International Journal of Contemporary Academic Research
Volume 6 Number 1, Jan-Mar. 2025. Doi: **10.5281/zenodo.15115883;** ISSN: 2782-8476

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Assessing the Effect of Arbuscular Mycorrhizal Fungi Colonization on Growth of Selected Leguminous and Non-leguminous Crops

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Abstract

The rhizosphere, or soil surrounding plant roots, is critical for microbial colonization, including mycorrhizae, which is necessary for plant growth and nutrient intake. This study investigated the relationship between arbuscular mycorrhiza fungi (AMF) colonization and growth parameters of cowpea, groundnut, cucumber, and melon. A Randomized Complete Block Design (RBCD) with the five plant species (treatments), replicated 3 times was adopted in this study, AMF spores were extracted from the rhizosphere of the plant species and subjected to morphological characterization, AMF percentage colonization was calculated, and correlation analysis was performed on the data obtained. Results showed that for leguminous plants, percentage AMF colonization had a strong positive correlation with leaf number (0.979), number of branches (0.949), a moderate positive correlation with canopy volume (0.567), and a weak positive correlation with total roots colonized (0.382). For non-leguminous plants, percentage AMF colonization had a strong positive correlation with canopy volume (0.930), leaf number (0.907), total roots colonized (0.897), and number of branches (0.752). Plant height, leaf length, leaf width, canopy height, and canopy volume were not affected by AMF colonization both in legumes and non-legumes.

Keywords: Arbuscular mycorrhiza fungi; cowpea; cucumber; groundnut; melon; colonization.

INTRODUCTION

Microbial community is an important component of every ecosystem and any disturbance in the microbial community can result in an imbalance of helpful bacteria, which can deteriorate the soil and reduce crop yields (Tsiknia *et al*., 2021; Xiao *et al*., 2022). Further soil degradation may arise from modifications in the makeup of microbial communities, which may also affect the cycling of nutrients in the soil and decrease its ability to retain water (O'Callaghan *et al*., 2022). In terms of nutrient cycling, insect control, and soil structure, microbes are crucial for sustainable soil fertility management (Xiao *et al*., 2022). Microbes in exchange for their role, get a steady stream of nutrients that help them thrive and create a healthier environment for plants to grow properly (Sangwan *et al*., 2022); for example, plant roots frequently give their associated fungi nitrogen and other vital minerals, and the fungi in turn give the plants water and other vital nutrients (Xiao *et al*., 2022). By utilizing microbes to their fullest potential, small holder farmers can not only increase crop yields but also improve the sustainability of their farming practices for future generations (Tsiknia *et al*., 2021).

There is a unique symbiotic association that fungi have with higher plants, especially those that are leguminous, and this unique symbiotic association is known as mycorrhiza (Barwant *et al*., 2025). Fossil evidence indicates that arbuscular mycorrhizal connections first appeared between 400 and 450 million years ago (Barwant *et al*., 2025), and the majority of AMF species belong to the Glomeromycotina subphylum of the Mucoromycota phyla (Spatafora *et al*., 2016). The Glomerales, Archaeosporales, Paraglomerales, and Diversisporales are among the numerous orders and 25 taxa that make up this subphylum and they eat lipids and products from photosynthetic plants to complete their life cycle (Dong *et al*., 2022; de Andrade *et al*., 2023).

Arbuscular Mycorrhizal Fungi (AMF) produce plant growth hormones, increase food availability, and inhibit root diseases by colonization of root and establishing a connection between the plant and the substrate (Barwant *et al*., 2025). These fungi are also known to offer important advantages to the plants they associate with, including enhanced nutrient and water uptake, increased resistance to disease (Chanclud & Morel, 2016), and nutrient mobilization from organic substrates (Wang *et al*., 2022). These interactions can result in significant changes in the composition, structure, and functioning of plant communities, hence, a thorough understanding of these interactions is necessary for successful ecosystem conservation and management (Bhattacharjee *et al*., 2022; Dong *et al*., 2022; Tiwari *et al*., 2022; Wild *et al*., 2022).

