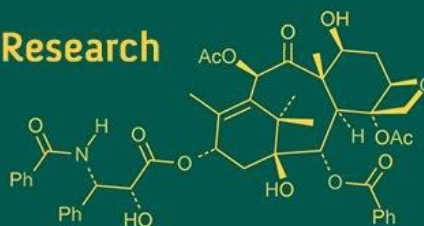


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(12): 248-254
www.biochemjournal.com
Received: 01-11-2025
Accepted: 02-12-2025

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Arbuscular mycorrhizal fungi diversity assessment and mass multiplication using maize and sorghum as trap crops

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i12Sd.6501>

Abstract

Amphibious lichens establish mutually beneficial partnerships with tree trunks' root systems, significantly impacting water uptake efficiency, resilience against environmental adversities, and overall ecosystem vitality enhancement. This research examined how efficiently Arbuscular Mycorrhizal fungi multiply when grown using traps and laboratory techniques, while also assessing their impact on the development of corn species (*Zea mays*) and sorghum plants (*Sorghum bicolor*), both within artificial environments designed for experimentation. Roots of maize and sorghum species along with their associated soil layers were sampled and examined in search of naturally occurring arbuscular mycorrhizal fungi; these included assessing their prevalence as well as measuring spore concentrations within them and quantifying how deeply they colonized plant roots. Traps of trap culture along with an *in vitro* AMF inoculum were subsequently introduced into pots containing plants to evaluate how these factors affect plant development.

Experimental outcomes indicated that airborne mycorrhizal fungi produced significantly higher numbers of spores (notably at rates of 265 per gram for maize and 249 for sorghum), as well as extensive root penetration into plant tissues. Forty percent of the crop was made up by maize; fifty-eight other crops were present as well. Approximately nine percent of sorghum is being utilized. Remarkable advancements in crop development were noted across all varieties tested; within-vessel arbuscular mycorrhizal fungi significantly boosted maize stalks by an additional 28%, while enhancing sorghums' heights by nearly 26%. Observations included early bloom onset, better ears/panicles for yield potential, and increased absorption of nutrients by plants. Studies indicate that when applied *in vitro*, AM fungi produce results superior to those obtained through trapping methods; this approach holds promise as an eco- friendly alternative to conventional fertilizer use, especially concerning its role in reducing reliance on phosphate-based chemicals.

The study focuses on enhancing agricultural productivity through techniques such as mycorrhizal colonization of maize and sorghum roots using spores for increased diversity in root systems while promoting sustainable farming practices.

Keywords: AMF, mycorrhiza, spore diversity, root colonization, trap culture, maize, sorghum, sustainable agriculture

Introduction

Near the base of plants, within what's termed the rhizosphere, diverse microbial communities thrive pathogens, decomposers breaking down organic materials, saprotrophs, and mutualistic fungi like mycorrhiza are all present (Andrade *et al.*, 2009; Nuccio E. E. 2013; Chauhan *et al.*, 2023) [4, 9, 23].

Mycorrhizae refers to beneficial symbiotic relationships where arbuscular mycorrhizal fungi link up with plant root systems for mutual benefit, often described as mutually advantageous interactions (Lanfranco *et al.*, 2016; Khaliq *et al.*, 2022) [15, 18]. In mutualistic relationships among various organisms, each party gains advantages through cooperation. Because this interaction happens between various kinds of organisms, we refer to it as an interspecies bond, while its positive impact on all parties makes it known as a harmonious connection. The term "mycorrhizae" is derived from fungal symbiosis with plant root systems. In recent years, researchers' interest in fungal-plant symbioses has grown due to their discovery's significance; specifically, how this mutualistic relationship

