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A Preliminary Seedling Growth Experiment for Assessing the Role of Mycorrhizae Species within Wheat Plant Tissue, Sand Culture along with Swiss AMF Inoculum Use

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Abstract

This study evaluates the impact of Arbuscular Mycorrhizal Fungi (AMF) on nutrient uptake and sand physicochemical properties in wheat (*Triticum aestivum* L.) seedlings along with Swiss AMF inoculum use. Conducted at the Agronomy Field Laboratory, University of Rajshahi, Bangladesh, the experiment employed a randomized complete block design with three treatments: control, mycorrhizal (+AMF), and non-mycorrhizal (-AMF), each replicated three times. *Rhizophagus irregularis* was used as the AMF species, with BARI Gom 28 as the host plant. The clay-textured soil had low organic matter and fertility, with a pH between 6.8 and 8.6. Results revealed significant improvements in nutrient uptake due to AMF inoculation. The +AMF treatment recorded the highest values for nitrogen (0.65%), calcium (8.077 mg kg^{-1}), copper (0.013 mg kg^{-1}), iron (5.246 mg kg^{-1}), potassium (22.66 me/100g), carbon (2.56%), magnesium (3.392 mg kg^{-1}), manganese (0.248 mg kg^{-1}), phosphorus (6.29 mg kg^{-1}), and zinc (0.094 mg kg^{-1}), compared to the control. Soil physicochemical properties were also affected, with the highest soil pH observed in the +AMF treatment. Soil nitrogen content was highest in the control, while phosphorus and sulfur levels decreased significantly in the +AMF treatment, indicating higher plant uptake. This study highlights the potential of AMF inoculation to enhance nutrient uptake and improve soil properties, suggesting a promising approach for boosting crop production in low-fertility soils.

Keywords: Arbuscular Mycorrhizal Fungi, Rhizophagus irregularis, nutrient uptake, sand physicochemical properties and wheat seedlings.

Introduction

Arbuscular mycorrhizal fungi (AMF) form a critical component of soil ecosystems and have garnered significant attention for their role in enhancing plant growth and soil health. These symbiotic fungi, belonging to the phylum Glomeromycota, establish mutualistic relationships with the roots of most terrestrial plants. The association between AMF and plants is characterized by the formation of arbuscules and vesicles within the root cortex, which facilitate nutrient and water exchange between the soil and the plant (Cakmak et al., 2023). This relationship has been widely documented to improve plant nutrient uptake, particularly for phosphorus (P), nitrogen (N), and other essential minerals (Bhantana et al., 2021). Given the global challenge of maintaining soil fertility and crop productivity, understanding and optimizing AMF interactions with crops is crucial for sustainable agricultural practices. The role of AMF in improving plant growth and soil health has been extensively documented. These fungi facilitate increased nutrient and water uptake by expanding the plant's root surface area through their hyphal networks (Püschel et al., 2020). This enhanced nutrient acquisition is particularly beneficial in nutrient-poor soils, where AMF can help mitigate the effects of nutrient deficiencies and improve crop yields (Khaliq et al., 2022). Additionally, AMF contributes to soil structure by promoting soil aggregation, which can enhance soil water retention and reduce erosion (Zhang et al., 2023). Recent studies have highlighted the significance of AMF in reducing the dependence on chemical fertilizers, thus supporting sustainable agricultural practices. For instance, a meta-analysis by (Qin et al., 2022) demonstrated that

AMF inoculation can reduce the need for phosphate fertilizers by up to 50%, while simultaneously increasing crop biomass and yield. This reduction in chemical inputs not only decreases production costs but also mitigates the environmental impact of agriculture by reducing nutrient runoff and soil degradation. The benefits of AMF extend beyond nutrient uptake. Recent research has shown that AMF can enhance plant resilience to abiotic stressors such as drought and soil salinity (Wahab et al., 2023). This increased resilience is particularly crucial under changing climatic conditions, where the frequency and intensity of such stressors are expected to rise. Despite these benefits, the use of AMF in agricultural practices remains underutilized. George and Ray 2023 argue that while the potential of AMF to enhance crop productivity and soil health is well-established, their application in conventional farming systems is limited. This is partly due to a lack of understanding of the specific interactions between different AMF species and crop varieties. In light of these considerations, this study aims to evaluate the effects of AMF inoculation on wheat seedling growth and nutrient uptake. Using a sand culture system and Swiss AMF inoculum, the experiment assesses the impact of different mycorrhizal treatments on wheat biomass and nutrient content. This research is intended to provide insights into the practical benefits of AMF inoculation for improving wheat productivity and promoting sustainable farming practices.

