Enhance the growth and yield of garden egg varieties (*Solanum aethiopicum* L.) through integrated arbuscular mycorrhizal fungi biochar and application

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Abstract

This study investigates the effects of Arbuscular Mycorrhizal Fungi (AMF), Biochar, and Nitrogen on the growth and yield of garden egg (*Solanum aethiopicum* L.) in both rain and dry seasons. The analysis of variance (ANOVA) for plant height revealed that AMF and Biochar significantly increased plant height in both seasons, while Nitrogen's impact was not significant. However, the interactions among AMF, Biochar, and Nitrogen were generally not significant for plant height, suggesting no synergistic effects among these factors during the entire growing period. The Tukey HSD test indicated that treatments combining AMF and Biochar, particularly at optimum levels, resulted in the tallest plants. For fresh fruit yield, significant three-way interactions (AMF.BIOCHAR.NITROGEN) were observed, particularly in the rain season, underscoring the importance of integrating these soil amendments to enhance yield. This interaction remained significant in the dry season, demonstrating the robustness of combined AMF, Biochar, and Nitrogen applications in improving plant performance under varying environmental conditions. Non-marketable yield was significantly reduced by the three-way interaction in both seasons, likely due to the combined benefits of enhanced nutrient uptake, improved soil structure, and optimized Nitrogen use. The significant interaction effects highlighted the complex synergy among AMF, Biochar, and Nitrogen, resulting in better crop productivity and reduced non-marketable yield. Chlorophyll content (SPAD units) was also significantly influenced by the three-way interaction in both seasons, with treatments involving AMF showing higher chlorophyll content. This finding aligned with previous research demonstrating the role of AMF in improving nutrient uptake and plant growth, while Biochar enhances soil properties and Nitrogen efficiency. The study concluded that AMF and Biochar significantly improve plant height and yield of garden egg in both rainy and dry seasons. The combined application of AMF, Biochar, and Nitrogen resulted in significant synergistic effects, enhancing crop productivity and reducing non-marketable yield. These findings emphasized the potential of integrating AMF and Biochar in soil management practices to achieve sustainable agricultural productivity. Further research should explore the long-term effects of AMF and Biochar on soil health and crop performance, as well as the economic feasibility of these amendments for large-scale agricultural practices. Additionally, investigating the molecular mechanisms underlying the synergistic interactions among AMF, Biochar, and Nitrogen can provide deeper insights into optimizing these factors for various crops and environmental conditions.

Keywords: arbuscular mycorrhizal fungi, biochar, nitrogen, garden egg, *Solanum* genus.

Melhoramento do crescimento e a produtividade de variedades de jiló (*Solanum aethiopicum* L.) por meio da aplicação integrada de fungos micorrízicos

arbusculares e biochar

Resumo

Este estudo avaliou os efeitos dos fungos micorrízicos arbusculares (FMA), do biochar e do nitrogênio sobre o crescimento e a produtividade do jiló (*Solanum aethiopicum* L.) nas estações chuvosas e secas. A análise de variância (ANOVA) indicou que FMA e biochar aumentaram significativamente a altura das plantas em ambas as estações, enquanto o nitrogênio não apresentou efeito isolado relevante. As interações entre os três fatores, no entanto, não foram significativas para a altura, sugerindo ausência de sinergia nesse parâmetro. O teste de Tukey HSD mostrou que a combinação de FMA e biochar, em níveis ótimos, promoveu maior crescimento vegetal. Quanto à produtividade de frutos frescos, observaram-se interações significativas entre FMA, biochar e nitrogênio, principalmente na estação chuvosa, evidenciando a importância da integração desses insumos para maximizar a produção. Essa interação também se manteve na estação seca, demonstrando a robustez do manejo integrado em diferentes condições ambientais. O rendimento não comercializável foi reduzido de forma significativa pela interação tripla em ambas as estações, resultado atribuído à maior absorção de nutrientes, à melhoria da estrutura do solo e à eficiência no uso do nitrogênio. O teor de clorofila (SPAD) também foi influenciado positivamente pela interação tripla, com destaque para os tratamentos contendo FMA, corroborando pesquisas anteriores que associam esses microrganismos à maior absorção de nutrientes e vigor vegetal. O biochar, por sua vez, contribuiu para melhorar as propriedades do solo e potencializar o aproveitamento do nitrogênio. De modo geral, o estudo demonstrou que a aplicação de FMA e biochar promove ganhos significativos no crescimento e na produtividade do jiló em ambas as estações. A integração com o nitrogênio, embora não tenha impactado a altura das plantas, resultou em efeitos sinérgicos importantes para a produção de frutos e redução das perdas não comercializáveis. Esses resultados reforçam o potencial do uso combinado de FMA e biochar como estratégias sustentáveis de manejo do solo, capazes de aumentar a eficiência nutricional, melhorar o desempenho das culturas e reduzir impactos ambientais. Pesquisas futuras devem aprofundar a avaliação dos efeitos de longo prazo desses insumos sobre a saúde do solo e a viabilidade econômica de sua adoção em escala comercial. Além disso, a investigação dos mecanismos moleculares que regulam a interação entre FMA, biochar e nitrogênio poderá fornecer subsídios para otimizar seu uso em diferentes culturas e condições ambientais.