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enhances plant nutrient uptake efficiency through increased root colonization (Lanfranco *et al.*, 2016; Bhupenchandra *et al.*, 2024) ^[5, 18]. For illustration, scientists discovered that certain types of mushrooms enhance plant absorption of phosphorous particularly when applied to depleted and nutrient-poor soil conditions (Miyasaka *et al.*, 2001; Madrid-Delgado *et al.*, 2021) ^[21, 22]. Understanding is crucial: while mycorrhizae won't boost soil's phosphate levels like some other microbes can; their presence helps improve root uptake through increased contact areas for plants (Hartmann *et al.*, 2009) ^[14]. Having both phosphorus (P), which plays crucial roles through its effects on plants' growth and development, alongside other vital nutrients ensures optimal nutrient availability for enhancing crop health and productivity. To achieve an optimal ecosystem, integrating both biotic and abiotic elements within soils proves crucial for fostering healthiness and equilibrium (Khan *et al.*, 2023) ^[16].

AMF entities fall under two primary categories. The initial type of mycorrhiza in question involves non-endophytic fungi known as ectomycorrhizal species (EMC) - these organisms lack penetration into either plants themselves or their root systems. Nearby plants reside in proximity to the plant's stem and root system, creating an umbrella-like "mantle" structure and Hartig web network. The mantle serves both as an absorber for nutrients while simultaneously safeguarding against certain diseases. A predominant form of ectomycorrhizal symbiosis is likely observed among tree varieties (Hartmann *et al.*, 2009) ^[14]. A distinct category within mycorrhiza includes those known as endomycorrhizal fungi. Their capability involves entering plant tissues via fungal filaments called hyphae, which reside in cell walls as well as extend between them. Within the cells, these filaments expand, acting as an energy reserve for the organism's metabolic processes (Andrade *et al.*, 2009) ^[4]. Consequently, the AMF absorbs sugars derived from root tissues whereas the mycorrhizal fungi transfer organic compounds along with moisture to the host plants' root systems (Cogo *et al.*, 2017) ^[10].

Endophytic fungi predominate among crop plants considered for agriculture. Due to their necessity for existence, these organisms rely on this way of life as an indispensable requirement. Thus, such fungi maintain their association through an intimate link involving both the plant's roots and the underlying soil substrate. Numerous fungal varieties facilitate such connections by individually possessing unique capabilities for absorbing particular nutrients. Therefore, each species selectively takes in specific nutrients; some may intake significantly higher amounts compared to other types (Andrade *et al.*, 2009) ^[4].

Arbuscular mycorrhizal fungi (AMF) enable host plants to thrive under adverse conditions by orchestrating intricate communication processes between the plant and the fungus, resulting in improved photosynthetic rates and other gas exchange-related characteristics (Birhane *et al.*, 2012) ^[6], along with augmented water absorption. Multiple studies indicate enhanced resilience to various stressors, such as drought, salt, herbivory, temperature fluctuations, metal exposure, and diseases, attributed to fungal symbiosis (Rodriguez *et al.*, 2008; Ahanger *et al.*, 2013; Abdel-Salam *et al.*, 2018) ^[1, 3, 28]. Approximately 90% of plant species, including flowering plants, bryophytes, and ferns, may establish symbiotic associations with arbuscular mycorrhizal fungi (Zhu *et al.*, 2010; Ahanger *et al.*, 2013) ^[3, 35]. AMF

produce vesicles, arbuscules, and hyphae inside roots, as well as spores and hyphae in the rhizosphere. The establishment of a hyphal network by arbuscular mycorrhizal fungi (AMF) with plant roots markedly increases root access to an extensive soil surface area, hence promoting plant development (Bowles *et al.*, 2016) ^[7]. AMF enhances plant nutrition by augmenting the availability and transport of several nutrients (Rouphael *et al.*, 2015) ^[29]. AMF enhances soil quality by affecting its structure and texture, hence promoting plant health (Zou *et al.*, 2016; Thirkell *et al.*, 2017) ^[32, 36]. Fungal hyphae may accelerate the breakdown of soil organic materials (Paterson *et al.*, 2016) ^[25]. Moreover, mycorrhizal fungi may influence ambient CO₂ fixation by host plants by enhancing the "sink effect" and the translocation of photo-assimilates from the aerial portions to the roots. This review emphasizes the significance of arbuscular mycorrhizal fungi (AMF) and recent research on their agricultural applications, specifically their function as bio-fertilizers in regulating plant growth and development, enhancing nutrient absorption in stressful conditions, and the extent to which AMF can promote plant growth under such environments.

