slightly alkaline, and non-saline, respectively. Bicarbonate and chloride were at high levels. In addition, soil soluble nitrate and total phosphorus were slightly at high levels. These high values were expected since pollution with cement dust is known to adversely affect soil physical and chemical properties (Ashraf et al., 2022). Based on the high bicarbonate and total phosphorus levels, it is expected that the available phosphorus in soil solution will more likely be high as will, since bicarbonate are well known to increase available P by releasing bound inorganic phosphorus into the soil solution, especially in neutral to alkaline soils (Björkman and Reiners, 2014).

Moreover, the concentrations of heavy metals (arsenic, cadmium, chromium, cobalt, lead, nickel, and zinc) were all below the soil maximum permissible levels (FAO, 2004). Although the soil heavy metal concentrations were at safe levels at the sampling time, these levels may fluctuate significantly in different seasons (wet vs dry). A recent study has shown that soil properties (physical and chemical) and wild plants morphological characters around the cement industry in Jazan city were significantly altered by cement dust pollution (El-Shabasy, 2021).

Table 1. Soil physical and chemical properties

Parameter	Value	Parameter	Value
Clay (%)	26	Total phosphorus (mg/kg)	765
Silt (%)	40	Arsenic (mg/kg)	<5
Sand (%)	34	Cadmium (mg/kg)	<1
Soil organic matter (%)	0.6	Chromium (mg/kg)	25
pH	7.8	Cobalt (mg/kg)	9
EC (dS/m)	1.1	Copper (mg/kg)	18
HCO_3^- (mg/L)	732	Lead (mg/kg)	5
Cl^- (mg/L)	70	Nickel (mg/kg)	20
Nitrate N (mg/kg)	33.5	Zinc (mg/kg)	44

Germination rate and percentage

Seed germination rate and percentage are shown in Figure 2. The results clearly indicated that AMF reduced both, germination rate and percentage. Similar results of negative effect of AMF on seed germination have previously been reported in some studies (Barber et al., 2013; Varga, 2015). These results are unexpected since the effects of AMF inoculation are generally believed to take place after seedlings start to grow and the fungus penetrate root cells (Smith and Read, 2010). Hence, these evidence highlights that AMF effects not only occur after colonization but can occur prior to the colonization stage as well. It is unclear how AMF propagules/spores can negatively affect seed germination. However, one could argue that the AMF inoculum may contain associated microorganisms, but this possibility is unlikely since in a study conducted by Varga (2015), there was a negative effect of AMF spores on seed germination even after treating the control samples with the washing water of spore extraction process to restore possible bacterial community associated with AMF spores. Another possible explanation is that AMF spore exudation could affect seed germination. This type of effect has been reported by Louarn et al. (2012) when *Orobanche cumana*, a root parasitic plant, inoculated with two distinct AMF. In addition, Maighal et al. (2016) has reported that AMF negatively affected seed viability. They suggested that this type of effect could possibly be related to either water content issue in the presence of AMF hyphae or due to allelochemical compounds translocated to the seeds from AMF hyphae. These mechanisms underlying the negative effects of AMF fungal hyphae or their spores are not yet understood and need further investigations.

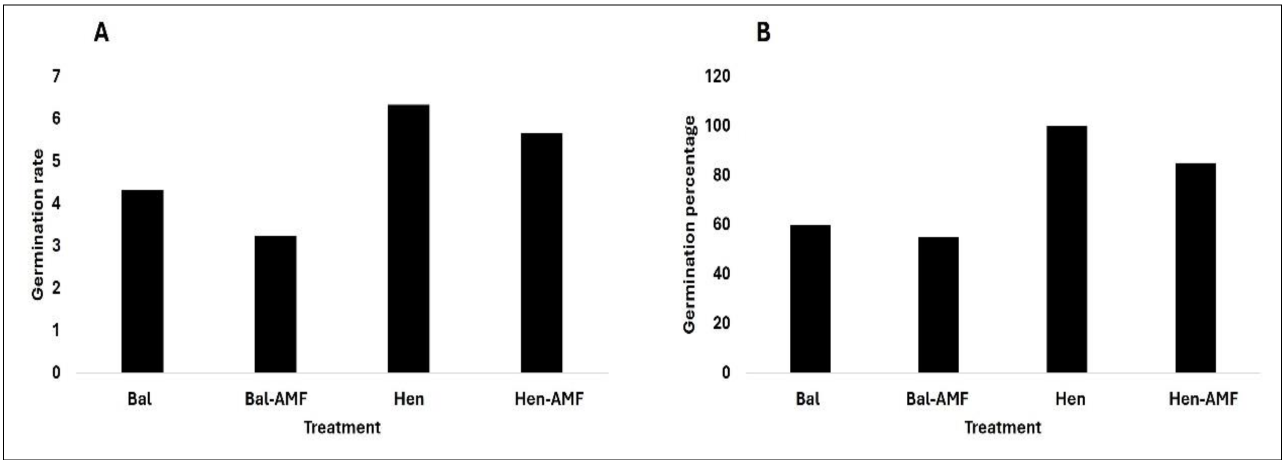

Figure 2. Seed germination rate (A) and percentage (B)

Mycorrhizal colonization and early plant growth parameters

Mycorrhizal colonization and plant growth parameters are shown in Figure 3. Hen-AMF had significantly higher AMF colonization compared to all other treatments (Bal-AMF, Bal, and Hen). However, in plant parameters, Hen-AMF had the lowest leaf number, shoot and root dry weights compared to all other treatments. In plant height, Hen-AMF was significantly (p value < 0.05) shorter than Bal-AMF. These results indicate that AMF colonization did not have detectable effects on the early growth of Bal variety but negatively affected the Hen variety. This indicates that the higher colonization of AMF found in the Hen variety may have resulted in a parasitic effect against plant growth.