Palavras-chave: fungos micorrízicos arbusculares, biochar, nitrogênio, jiló, gênero *Solanum*.

1. Introduction

The cultivation of garden egg (*Solanum aethiopicum* L.), a vital crop in many tropical and subtropical regions, plays a crucial role in food security and nutritional health. This crop, known for its high levels of vitamins, minerals, and antioxidants, is increasingly essential in addressing malnutrition and dietary deficiencies (Dadzie et al., 2021). However, achieving optimal growth and yield of garden egg varieties remains a significant challenge for farmers, primarily due to soil fertility issues and nutrient management practices (Odedina et al., 2011).

In recent years, sustainable agricultural practices have gained attention as viable solutions for improving crop productivity while minimizing environmental impact. Among these practices, the integration of Biochar and Arbuscular Mycorrhizal Fungi (AMF) has emerged as a promising strategy. Biochar, a carbon-rich product obtained from the pyrolysis of organic materials, enhances soil physical properties, retains soil moisture, and increases nutrient availability (Arafat et al., 2018). Its application has been shown to improve soil structure, reduce leaching of nutrients, and enhance microbial activity, all of which contribute to better crop performance (Laird et al., 2010).

Arbuscular Mycorrhizal Fungi, on the other hand, form symbiotic associations with plant roots, facilitating the uptake of essential nutrients, particularly nitrogen and phosphorus, which are often limiting factors in many soils (Smith; Read, 2010). These fungi extend the root system's reach, allowing plants to access nutrients and water more efficiently. Additionally, AMF inoculation can improve plant stress tolerance, increase disease resistance, and promote overall plant health and vigor (Jeffries et al., 2003).

The combined use of Biochar and AMF represents a synergistic approach that leverages the benefits of both soil amendments to enhance plant growth and yield. While Biochar improves soil conditions and nutrient availability, AMF enhances nutrient uptake and utilization by plants (Hammer et al., 2015). This integrated approach has the potential to significantly improve the productivity of garden egg varieties, ensuring sustainable and resilient agricultural systems.

Despite the promising potential of Biochar and AMF, there is a need for more comprehensive studies to understand their interactive effects on garden egg cultivation. Most existing research has focused on the individual effects of Biochar or AMF, with limited investigations into their combined application (Biederman & Harpole, 2013). This study aims to fill this gap by evaluating the impact of integrated Biochar and AMF application on the growth and yield of garden egg. Through seasonal open pot experiments, this research will assess the effects of various Biochar and AMF treatments on plant growth parameters and fruit yield.

The integration of Biochar and AMF holds great promise for enhancing the growth and yield of the garden egg. This study aimed to explore this potential and provide practical recommendations for farmers, contributing to the development of sustainable and productive agricultural systems.

2. Materials and Methods

2.1 Site description

The research was conducted at the research farm of the West African Centre of Excellence for Water, Irrigation and Sustainable Agriculture (WACWISA) situated in Tamale in the Northern region of Ghana. The location is at an altitude of approximately 180 m above sea level (Ghana Meteorological Agency, 2018). The region has one rainy season, which starts from May and ends in October, with a dry season covering November to April. The annual average rainfall is about 1100 mm (Owusu, 2009), whilst the average temperature is within 24 °C and 35 °C (Buri et al., 2010). The soil texture of the site is sandy loam, which is slightly acidic (soil pH of 5.5 to 6.9). The test crop was garden egg, which is suitable for the climate and soil characteristics of the region.