Arbuscular mycorrhizal fungi as a biofertilizer

Bio-fertilizers consist of naturally occurring compounds used to enhance soil fertility. These fertilizers are beneficial for soil health and promote plant growth and development (Sadhana, 2014; Madawala, 2021; Kuila *et al.*, 2022) ^[17, 20, 30]. Numerous research studies on AMF completed over the last two decades have shown its myriad advantages for soil health and crop output (Wahab *et al.*, 2023; Chaudhary *et al.*, 2025) ^[8, 33]. Consequently, it is well accepted that AMF may serve as a substitute for inorganic fertilizers in the foreseeable future, since mycorrhizal treatment may significantly reduce the quantitative reliance on chemical fertilizer inputs, particularly phosphorus (Ortas, 2012; Adeyemi *et al.*, 2020) ^[2, 24]. The persistent use of inorganic fertilizers, herbicides, and fungicides has resulted in several issues for soil, plants, and human health due to their detrimental effects on food quality, soil integrity, and air and water systems (Pathak *et al.*, 2022) ^[26]. AMF is expected to potentially reduce chemical fertilizer use by up to 50% for optimal agricultural output (Qian *et al.*, 2024) ^[27].

This research aimed to evaluate the effectiveness of trap culture inoculum in improving plant growth, as well as to compare the efficiency of arbuscular mycorrhizal fungi (AMF) propagation using different host plants, specifically maize (*Zea mays* L.) and sorghum (*Sorghum bicolor*). Additionally, the study explored alternative AMF inoculation methods to enhance root colonization and promote the growth of maize and sorghum under soil conditions.

Materials and methods

Study area and sample collection

The research was carried out at Arihant Bioscience (India) Pvt. Ltd.'s Department of Research and Development in Ankleshwar, Gujarat, between March and August of 2025. The department's research center used controlled pot and bag trials to cultivate maize and sorghum plants for the root and rhizosphere soil samples.

A soil borer was used to dig each plant to a depth of 10 to 15 cm in order to obtain around 500 g of soil for rhizosphere

sampling. Carefully separated and gathered was the dirt that stuck to the root zone. The same plants were used to gather root samples at the same time. The roots were cleaned of dirt and divided into segments of two to three centimeters for further processing.

The rhizosphere soil samples and root segments were promptly tagged, put in airtight, sterile plastic bags, and sent to the lab for further examination of the AMF spore count and root colonization.

Root clearing, staining, and assessment of mycorrhizal colonization

To remove adherent soil particles, roots (2-3 cm segments) from sorghum and maize plants were gathered and carefully cleaned under running tap water. To remove the cytoplasmic contents, the cleaned root pieces were put into glass vials with 10% potassium hydroxide (KOH) and heated until almost boiling, which took two to three minutes. To get rid of any remaining KOH, the roots were washed four or five times with tap water after clearing.

After five minutes of acidification in 1% hydrochloric acid (HCl), the roots were thoroughly cleaned. In order to enable the stain to enter fungal structures, the root segments were submerged in acidic glycerol containing 0.05% Trypan Blue (or as available; 1 mL staining solution was employed in this work) for around ten minutes.

Following staining, the roots were briefly submerged in either IPA (isopropyl alcohol) or acidified glycerol to remove any remaining color. The thickness of the root and the strength of the stain determined how long it took to remove. The roots were put on glass slides for microscopic examination after being cleaned with tap water to create the ideal contrast.