Materials and Methods

Experimental location: The experimental location was the Agronomy Field Laboratory, Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh. Geographically, the Agronomy field laboratory is located at 24°22′36″N latitude and 88°38′27″ E longitude at an elevation of 20 m above sea level, belonging to the Agro-Ecological Zone-11 (AEZ-11) of Bangladesh.

Experimental Soil: The soil was collected from cultivable plots in the agronomy farms situated in the Rajshahi region in which paddy rice was grown before. The texture of the soil was clay, the organic matter status and soil fertility were low, and the pH of the soil was between 6.8 and8.6 (Bhuiyan et al., 2008). The soil was passed through a 4-mm sieve to eliminate coarse rock and plant material, thoroughly mixed to ensure uniformity, and stored at 4°C before use (not more than 2 weeks). Before the experiment, unwanted materials such as dry roots, grasses, and hard stones were removed from the soil. The soil was mixed thoroughly before starting the incubation experiment. A subsample of approximately 0.5 kg was taken, air dried, passed through a 2-mm sieve and used for the determination of physicochemical characteristics of soil.

Table 1: Pedigree of BARI Gom 28

Variety	Accession No.	Pedigree/Cross	Year of Release
BARI Gom 28	BAW 1141	CHIL/2*STAR/4/BOW/CROW// BUC/PVN/3/2*VEE#10 CMSS95Y00624S-0100Y-0200M-17Y-010M-5Y-0M	2012

Experimental plant: Wheat (***Triticum aestivum*** L.) was selected as the host plant for this study. In Bangladesh, after rice, wheat is the second most essential food crop. The most commonly grown wheat variety is BARI Gom 28 (CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10)

CMSS95Y00624S-0100Y-0200M-17Y-010M-5Y-0M) was used. The pedigree of BARI Gom 28 is shown in Table 1.

Table 2. Isolate used in this study and their origin.

AM Fungus	AMF spores /10 g soil	BEG Code	Local code	Source	Country of Origin
Rhizophagus irregularis (formerly Rhizophagus irregularis)	105	BEG 145	SAM P7	J.C. Dodd	United Kingdom

Source and specification of collected AMF inocula for the seedling growth experiment:
AMF inocula were collected from Agroscope-Switzerland. The AMF species was *R. irregularis*, Thiazoliums intracardiac, which belong to the Glomeromycota. Commercial AM fungus species such as Rhizophagus irregularis (isolate BEG 21) were provided by the rhizosphere laboratory of Cukurova University, Turkey. The collected mycorrhizal fungi species such as *Rh irregularis* (isolate BEG 21, Genbank accession number DQ377990) were propagated separately in the greenhouse in pots containing a 3:17 (v/v) soil: sand mixture planted with *Zea mays*. The soil: sand mixture was inoculated with 5% inoculum of the respective fungal isolates. Every 2nd week, pots received 20 ml of a modified Hoagland solution (Hoagland & Arnon, 1938), which contained one-quarter of the original P concentration. A control inoculum, not containing AMF propagules, was produced under the same conditions. After three months of growth, pots were dried, emptied, and roots were cut into <5 cm pieces and homogeneously mixed with the experimental substrate. The other properties of these AMF species are also shown in Table 2.

Experimental design and treatment: The experiment was a randomized complete block design (RCBD). The experiment consisted of three treatments: the control treatment, the mycorrhizal (+AMF) treatment, and the non-mycorrhizal (-AMF) treatment, each being replicated 3 times and set up in a plant growth chamber.