2.2 Experimental design and treatments

The experimental was an asymmetrical 2 x 2 x 3 factorial study laid out in a Randomized Complete Block Design (RCBD), with three replications; with Arbuscular Mycorrhizal Fungi (AMF) MycoPep (Glomus intraradices) at (0 and 8 t ha^{-1}) Biochar (0, 10 t ha^{-1}), and Nitrogen (N) (0, 150, and 200 kg N ha^{-1}). The test crop was the Kotobi+ variety of garden egg.

Two experiments were conducted: an open field pot and a field experiment. For the pot experiment, plastic buckets measuring 35 cm in both diameter and height were used. Each pot had a base cover with ten 15 mm drainage holes (Figure 3). A 15 mm wide, 35 mm long PVC drainage outlet was attached to the cover to help collect leachate. To aid in drainage and prevent soil loss, a 200 g layer of washed sand was placed at the bottom of each pot, on top of a filter paper (Figure 3). Each pot was filled with 20 kg of soil and sand, mixed in the ratio 3:1, and one plant was grown in each container.

2.3 Nitrogen fertilization

Nitrogen was applied using urea, which contains 46% Nitrogen. 200 kg N ha^{-1} is a recommended rate of Nitrogen application with organic fertilizer for optimal production of garden egg in Ghana (Adjei et al., 2023). The fertilizer was administered in four equal split applications at 1, 4, 8, and 12 weeks after transplanting (WAT).

2.4 Source of inoculant and biochar

MycoPep (Vascular Arbuscular Fungi Glomus intraradices) is a biofertilizer produced by Peptech Bioscience Ltd in New Delhi, India, and distributed by Agromonti Limited in Accra, Ghana. Biochar was produced from rice husks obtained from the Avnash Rice Processing Factory in Nyankpala, Ghana, through a process of high-temperature pyrolysis.

2.5 Data collection

Table 1 outlines the methods used for data collection in this study, along with references to the methodologies applied.

Table 1. Methods for collecting yield and yield components

Parameter	Method	References
Height	Measured using a ruler or measuring tape from the base of the plant to the tip of the highest leaf or stem.	(Akinbile; Yusuff, 2020)
Total Fruit Yield	Weighed using a digital scale. Harvested fruits are collected, and their fresh weight is recorded.	Akinbile; Yusuff, 2020
Non-marketable Yield	Weighed using a digital scale. Non-marketable fruits are those that do not meet quality standards and are weighed separately from marketable fruits.	Ndereyimana et al., 2013
Chlorophyll Content	Measured using a SPAD chlorophyll meter (Soil Plant Analysis Development). SPAD readings are taken from the topmost fully expanded leaves.	Uddling et al., 2007

Source: Authors, 2025.

Before incubation, the pH, cation exchange capacity (CEC), total organic carbon (TOC), total Nitrogen (N), ammonium ions (NH_4^+), nitrate ions (NO_3^-), and available phosphorus (P) in the Biochar and soil samples were analysed using the methods that are presented (Table 2). Each measurement was performed in triplicate, and the average value was recorded in (Table 3).

Table 2. Methods of measuring preliminary physico-chemical characteristics of soil and biochar.

Parameter	Method	Reference
pH	Measured in a soil-water suspension (1:1 or 1:2.5) using a pH meter.	Thomas, 1996.
CEC (Cmol (+) kg^{-1})	Extracted with ammonium acetate (pH 7.0), then measured using atomic absorption spectrometry.	Rhoades, 1982.
TOC (mg kg^{-1})	Determined by dry combustion using a CHN analyzer.	Nelson; Sommers, 1996.
Total N (g kg^{-1})	Measured by dry combustion using the Kjeldahl method.	Bremner, 1960.
NH_4^+ (mg kg^{-1})	Extracted with potassium chloride (KCl) and measured using spectrophotometry.	Mulvaney, 1996.
NO_3^- (mg kg^{-1})	Nitrate concentrations in the soil samples were measured using the LaquaTwin nitrate meter (Model B-743, Horiba, Japan) following the manufacturer's instructions.	Instruction Manual for LaquaTwin Nitrate Meter Model B-743.
Available P (mg kg^{-1})	Extracted using the Bray-1 and measured using spectrophotometry.	Olsen; Sommers, 1982.
Soil texture	Determined using the hydrometer method	Gee; Bauder, 1986.

Table 3. Preliminary physical and chemical properties of soil and biochar.