The Grid-Line Intersect Method, originally described by Giovannetti and Mosse (1980) [13] and further standardized by INVAM (International Culture Collection of Arbuscular Mycorrhizal Fungi), is a commonly used technique for quantifying vesicular-arbuscular mycorrhizal (VAM) infection in plant roots. By noting the existence of AMF

structures (hyphae, vesicles, and arbuscules) at each junction point and expressing the colonized crossings as a percentage of all intersections investigated, the percentage of root colonization was determined.

Isolation of amf spores from rhizosphere soil

Using the wet sieve and decanting method of Gerdemann and Nicolson (1963) [12] and the floating adhesion technique of Sutton and Barron (1972) [31], arbuscular mycorrhizal fungus (AMF) spores were separated from the rhizosphere soil. The Department of Research and Development at Arihant Bioscience (India) Pvt. Ltd., Ankleshwar, Gujarat, collected rhizosphere soil (10-15 cm depth) from maize plants cultivated in pot and bag trials. To guarantee that only rhizosphere soil was obtained, surface dirt was removed before sampling.

A blender was filled with 500 mL of tap water and around 50 g of rhizosphere soil. The blender was run at high speed for five seconds. A series of sieves with decreasing mesh sizes (250 mm, 0.106 mm, and 0.045 mm) were filled with the resultant soil suspension. To ensure that the spores moved through the sieves properly, a constant stream of tap water was administered.

The coarse material that remained on the top sieve (250 mm) was thrown away. A little stream of water was used to wash the fractions that were retained on 0.106 mm and 0.045 mm sieves which usually contain AMF spores into other Petri plates. A stereomicroscope was used to look for AMF spores in these preparations.

Using a micropipette or tiny forceps, individual spores were carefully selected and placed on sterile Petri plates for counting and identification. The final sample did not include the bulk of the sand particles that were still in the blender.

Assessment of root colonization and spore populations

The International Culture Collection of Vesicular Arbuscular Mycorrhiza, or INVAM, dyed the maize root samples before estimating the percentages of vesicular mycorrhizal colonization using the grid line technique.

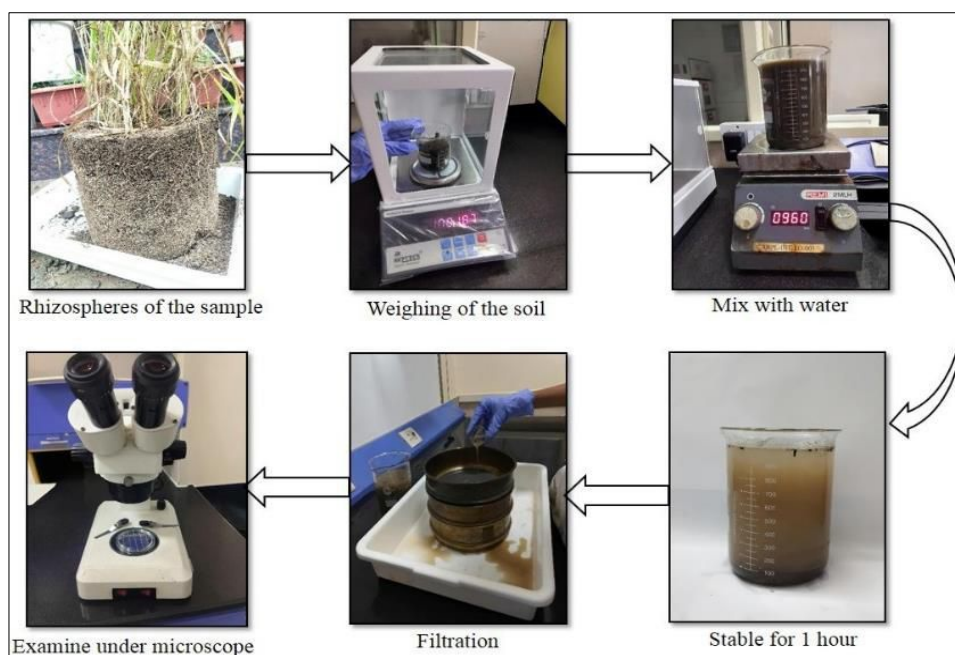


Fig 1: Sequential steps of the AMF spore isolation process using the INVAM wet-sieving method, including: (A) collection of maize rhizosphere soil, (B) weighing of soil samples,

(C) mixing with water, (D) sedimentation, (E) filtration through sieves, and (F) microscopic examination for AMF spore quantification.