Experimental procedure: Six pots each filled with 300 g sand were taken. The pots were labeled as Arabic numerals 1 to 6. A 20 g *Rhizophagus irregularis* inoculum was added to pot number 3 and 4 and mixed thoroughly with the sand. Pot 1 and 2 were control and nothing was added. Pot 5 and 6 were non-mycorrhiza, the medium where no mycorrhiza was added. All the pots were incubated at room temperature for 4 weeks to allow stabilization of the chemical properties of the sand before the experiment was initiated. During the incubation period water was maintained to field capacity by weighing the pots. Sand was not sterilized for this experiment because it was expected that sand would not have any microbial activities. The experiment consisted of three treatments: the control treatment, the mycorrhizal (+AMF) treatment, and the non-mycorrhizal (-AMF) treatment, each being replicated 3 times and set up in a plant growth chamber. In the sand culture experiment, the relative humidity was 50% at 5 days of sampling and reached 70% at 8 days of sampling. On average, the maximum and minimum temperature varied 28 to 32°C during the seedling growth experiment period (13 days). Seedlings were regularly phonologically observed for nutrient deficiency and any other issues during the growth period.

Data collection: Initial sand physicochemical properties were studies and data collected. After application of AMF, bulk sand physicochemical properties, wheat seedling growth response and nutrient content within plant tissue were studied at 13 days after sowing (DAS).

Measurement of bulk sand physical and chemical properties: The pH of the bulk soil was determined in deionized water using a soil-to-solution ratio of 1:5. Organic carbon of the bulk soil samples was determined by wet oxidation method (Walkley & Black, 1934). Bulk soil organic matter content was determined by multiplying the percent value of organic carbon with the conventional Van-Bemmelen's factor of 1.724. The nitrogen content of the bulk soil sample was determined by distilling soil with alkaline potassium permanganate solution (Subbaiah, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2SO_4). Bulk soil available S (ppm) was determined by calcium phosphate extraction method with a spectrophotometer at 535 nm (Petersen et al., 1996). The soil available K was extracted with 1N NH_4OAC and determined by an atomic absorption spectrometer. The available P of the bulk soil was determined by spectrophotometer at a wavelength of 890 nm. The bulk soil sample was extracted by Olsen method with 0.5 M $NaHCO_3$. The Zn, Fe, Mn, Cu, Mg, B in the bulk soil sample was measured by an atomic absorption spectrophotometer (AAS) after extracting with DTPA (Soltanpour & Schwab, 1977).

Measurement of plant biomass: After both harvests, shoots were dried at 70°C for 48 h and weighed for dry weight (DW) determination. Dried shoot tissue was ground, homogenized, and digested with nitric and perchloric acid. For nutrient concentration analysis, ICP-OES was performed by dry ashing. The C and N percentages in whole plant samples and soils were determined using an element analyzer (Vario EL cube; Elementar, Hanau, Germany).

Statistical analysis: The collected data were analyzed statistically using the analysis of variance technique and the mean differences were adjudged by Duncan's New Multiple Range Test ($P \leq 0.05$) with the help of STATVIEW software.

Results

The experiment demonstrated that the addition of Arbuscular Mycorrhizal Fungi (AMF) significantly influenced nutrient uptake and soil physicochemical properties in wheat seedlings. The addition of Arbuscular Mycorrhizal Fungi (AMF) significantly enhances wheat plant biomass, with the +AMF treatment showing the highest biomass at 0.033g pot^{-1}, compared to 0.025g pot^{-1} in the control group. This indicates that AMF contributes to improved plant growth, likely through better nutrient uptake and water absorption, highlighting its potential benefits for agricultural practices (Figure 1). The +AMF treatment, which included both nutrients and *Rhizophagus irregularis*, resulted in the highest nitrogen content in plant tissues at 0.65%, while the control group had the lowest at 0.425%. Calcium content was markedly higher in the +AMF treatment at 8.077 mg kg^{-1}, representing a 195% increase compared to the control. Copper content also peaked in the +AMF group at 0.013 mg kg^{-1}, while the control had only 0.008 mg kg^{-1}. Similarly, the iron content in the +AMF treatment was 5.246 mg kg^{-1}, significantly higher than the 1.868 mg kg^{-1} observed in the control group. Potassium uptake showed the most substantial increase, with the +AMF treatment reaching 22.66 me/100g, which is a 701% increase over the control. The carbon content in plant tissues was highest in the +AMF treatment at 2.56%, compared to 1.56% in the control. Magnesium content also saw an increase, with the +AMF treatment at 3.392 mg kg^{-1}, while the control had 2.327 mg kg^{-1}. Manganese content was slightly higher in the +AMF treatment at 0.248 mg kg^{-1} compared to the control at 0.123 mg kg^{-1}. The phosphorus content in the +AMF treatment was 6.29 mg kg^{-1}, a 367% increase over the control, while zinc content in the +AMF treatment reached 0.094 mg kg^{-1}, a 754% increase over the control (Table 3).