Properties	Soil	Rice husk biochar

pH	6.32	9.74
CEC (Cmol (+) kg^{-1})	22.00	32.41
TOC (mg kg^{-1})	8.86	25.5
DOC (mg kg^{-1})	11.12	44.46
Total N (g kg^{-1})	1.27	4.21
NH_4^+ (mg kg^{-1})	8.00	1.26
NO_3^- (mg kg^{-1})	16.66	3.13
Available P (mg kg^{-1})	22.54	195.99
Soil texture	Sandy loam	

Source: Authors, 2025.

2.6 Statistical data analysis

All data were analyzed using Analysis of Variance (ANOVA) to identify significant differences among treatments. Mean separations were conducted using the Tukey Honestly Significant Difference (HSD) test at a 5% significance level. Statistical analyses were performed with GenStat software, 12th edition.

3. Results

3.1 Plant height in the rainy season

According to Table 3.1, the effect of AMF was highly significant, indicating a substantial impact on plant height during the rainy season. Additionally, (Table 3.1) suggests that the impact of Biochar on plant height during the rainy season was also highly significant. $P > 0.05$ was found for all AMF x Biochar, AMF x Nitrogen, Biochar x Nitrogen, and AMF x Biochar x Nitrogen interactions, indicating that they were not significant (Table 3.1). The combined effects of AMF, Biochar, and Nitrogen on plant height were not statistically significant during the rain season, as demonstrated by the lack of significant interactions.

Table 3.1. Analysis of variance (ANOVA).

Variate: Plant height in the rainy season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.28	1.14	0.02	
AMF	1	3791.48	3791.48	54.26	<.001
BIOCHAR	1	900	900	12.88	<.001
NITROGEN	2	207.16	103.58	1.48	0.231
AMF.BIOCHAR	1	3.74	3.74	0.05	0.817
AMF.NITROGEN	2	4.73	2.36	0.03	0.967
BIOCHAR.NITROGEN	2	38.54	19.27	0.28	0.759
AMF.BIOCHAR.NITROGEN	2	6.03	3.02	0.04	0.958
Residual	130	9084.01	69.88		
Total	143	14037.97			

Source: Authors, 2025.

However, Tukey post-hoc tests for the three-way interaction model were conducted as an exploratory analysis to identify potential trends or interactions that might have biological significance, even if they were not statistically significant (Table 3.2). The treatments that are not significantly different from one another are indicated by the grouping (a, ab, abc, etc.). The mean of plant height of Mm Bb N200 was the greatest at 39.62 cm, which indicated a significant difference from MoBoNo (20.34 cm). Plant heights were generally higher in treatments combining AMF (Mm) and Biochar (Bb) than in treatments without AMF and Biochar (Table 3.2).

Table 3.2. The results of the Tukey HSD test for plant height during the rainy season.

Treatments	Means (cm)	Significant groups
MoBoNo	20.34	a
Mo Bo N150	24.32	ab
Mo Bo N200	24.35	ab
Mo Bb No	26.75	abc
Mo Bb N150	28.1	abc
Mo Bb N200	28.19	abc
Mm Bo No	30.88	abcd
Mm Bo N150	33.97	bcd
Mm Bo N200	33.99	bcd
Mm Bb No	37.22	cd
Mm Bb N150	37.96	cd
Mm Bb N200	39.62	d

Source: Authors, 2025.

3.2 Plant height in the dry season

AMF had a significant impact on plant height ($P < 0.05$), indicating that AMF influenced the plant height throughout the dry season (Table 3.3). Biochar was also highly significant ($P < 0.05$). Similar to the findings during the rain season, none of the interactions were significant (Table 3.3).

Table 3.3. Analysis of variance (ANOVA).

Variate: Plant height in dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.42	1.21	0.02	
AMF	1	3174.57	3174.57	45.15	<.001
BIOCHAR	1	789.7	789.7	11.23	0.001
NITROGEN	2	177.11	88.56	1.26	0.287
AMF.BIOCHAR	1	1.84	1.84	0.03	0.872
AMF.NITROGEN	2	4.75	2.37	0.03	0.967
BIOCHAR.NITROGEN	2	33.4	16.7	0.24	0.789
AMF.BIOCHAR.NITROGEN	2	6.67	3.34	0.05	0.954
Residual	130	9139.54	70.3		
Total	143	13330.01			

But, Tukey post-hoc tests for the three-way interaction model were conducted as an exploratory analysis to examine the potential trends or interactions that could have biological significance, even if they were not statistically significant (Table 3.4). Mm Bb N200 (35.03 cm) differed significantly from MoBoNo (17.13 cm) in terms of mean plant height (Table 3.4). Treatments with AMF (Mm) and Biochar (Bb) tended to grow taller plants, just like during the rainy season.