Additionally, AMF can help plants withstand environmental stressors like drought and soil salinity, leading to higher levels of plant growth and an increased rate of survival (Tsiknia *et al*., 2021). AM fungus can improve plant nutrient absorption (Jiang *et al*., 2017), improve the uptake of water and mineral nutrients from nearby soils and protect plants from fungal infections (Astapati & Nath, 2023). AMFs are therefore necessary for both plant production and the ecology, and their importance in sustainable agricultural development cannot be overstated (Woo *et al*., 2023).

METHODOLOGY

2.1 Study Area

Between April and September of 2024, this study was carried out at the University of Port Harcourt's faculty of agriculture's teaching and research farm in Rivers State, Nigeria. The research region has an average annual rainfall of 3000 to 4500 mm, with an average lowest temperature of 22°C and a maximum temperature of 31°C.

2.2 Experimental Design

The investigation was conducted in a 26 m × 16 m experimental area using a Randomised Complete Block Design (RBCD) with four plant species (treatments) replicated 3 times. Raised beds of 3 x 4 meters were created on each plot with a 1 m allay way after the trial unit was manually cleared and the layout was measured out with tape and marked with pegs and ropes.

2.3 Source of Planting Materials

The seeds of cowpea, groundnut, melon, and cucumber were purchased from the local market in Choba, Rivers State, Nigeria.

2.4 Soil Sampling

Samples of soil were taken both prior to planting and at maturity, while and roots were taken at maturity. Using a hand trowel or shovel, soil samples were taken from the plant roots' rhizosphere. The samples were then put into clearly marked polythene bags and brought to the lab for examination.

2.5 AMF Spores Extraction

Using a wet sieving and decanting technique, 50g of rhizospheric soil sample was combined with 200ml of distilled water in a large beaker. After an hour, the contents of the beaker were decanted through sieves arranged in descending order from 200um to 25um size in order to separate the AMF spores from the soil. Until the top layer of the soil suspension was transparent, the procedure was repeated three times. The material that was kept on the sieve was then decanted into a beaker with a stream of water, and the number of spores was estimated.

2.6 AMF Root Colonization

The root samples were cleaned of soil and cut into 1-cm-long pieces. They were then cleansed in 2.5% KOH at 90^0C for 20 to 30 minutes, rinsed in water, acidified with 5N HCl, and stained with lactophenol containing 0.05% tryphan blue in order to analyze mycorrhizal colonization in the plants. About 50 segment-stained root samples were put on slides and viewed at 10x10 magnification using a compound microscope to check for AM colonization. Percent root colonization was calculated using the following formula,

$$\%\ \text{Root colonization} = \frac{\text{No of positive segments}}{\text{No of segments observed}} \times 100$$

2.7 Growth Parameters of Plants

Plant growth parameters such as plant height, number of leaves, branches, leaf length and leaf width were taken at weekly intervals following the procedures by Alfred *et al*., (2019) and Alfred & Paul, (2024). At the point of harvest, destructive sampling was carried out on selected roots of the leguminous, non-leguminous crops and weeds.

2.8 Statistical Analysis

All the data collected were represented using graphs and correlation analysis as reported by Alfred & Paul, (2024) was performed to determine the relationship between AMF colonization and different growth parameters. All analysis were performed using Minitab statistical software version 21.

RESULT

3.1 AMF Colonization and Growth Parameters of Leguminous Plants

The result for AMF percentage colonization and growth parameters of leguminous plants is shown in Fig. 1. Total roots colonized was highest in groundnut (16.3), as well as percentage colonization (39.7), while percentage colonization was lowest for cowpea (27.4), and percentage bare ground coverage was highest for cowpea (85.9). Plant height was highest in cowpea (20.0), leaf length was highest for cowpea (28.8), leaf width was highest for cowpea (15.3), highest number of branches was recorded for groundnut, leaf number was highest for groundnut (71.4), and canopy height was highest for cowpea, among others.