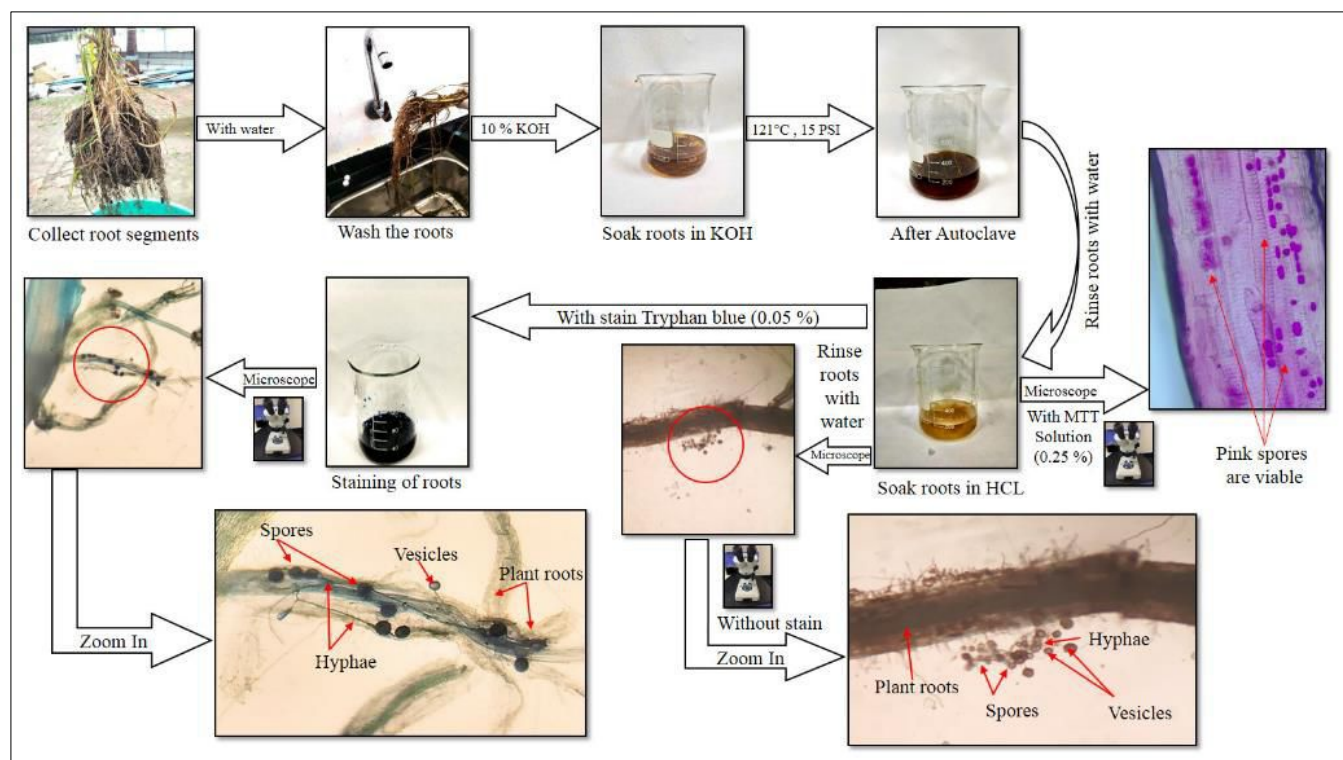


Fig 2: Microscopic visualization of mycorrhizal colonization in maize (*Zea mays* L.) roots, showing characteristic AMF structures including hyphae, vesicles, and arbuscules within the cortical cells, confirming successful root infection.