Table 3.4. The results of the Tukey HSD test for plant height during the dry season.

Treatments	Means (cm)	Significant groups
MoBoNo	17.13	a
Mo Bo N150	20.85	ab
Mo Bo N200	20.9	ab
Mo Bb No	23.25	abc
Mo Bb N150	24.46	abcd
Mo Bb N200	24.54	abcd
Mm Bo No	26.92	abcd
Mm Bo N150	29.72	bcd
Mm Bo N200	29.74	bcd
Mm Bb No	32.72	cd
Mm Bb N150	33.36	cd
Mm Bb N200	35.03	d

Source: Authors, 2025.

3.3 Fresh fruit yield in the rainy season

Table 3.5 shows that there was a strong interaction effect among AMFxBiocharxNitrogen that was highly significant ($p < 0.05$) on fresh fruit.

Table 3.5. Analysis of variance (ANOVA).

Variate: Fresh fruit yield in the rainy season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5716441	2858221	135.62	
AMF	1	496085	496085	23.54	<.001
BIOCHAR	1	91017	91017	4.32	0.04
NITROGEN	2	1367	684	0.03	0.968
AMF.BIOCHAR	1	48421	48421	2.3	0.132
AMF.NITROGEN	2	928463	464232	22.03	<.001
BIOCHAR.NITROGEN	2	1171418	585709	27.79	<.001
AMF.BIOCHAR.NITROGEN	2	2165063	1082531	51.36	<.001
Residual	130	2739831	21076		
Total	143	13358108			

Source: Author, 2025.

The treatments with the lowest mean yields were Mo Bo No, Mo Bo N150, and Mo Bb N150, indicating that the lack of AMF and Biochar led to the lower yields (Table 3.6). However, the treatments with the highest yields, such as Mm Bo N150 and Mm Bb N200, indicated the beneficial effects of the presence of AMF and Biochar.

Table 3.6. The results of the Tukey HSD test for fruit fresh yield during the rainy season

Treatments	Means (g)	Significant groups

MoBoNo	582.2	a
Mo Bo N150	759.7	ab
Mo Bo N200	886.3	bcd
Mo Bb No	1054.1	de
Mo Bb N150	599.9	a
Mo Bb N200	835.1	bc
Mm Bo No	967.9	cde
Mm Bo N150	1139.5	e
Mm Bo N200	583	a
Mm Bb No	770.2	ab
Mm Bb N150	897.6	bcd
Mm Bb N200	1063.5	de

Source: Authors, 2025.

3.4 Fresh fruit yield in the dry season

The three-way interaction (AMF x Biochar x Nitrogen) remained extremely significant ($p < 0.05$) throughout the dry season (Table 3.7).

Table 3.7. Analysis of variance (ANOVA);

Variate: Fresh fruit yield Dry Season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	3722389	1861194	55.66	
AMF	1	499533	499533	14.94	<.001
BIOCHAR	1	93448	93448	2.79	0.097
NITROGEN	2	1640	820	0.02	0.976
AMF.BIOCHAR	1	46002	46002	1.38	0.243
AMF.NITROGEN	2	867551	433775	12.97	<.001
BIOCHAR.NITROGEN	2	1092650	546325	16.34	<.001
AMF.BIOCHAR.NITROGEN	2	2222203	1111101	33.23	<.001
Residual	130	4347281	33441		
Total	143	12892696			

Source: Authors, 2025.

The treatments with the lowest mean yields during the dry season were Mo Bo No, Mo Bb N150, and Mm Bo N200; these treatments differed significantly from those with greater yields, such as Mm Bo N150 and Mm Bb N200 (Table 3.8).

Table 3.8. The results of the Tukey HSD test for fruit fresh yield during the dry season.

Treatments	**Means (g)**	**Significant groups**

Mo Bo No	433	a
Mo Bo N150	585.2	ab
Mo Bo N200	716.8	bc
Mo Bb No	903	cd
Mo Bb N150	448.6	a
Mo Bb N200	643.4	ab
Mm Bo No	782.3	bcd
Mm Bo N150	978.1	d
Mm Bo N200	435.1	a
Mm Bb No	593.6	ab
Mm Bb N150	729.5	bc
Mm Bb N200	918.2	cd

Source: Authors, 2025.