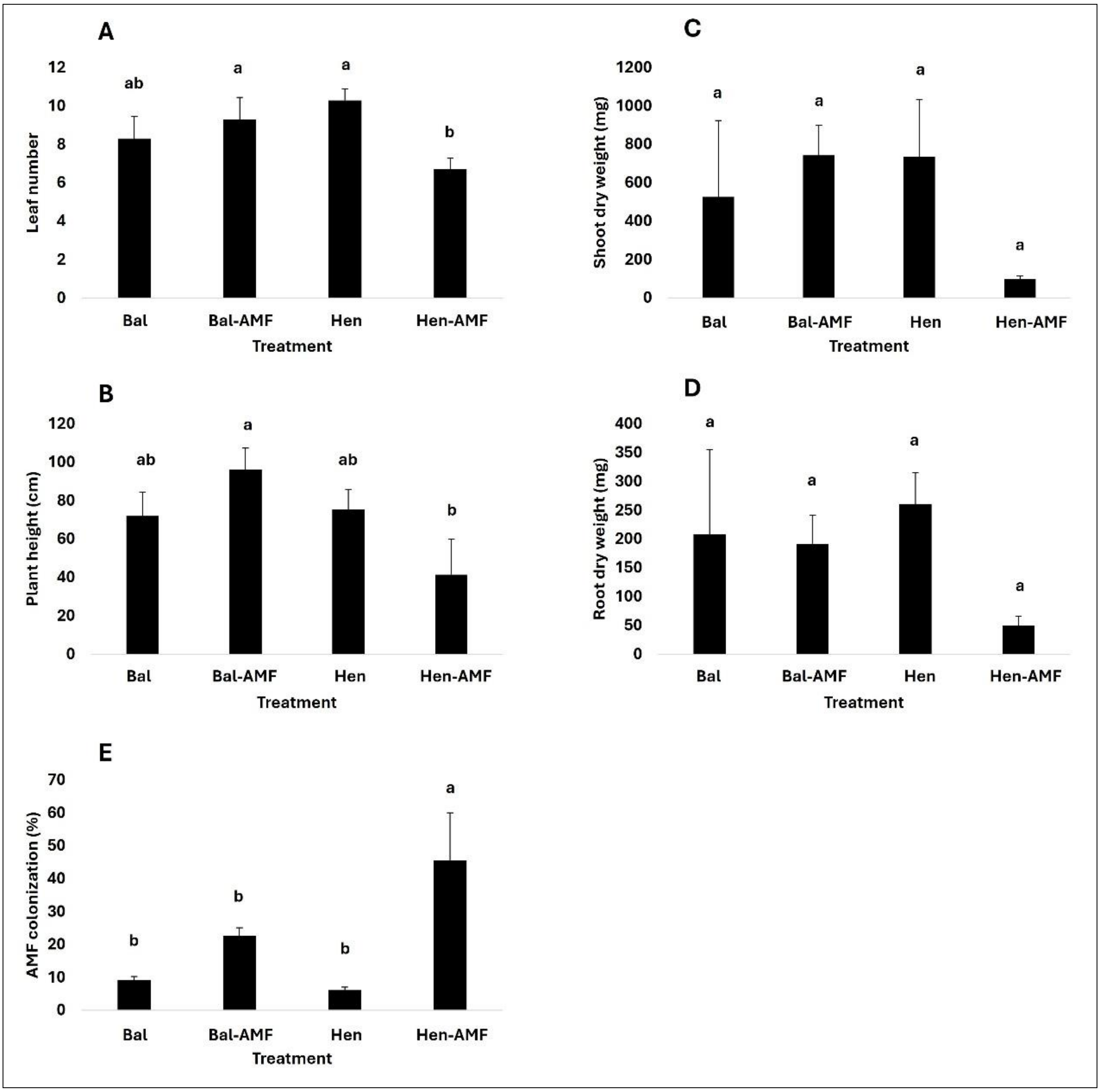

Figure 3. Plant growth parameters (A-D) and percentage of AMF colonization (E)

Depending on the availability of resources outside of the symbiosis, the relationship between plants and AMF might change from mutualism to parasitism (Guo et al., 2022; Duan et al., 2025). Plants that have easy access to soil resources may provide less carbon to their fungal partners, which could make ongoing fungal colonization metabolically expensive. Therefore, when soil nutrient availability increases, the fungal-plant association may turn parasitic, decreasing the plant's reliance on fungal partners while the metabolic costs of maintaining the symbiosis remain constant (Zhang et al., 2024). Moreover, it has been shown that plant genotype, AMF strain, and environmental context all have a significant impact on the fitness implications of mycorrhizal-plant interactions (Guo et al., 2022). Therefore, this supports the findings in our study that early growth of pearl millet varieties may exhibit different interactions with AMF.

CONCLUSIONS

The study findings revealed that AMF inoculation negatively affected germination percentage and germination rate of pearl millet under cement-contaminated soil conditions. Contrary to common beliefs that AMF effects occur post-colonization, evidence suggests that they can also influence germination before the establishment of mycorrhizal colonization. In addition, AMF did not significantly affect the early growth of the Baladi variety, but negatively impacted the Hendi variety, indicating a possible parasitic effect against Hendi variety that resulted in higher AMF colonization. This suggests potentially varying interactions between AMF and pearl millet varieties in the early growth stage under controlled conditions.

To better understand the mechanisms driving AMF's detrimental effects on seed germinations, future research with more replicates is necessary, as shown by this study's results as well as the scant results of previous investigations. In addition, field trials with more replicates under diverse biotic and abiotic conditions are also important to fully unravel how crop varieties possibly differ in their responsiveness to AMF. Consequently, this will maximize benefits of AMF application in agricultural fields.

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