Staining procedure for maize and sorghum roots

Samples of the roots of maize and sorghum were first properly cleaned under running water to get rid of any sand and dirt particles that could have stuck to them. Clean roots were divided into 2-3 cm pieces and put into glass vials with 10% KOH. After that, the samples were boiled for three minutes at around 100°C, with the length of time varying according to the thickness of the roots; older and thicker roots needed a little more time to be cleared. The root tissue's browning served as a sign that the cytoplasmic contents had been adequately removed.

The roots were cleaned and then repeatedly cleansed with tap water. The ink- vinegar technique was then used to dye the cleared roots. To enable fungal structures to penetrate, root pieces were submerged in the stain for around ten minutes. After staining, the roots were carefully cleaned with water to remove any remaining stain.

In order to detect AMF structures such hyphae, vesicles, and arbuscules, the dyed roots were placed on microscope slides and inspected under a light microscope. Using the Grid-Line Intersect technique (INVAM technique), the percentage of root colonization was calculated by dividing the total number of observed intersections by the number of colonized junctions. Before each staining session, a new combination of water, glycerin, and lactic acid in a 1:1:1 ratio (v/v/v) was made as the stain.

Data collection

To record observations on each plant, three sorghum and maize plants were chosen at random. Sorghum and maize plants were sampled for the replication in each population. The majority of the data records are based on character measurements and the following (Maize: local variety, Sorghum: local variety):

Table 1: The collected data were recorded on three randomly selected plants from each, and the quantitative characters were recorded by using descriptors for maize and sorghum.

Parameter	Treatment	Maize (Mean $\pm$ SD)	Sorghum (Mean $\pm$ SD)
AMF Spore Count (spores / 50 g soil)	Control (no AMF)	45 $\pm$ 4	52 $\pm$ 5
	Trap Culture AMF	210 $\pm$ 12	198 $\pm$ 15
	<i>In vitro</i> AMF	265 $\pm$ 18	249 $\pm$ 20
Root Colonization (%)	Control (no AMF)	5.2 $\pm$ 1.0	7.1 $\pm$ 1.4
	Trap Culture AMF	48.6 $\pm$ 4.3	44.2 $\pm$ 3.9
	<i>In vitro</i> AMF	62.4 $\pm$ 5.1	58.9 $\pm$ 4.6
Plant Height (cm)	Control	132.4 $\pm$ 6.2	148.1 $\pm$ 5.9
	Trap Culture AMF	158.9 $\pm$ 7.3	165.7 $\pm$ 7.0
	<i>In vitro</i> AMF	170.3 $\pm$ 8.1	174.3 $\pm$ 7.4
Ear Height (cm) (Maize only)	Control	62.8 $\pm$ 3.8	—
	Trap Culture AMF	73.4 $\pm$ 4.2	—
	<i>In vitro</i> AMF	79.6 $\pm$ 3.9	—

Ear Length (cm) (<i>Maize only</i>)	Control	11.2±0.7	—
	Trap Culture AMF	14.5±1.1	—
	<i>In vitro</i> AMF	16.1±1.3	—
Leaf Number (<i>Sorghum only</i>)	Control	—	9.2±0.4
	Trap Culture AMF	—	10.4±0.6
	<i>In vitro</i> AMF	—	11.0±0.6
Panicle Length (cm) (<i>Sorghum only</i>)	Control	—	19.4±1.2
	Trap Culture AMF	—	23.7±1.5
	<i>In vitro</i> AMF	—	26.1±1.6

Results

1. Production of AMF Spores

- The AMF spore density was much greater in the sorghum and maize trap cultures than in the control.
- The *in vitro*-produced inoculum had the highest spore count, five to six times higher than the control, with 265 spores/50 g soil in maize and 249 spores/50 g soil in sorghum.

2. Root Colonization

- The control group had a relatively low natural colonization rate (5-7%).
- *In vitro* AMF inoculum obtained the maximum colonization, with 62% in maize and 58% in sorghum, but trap culture inoculation increased colonization to 48-44%.
- This suggests that the *in vitro* AMF inoculant was more successful in establishing symbiosis than trap culture.