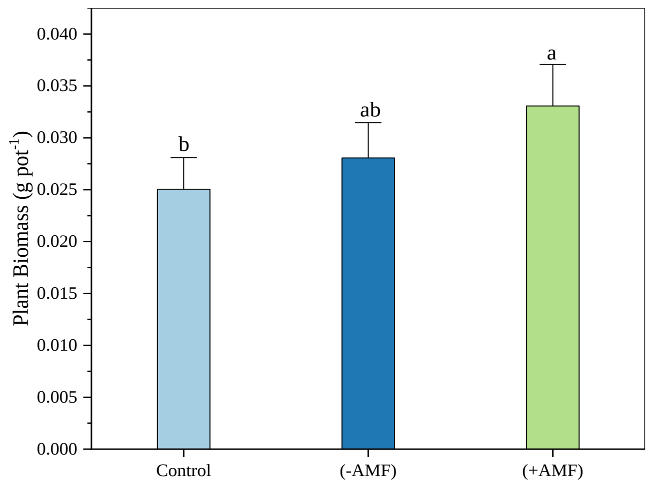

Figure 1. Dry wheat seedling biomass after harvest for several treatments. In the control treatments, nothing was added. The –AMF means the same nutrients were applied but no AMF was added. +AMF means the same nutrients and *Rh. irregularis* addition. Data are the mean of three replicates. Small bars on mean showing standard errors; mean followed by different letter differed significantly.

In terms of soil physicochemical properties, soil pH was highest in the +AMF treatment at 8.497, although this increase was not statistically significant. The control group had the highest soil nitrogen content at 0.083%, while the +AMF and -AMF treatments had significantly lower nitrogen contents of 0.021% and 0.02%, respectively. Soil phosphorus content was highest in the control at 14.7 mg kg^{-1}, with the +AMF treatment showing a decrease to 4.6 mg kg^{-1}, indicating higher phosphorus uptake by plants in the presence of AMF. Potassium levels remained constant across all treatments at 0.039 me/100g. Sulfur content was highest in the control group at 5.403 mg kg^{-1}, while the +AMF treatment had a significantly lower sulfur content of 2.903 mg kg^{-1}, reflecting increased sulfur uptake. Soil zinc concentration was significantly higher in the +AMF treatment at 0.509 mg kg^{-1} compared to 0.111 mg kg^{-1} in the control and -AMF treatments. Organic matter content was highest in the control at 1.339%, while the +AMF treatment had a much lower content at 0.308%, indicating enhanced organic matter utilization by AMF. Finally, the carbon content in the soil was highest in the +AMF treatment at 0.385%, compared to 0.225% in the -AMF treatment and 0.255% in the control, highlighting the positive effect of AMF on soil carbon content (Table 4).

Correlation

Correlation analysis was performed among wheat tissue nutrient content and bulk soil properties like Biomass (g pot^{-1}), N (%), Ca (mg kg^{-1}), Cu (mg kg^{-1}), Fe (mg kg^{-1}), K (me/100g), C (%), Mg (mg kg^{-1}), Mn (mg kg^{-1}), P (mg kg^{-1}), Zn (mg kg^{-1}), Soil pH (in water), Soil N (%), Soil P (mg kg^{-1}), Soil K (me/100g), Soil S (mg kg^{-1}), Zn (mg kg^{-1}), Soil OM (%), Soil C (%).