3.2 AMF Colonization and Non-leguminous Plants

Fig. 2 shows the result of AMF colonization and growth parameters of non-leguminous plants. For percentage AMF colonization, 21.0 was recorded for melon, 67.4% bare ground coverage was recorded for melon, total roots colonized was highest in cucumber (7.0), melon had the highest plant height (79.2), leaf length was highest for melon (25.0), cucumber had the highest leaf width (43.9) followed by melon (16.2), the highest number of branches (6.9) was recorded for melon (6.9), melon had the highest leaf number (27.9), canopy height was highest for melon (142.7) followed by cucumber (125.9), among others.

3.3 Relationship between AMF Colonization and Growth Parameters of Leguminous Plants

The result of correlation analysis showed for AMF colonization and growth parameters of leguminous plants is shown in Table 1. Percentage AMF colonization had a strong positive correlation with leaf number (0.979), number of branches (0.949), a moderate positive correlation with canopy volume (0.567), and a weak positive correlation with total roots colonized (0.382). Additionally, total roots colonized had a strong positive relationship plant height (0.704) and a weak positive correlation with leaf number (0.185), and number of branches (0.071), among others.

3.4 Relationship between AMF Colonization and Growth Parameters of Non-leguminous Plants

The result of correlation analysis showed for AMF colonization and growth parameters of non-leguminous plants is shown in Table 2. Percentage AMF colonization had a strong positive correlation with canopy volume (0.930), leaf number (0.907), total roots colonized (0.897), and number of branches (0.752), and a weak positive correlation with leaf length (0.147). Additionally, total roots colonized had a strong positive correlation with percentage bare ground cover (0.672), and leaf number (0.629), and weak positive correlation with number of branches (0.384), among others.

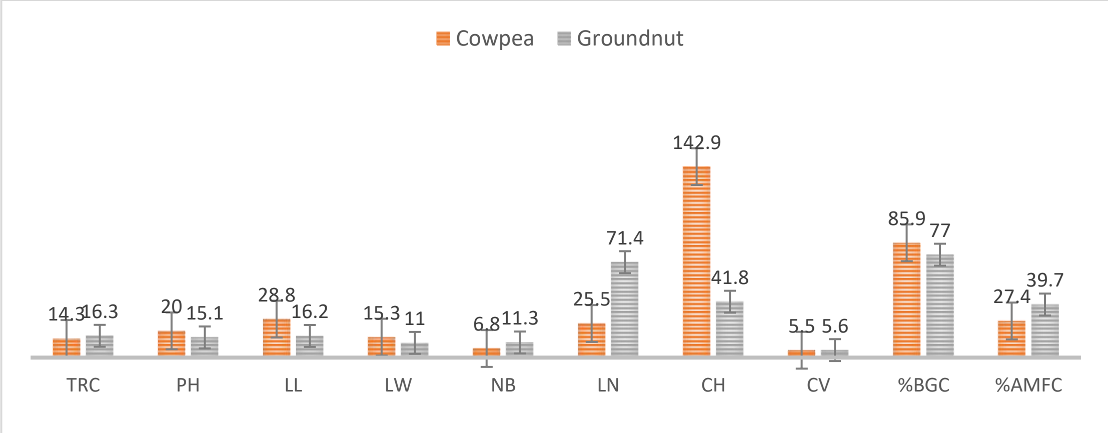

Fig. 1. AMF colonization and leguminous plants

Key: TRC = Total root colonized, PH = Plant height, LL = Leaf length, LW = Leaf width, NB = Number of branches, LN = Leaf number, CH = Canopy height, CV = Canopy volume, %BGC = % Bare ground coverage, and %AMFC = % Arbuscular mycorrhiza fungi colonization