3. Effect on Maize Growth

- Growth metrics were greatly enhanced in maize plants treated with AMF.
- Plant height rose by 28% *in vitro* and 20% in trap culture.
- *In vitro*, ear length grew from 11.2 cm to 16.1 cm.

4. Effect on Sorghum Growth

- AMF injection also significantly enhanced sorghum growth.
- Plant height rose by 26% *in vitro* and 18% in trap culture.
- The length of the panicle grew from 19.4 cm (control) to 26.1 cm (*in vitro*).
- The number of leaves grew by one or two, suggesting improved photosynthesis and nutritional intake.

Discussion

The findings unequivocally demonstrate that in maize and sorghum, both trap culture and *in vitro* AMF injection greatly increased plant growth, root colonization, and spore output. But *in vitro* AMF proved to be the most successful, exhibiting better colonization and growth increase.

1. Mycorrhizal colonization

Higher colonization in *in vitro* AMF is expected due to:

- High purity and viability
- Absence of competing microorganisms
- Greater propagule density

This is consistent with previous research by Birhane *et al.* (2012) and Colonna *et al.* (2015) Leventis *et al.* (2021)^[11, 19] and Xiao *et al.* (2023)^[16, 34], which demonstrated improved

nutrient absorption and stress tolerance in AMF-colonized plants.

2. Plant growth improvement

AMF enhanced several key physiological processes:

- Phosphorus acquisition
- Water-use efficiency
- Root branching
- Early flowering

3. Species response variation

Although both plants responded well, maize demonstrated somewhat more colonization and growth stimulation than sorghum, most likely because of:

- Root architecture differences
- Host specificity of AMF strains
- Variation in rhizosphere exudates

Conclusion

The research showed that viable inoculum capable of boosting plant development in maize and sorghum was effectively created using both trap culture and *in vitro* AMF propagation techniques. The greatest spore density, root colonization, and plant growth response were obtained using an *in vitro* AMF inoculum.

Key conclusions

- The development of maize and sorghum is much enhanced by AMF; *in vitro* inoculum is more effective than trap culture; AMF shortens blooming time and enhances nutrient absorption.
- AMF may be used as a biofertilizer to lessen reliance on artificial fertilizers.

Recommendations

1. Compared to trap culture, *in vitro* AMF inoculum provides greater purity, viability, and consistent colonization, making it the preferable option for commercial usage.
2. To promote sustainable crop production, AMF should be used with less chemical fertilizer, particularly phosphorus.
3. To assess AMF establishment in agricultural settings, routine root colonization monitoring is advised.
4. In cereals like maize and sorghum, the use of host-specific AMF strains may improve growth response even more.
5. To promote long-term AMF survival, soil health improvement techniques including decreased tillage and the addition of organic matter should be used.
6. To confirm the findings of the pot experiment, larger field experiments should be carried out in various agroclimatic zones and soil types.

Future scope

1. Molecular identification of AMF species to determine colonization effectiveness and host choice.
2. The creation of AMF biofertilizers in granular, liquid, and capsule forms that are appropriate for mechanical farming.
3. Investigating AMF-microbial consortia by mixing AMF with helpful bacteria like *Trichoderma*, *Azotobacter*, and *PSB*.
4. Using AMF to reduce stress, particularly heavy-metal stress, salinity, and drought in plants and grains.
5. Extensive research on soil carbon sequestration to assess how AMF enhances soil aggregation and organic carbon storage.
6. Genotype-specific research to determine which sorghum and maize cultivars react best to AMF inoculation.

Acknowledgment

The authors express their sincere gratitude to the Department of Research and Development, Arihant Bioscience (India) Pvt. Ltd., Ankleshwar, Gujarat, for providing laboratory facilities, plant material, and technical support to conduct this research. Special thanks are extended to the research staff for assistance in AMF staining, microscopy, and soil analysis.

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