A significant positive relationship was observed between biomass (g pot^{-1}) with wheat tissue nutrient content and bulk soil properties (Figure 2). Among all the parameters, biomass was positively correlated with N (0.799**), Ca (0.679*), Cu (0.870**), Fe (0.641), K (0.747*), C (0.695*), Mg (0.626), Mn (0.632), P (0.753*), Zn (0.816**), Soil pH (0.893**), Soil K (0.602), Zn (0.767*), Soil C (0.57) and negatively correlated with Soil N (-0.588), Soil P (-.674*), Soil S (-.740*), Soil OM (-0.638).

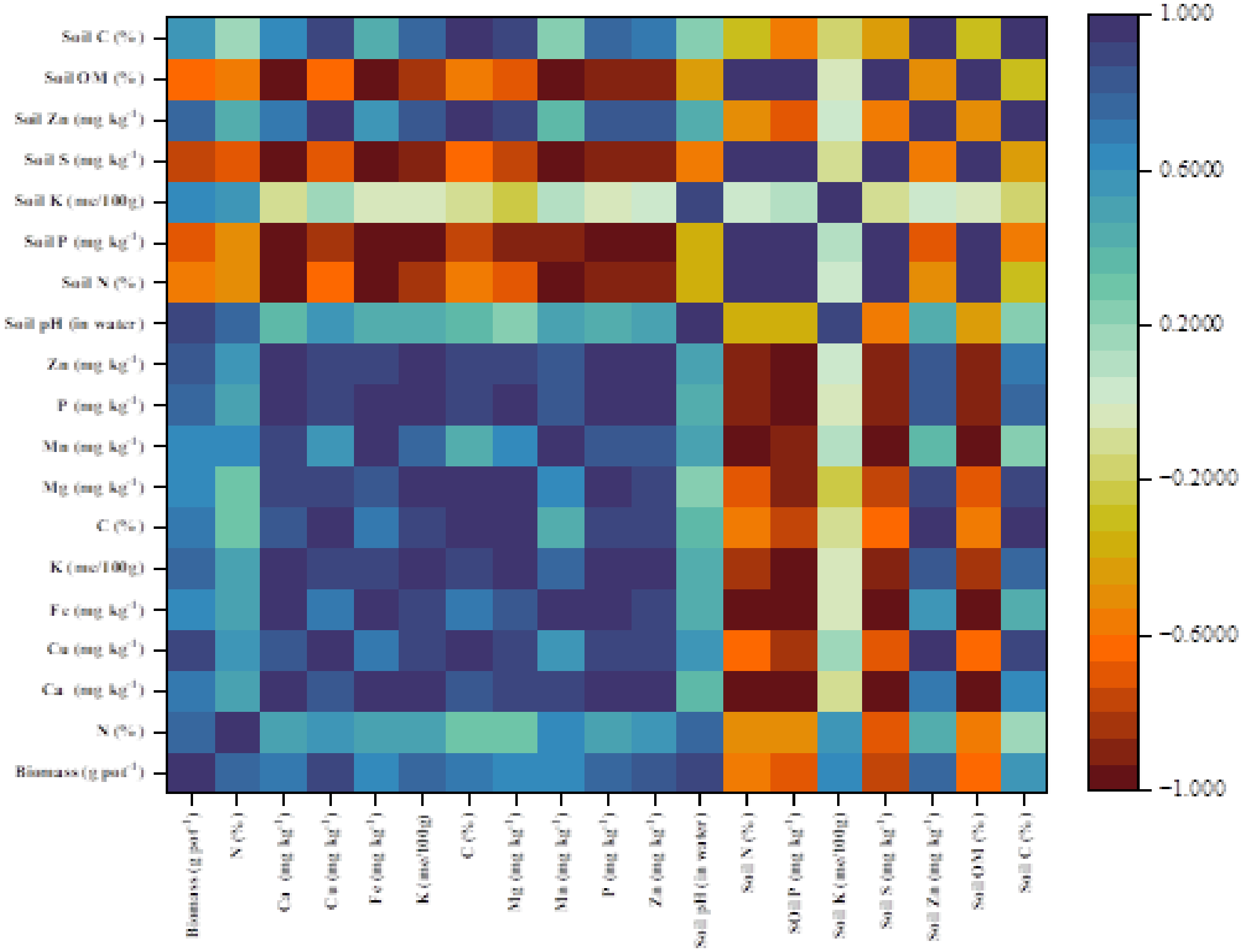

Figure 2. Correlation coefficient of different parameters of wheat tissue and bulk soil under different treatments