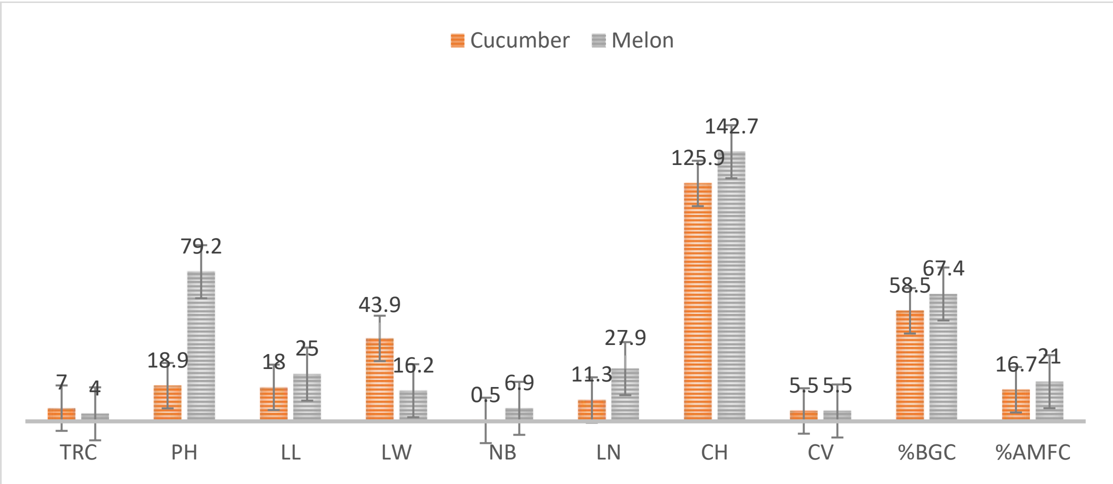

Fig. 2. AMF Colonization and Growth Parameters for Non-leguminous Plants

Key: TRC = Total root colonized, PH = Plant height, LL = Leaf length, LW = Leaf width, NB = Number of branches, LN = Leaf number, CH = Canopy height, CV = Canopy volume, %BGC = % Bare ground coverage, and %AMFC = % Arbuscular mycorrhiza fungi colonization

Table 1. Relationship between AMF colonization and growth parameters of leguminous plants

	TRC	PH	LL	LW	NB	LN	CH	CV	%BGC
PH	0.704								
LL	-0.586	0.164							
LW	-0.393	0.376	0.976						
NB	0.071	-0.659	-0.850	-0.945					
LN	0.185	-0.568	-0.905	-0.976	0.993				
CH	-0.199	0.557	0.911	0.979	-0.992	-1.000			
CV	-0.545	-0.979	-0.361	-0.557	0.798	0.723	-0.714		
%BGC	-0.959	-0.474	0.791	0.637	-0.349	-0.455	0.467	0.286	
%AMFC	0.382	-0.388	-0.973	-1.000	0.949	0.979	-0.982	0.567	-0.627

Key: TRC = Total root colonized, PH = Plant height, LL = Leaf length, LW = Leaf width, NB = Number of branches, LN = Leaf number, CH = Canopy height, CV = Canopy volume, %BGC = % Bare ground coverage, and %AMFC = % Arbuscular mycorrhiza fungi colonization

Table 2. Relationship between AMF colonization and growth parameters of non-leguminous plants

	TRC	PH	LL	LW	NB	LN	CH	CV	%BGC
PH	-0.512								
LL	-0.304	0.974							
LW	-0.401	-0.582	-0.751						
NB	0.384	0.597	0.763	-1.000					
LN	0.629	0.346	0.549	-0.964	0.959				
CH	-0.879	0.859	0.721	-0.084	0.102	-0.183			
CV	-0.981	0.336	0.115	0.570	-0.555	-0.767	0.771		
%BGC	0.672	0.293	0.501	-0.948	0.942	0.998	-0.238	-0.802	
%AMFC	0.897	-0.080	0.147	-0.764	0.752	0.907	-0.579	0.930	

Key: TRC = Total root colonized, PH = Plant height, LL = Leaf length, LW = Leaf width, NB = Number of branches, LN = Leaf number, CH = Canopy height, CV = Canopy volume, %BGC = % Bare ground coverage, and %AMFC = % Arbuscular mycorrhiza fungi colonization

4. DISCUSSION

Arbuscular mycorrhizal fungi have the potential to function as "bio-fertilizers and bio-protectors" in environmentally sustainable agriculture by colonizing the roots of many agriculturally important food and bioenergy crops (Barwant *et al*., 2025). AMF colonization have been reported to alter a number of morpho-physiological characteristics in plants (Hashem *et al*., 2015), hence, this study investigated the relationship between AMF colonization and growth of selected legumes and non-legumes.

In this study, total roots colonized was highest in the control, followed by groundnut, while percentage colonization was highest for groundnut and followed by cowpea (Fig. 1). This clearly demonstrates that AMF colonization is more in legumes than in non-legumes. AMF association with cowpea have been widely reported (Kyei-Boahen *et al*., 2017; Gondwe *et al*., 2019; Wahab *et al*., 2023). The result for AMF percentage colonization and growth parameters of leguminous plants shows that percentage bare ground coverage was highest for cowpea, leaf length and leaf width was highest for cowpea, highest number of branches and leaf number was recorded for groundnut, and canopy height was highest for cowpea (Fig. 1). AMF colonization and growth parameters of non-leguminous plants showed that total roots colonized was highest in cucumber, melon had the highest plant height and leaf length, cucumber had the highest leaf width, and canopy height was highest for melon (Fig. 2). These variations could be due to different species of legumes and non-legumes used in this study. Moreover, research has shown that even when legume plants are grown on relatively poor soils, their association with arbuscular mycorrhizal fungi (AMF) can increase hydro mineral nutrition and significantly reduce parasitic infection, allowing an increase in growth and productivity (Zhao *et al*., 2015; Frosi *et al*., 2016; Yooyongwech *et al*., 2016).

AMF can greatly increase crop productivity and ecosystem sustainability (Barwant *et al*., 2025), and cowpeas inoculated with AMF have been reported to show a notable four-fold increase in shoot and root biomass when compared to control plants (Haro *et al*., 2016). The result of correlation analysis showed for AMF colonization and growth parameters of leguminous plants showed positive correlation between percentage AMF colonization and leaf number, number of branches, and canopy volume (Table 1). For non-leguminous plants, positive correlation was reported between percentage AMF colonization and canopy volume, leaf number, total roots colonized, number of branches, and leaf length (Table 2). However, percentage AMF colonization had no relationship with plant height, leaf length, and canopy height, and canopy volume (Tables 1 and 2). These findings is supported by Mensah *et al*., (2015), who posited that plants' reactions to AMF might range from extremely advantageous to detrimental, and Wahab *et al*., (2023) who maintained that the effects of AMFs on plant growth and ecological stability are species- and environment-specific.

Contrary to the findings in Tables 1 and 2, AMF abundance and community composition were significantly impacted by the relationship between density and legume species, available phosphorus (P) and microbial biomass nitrogen (N) showed significant negative and positive correlations with AMF abundance and variety, respectively, implying that various legume species and densities may enhance the amount of accessible N, hence enhancing AMF abundance and mitigating soil P deficits (Xiao *et al*., 2019). Also, the results by Sameer & Sapan, (2020) demonstrated that AMF significantly increased the biochemical constituents such as chlorophyll-a, chlorophyll-b, total chlorophyll, protein, carbohydrate, reducing sugar, non-reducing sugar, and total phenol, as well as growth parameters such as root and shoot length, total leaf area, and fresh and dry weight of roots and shoots in cowpea, implying that AMF colonization enhanced the general growth and development of cowpea plants.

CONCLUSION

AMF are important in sustainable agriculture as they contribute greatly to general plant growth and development and ecosystem stability. However, the impacts of AMF seems to be intricate, and goes beyond just AMF colonization of roots to complex interactions with environmental factors and soil nutrient availability. While the impacts of AMF colonization on growth and yield of crops have been widely reported, this study found no relationship between AMF colonization and plant height, leaf length, leaf width, canopy cover, and canopy volume.

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