Discussions

Effect of mycorrhizae inoculation on wheat plant tissue nutrient content

The results of this study demonstrate a significant enhancement in nutrient uptake by wheat seedlings inoculated and sand culture with Arbuscular Mycorrhizal Fungi (AMF), particularly *Rhizophagus irregularis*. This finding aligns with recent literature indicating that AMF can substantially improve nutrient acquisition and plant health (Figure1). In this study, the (+AMF) treatment showed significantly higher levels of calcium, copper, iron, potassium, phosphorus, and zinc compared to both the control and -AMF treatments (Table 3). This is consistent with recent research by (Aslanpour et al., 2019), which highlights that AMF enhance the uptake of essential nutrients by expanding the root surface area and forming hyphal networks in the soil, which facilitate more efficient nutrient absorption (Aslanpour et al., 2019). The increase in potassium content in the (+AMF) treatment, reaching 22.66 me/100g, is particularly noteworthy as it is nearly 700% higher than the control (Table 3). This substantial increase corroborates findings by (Bhantana et al., 2021), who demonstrated that AMF can significantly boost potassium uptake, which is critical for various physiological processes in plants (Bhantana et al., 2021). The enhanced phosphorus content in the (+AMF) treatment, reaching 6.29 mg kg^{-1}, confirms the role of AMF in improving phosphorus acquisition. Recent studies have shown that AMF can mobilize phosphorus from insoluble forms in the soil, making it more available to plants (Ibrahim et al., 2022) (Table 3). Similarly, the increase in zinc content observed in the (+AMF) treatment, 0.094 mg kg^{-1} (Table 3), aligns with findings from a study by (Bhantana et al., 2021), which reported that AMF inoculation can significantly enhance zinc availability and uptake in crops. These results underscore the potential of AMF to improve nutrient availability and uptake in crops, contributing to better plant growth and productivity (Table 3). The observed increases in nutrient levels, particularly in phosphorus and potassium, suggest that AMF inoculation could be a valuable strategy for improving soil fertility and crop yield, especially in nutrient-deficient soils.

Mycorrhizae inoculation enhances soil nutrient

The impact of AMF on the physicochemical properties of the sand culture was also significant, though with varying effects across different parameters. The observed increase in soil pH from 7.993 in the control to 8.497 (Table 4) in the (+AMF) treatment is consistent with other findings, in which reported that AMF can influence soil pH by affecting microbial activity and nutrient cycling (Saia et al., 2020). However, the differences in pH among treatments were not statistically significant, suggesting that the

Table 3. Nutrient content of wheat selling tissue

Treatment	N (%)	Ca (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	K (me/100g)	C (%)	Mg (mg kg^{-1})	Mn (mg kg^{-1})	P (mg kg^{-1})	Zn (mg kg^{-1})
Control	0.425± 0.072	2.744± 0.086c	0.008± 0c	1.868± 0.05c	2.83± 0.088c	1.56± 0.041b	2.327± 0.073c	0.123± 0.003b	1.346± 0.036c	0.011± 0c
(-AMF)	0.589± 0.101	6.422± 0.201b	0.009± 0b	4.788± 0.127b	12.73± 0.397b	1.67± 0.044b	2.668± 0.083b	0.266± 0.007a	3.951± 0.105b	0.055± 0.001b
(+AMF)	0.65± 0.111	8.077± 0.252a	0.013± 0a	5.246± 0.139a	22.66± 0.708a	2.56± 0.068a	3.392± 0.106a	0.248± 0.006a	6.29± 0.167a	0.094± 0.002a
LS	NS	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV (%)	25.5	5.77	4.36	4.91	6.38	4.63	5.42	4.54	5.18	5.13

Data are means of three replicates. Mean values in a column having a different letter (s) differed significantly, NS= Non-significant, CV= Co-efficient of variation, LS= Level of significance, Control = Nothing was added, (-AMF) = Same nutrients were applied but no AMF was added, (+AMF) = Mycorrhizae added to pot

impact of AMF on soil pH may be less pronounced compared to its effect on nutrient uptake. The reduction in soil nitrogen content from 0.083% in the control to 0.021% in the (+AMF) treatment reflects increased nitrogen uptake by the plants (Table 4), a phenomenon supported by recent research. Studies by Yangyang et al., (2024) have shown that AMF can enhance nitrogen uptake and reduce nitrogen losses from the soil . This reduction in soil nitrogen, despite the higher uptake by plants, highlights the efficiency of AMF in mobilizing and utilizing soil nutrients. Phosphorus content in the sand was significantly reduced in the (+AMF) treatment, from 14.7 mg kg^{-1} in the control to 4.6 mg kg^{-1} (Table 4). This reduction aligns with the findings of Adeyemi et al., (2021), who reported that AMF inoculation can significantly decrease soil phosphorus levels as a result of enhanced phosphorus uptake by plants . The results also indicate a decrease in soil organic matter and carbon content with AMF addition, which could be attributed to the increased microbial activity and decomposition processes stimulated by the fungi (Table 4). Recent research by Zhou et al., (2020), supports this observation, suggesting that AMF can influence soil organic matter dynamics by enhancing microbial decomposition . Overall, while AMF significantly impacted nutrient uptake and some soil physicochemical properties, the variations observed highlight the complexity of AMF interactions in soil environments. These findings contribute to our understanding of AMF's role in soil nutrient dynamics and plant growth, offering valuable insights for optimizing their use in agricultural practices.

Table 4. Bulk sand physicochemical properties used in this experiment

Treatment	pH (in water)	N (%)	P (mg kg^{-1})	K (me/100g)	S (mg kg^{-1})	Zn (mg kg^{-1})	OM (%)	C (%)
Control	7.993±0.26	0.083±0.002a	14.7±0.478a	0.039±0.001	5.403±0.169a	0.111±0.003b	1.339±0.036a	0.255±0.008b
(-AMF)	8.299±0.27	0.02±0.001b	7.367±0.24b	0.039±0.001	3.203±0.1b	0.111±0.003b	0.303±0.008b	0.225±0.007b
(+AMF)	8.497±0.276	0.021±0.001b	4.6±0.15c	0.039±0.001	2.903±0.091b	0.509±0.013a	0.308±0.008b	0.385±0.012a
LS	NS	0.05	0.05	NS	0.05	0.05	0.05	0.05
CV (%)	5.64	5.3	6.25	4.29	5.65	5.35	4.87	5.48

Data are means of three replicates. Mean values in a column having a different letter (s) differed significantly, NS= Non-significant, CV= Co-efficient of variatior LS= Level of significance, Control = Nothing was added, (-AMF) = Same nutrients were applied but no AMF was added, (+AMF) = Mycorrhizae added to pot sand

Conclusion

The study demonstrates that the application of Arbuscular Mycorrhizal Fungi (AMF), specifically *Rhizophagus irregularis*, significantly enhances the growth and nutrient uptake of wheat seedlings in sand culture. AMF inoculation resulted in a notable increase in plant biomass and improved nutrient concentrations, particularly in phosphorus, potassium, calcium, and zinc. Additionally, the presence of AMF influenced soil nutrient dynamics by reducing soil nitrogen and phosphorus levels, which reflects the fungi's efficient utilization of these resources. While soil pH showed slight increases, it was not statistically significant. These findings underscore the potential of AMF as a beneficial practice in agriculture, offering a sustainable approach to improving crop performance and soil fertility. Further research could provide deeper insights into optimizing AMF use across different soil types and environmental conditions.

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