3.5 Non-marketable yield (Rain season)

Table 3.9 shows that the three-way interaction between AMF, Biochar, and Nitrogen was extremely significant ($p < 0.05$). Table 3.9 shows that the two-way interactions were likewise quite significant.

Table 3.9. Analysis of variance (ANOVA).

Variate: Non-marketable Yield (g per plant) in rainy season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	108.6294	54.3147	65.88	
AMF	1	7527.453	7527.453	9130.64	<.001
BIOCHAR	1	2636.436	2636.436	3197.94	<.001
NITROGEN	2	448.3574	224.1787	271.92	<.001
AMF.BIOCHAR	1	376.9493	376.9493	457.23	<.001
AMF.NITROGEN	2	35.5424	17.7712	21.56	<.001
BIOCHAR.NITROGEN	2	108.9412	54.4706	66.07	<.001
AMF.BIOCHAR.NITROGEN	2	75.7197	37.8599	45.92	<.001
Residual	22	18.1372	0.8244		
Total	35	11336.17			

Source: Authors, 2025.

A Tukey HSD (Honestly Significant Difference) test was performed to compare the means of different treatment combinations for non-marketable yield during the rainy season. The findings are shown in (Table 3.10). The treatments that had the same letter did not significantly differ from each other, and the significant groups were indicated by letters. Mm Bb N200 produced the least amount of non-marketable yield, whereas MoBoNo produced the most. In general, there was a significant decrease in non-marketable yield when Biochar and the AMF were used, especially when the Nitrogen levels were at their optimal values Table 3.10. The findings showed how crucial it is to choose the ideal application rates of AMF, Biochar, and Nitrogen in order to maximize the quality of the yield during the rainy season.

Table 3.10. The results of the Tukey HSD test for Non-marketable yield during the rain

Treatments	Means (g)	Significant groups
Mo Bo No	108.22	i
Mo Bo N150	98.51	h
Mo Bo N200	89.37	g
Mo Bb No	76.7	f
Mo Bb N150	75.19	ef
Mo Bb N200	73.45	e
Mm Bo No	67.09	d
Mm Bo N150	62.66	c
Mm Bo N200	60.17	c
Mm Bb No	55.52	b
Mm Bb N150	52.49	a
Mm Bb N200	49.99	a

Source: Authors, 2025.

3.6 Non-marketable yield (Dry season)

The combined influence of these three factors on non-marketable yield was complex and significant ($P < 0.05$), as indicated by the significant three-way interaction among the components of AMF, Biochar, and Nitrogen (Table 3.11).

Table 3.11. Analysis of variance (ANOVA).

Variate: Non-marketable Yield (g per plant) in dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.3905	0.6952	0.77	
AMF	1	11656.6194	11656.62	12900.77	<.001
BIOCHAR	1	4237.7594	4237.759	4690.07	<.001
NITROGEN	2	696.067	348.0335	385.18	<.001
AMF.BIOCHAR	1	585.5444	585.5444	648.04	<.001
AMF.NITROGEN	2	52.3796	26.1898	28.99	<.001
BIOCHAR.NITROGEN	2	155.1942	77.5971	85.88	<.001
AMF.BIOCHAR.NITROGEN	2	117.74	58.87	65.15	<.001
Residual	22	19.8783	0.9036		
Total	35	17522.5728			

Source: Authors, 2025.

The mean of non-marketable yield for each combination of treatments is shown in (Table 3.12). Significant differences between the treatments were indicated by the different letters adjacent to the mean values. The treatments that did not share a letter were significantly different. The highest non-marketable yield (127.34 g) of the treatment MoBoNo (no AMF inoculation, no Biochar, and no Nitrogen) differed significantly from the other treatments (group "k"). This indicated that during the dry season, the combination of these factors produced the greatest quantity of unmarketable yield (Table 3.12). With the lowest non-marketable yield (54.25 g) of treatment Mm Bb N200 (AMF inoculation, Biochar, and optimal Nitrogen) was classified as "a." This indicated that during the dry season, this combination was the most successful in reducing non-marketable yield.

Table 3.12. The results of the Tukey HSD test for Non-marketable yield during the dry season.

Treatments	Means (g)	Significant groups
MoBoNo	127.34	K
Mo Bo N150	114.74	J
Mo Bo N200	104.15	I
Mo Bb No	87.7	H
Mo Bb N150	85.74	Gh
Mo Bb N200	83.49	G
Mm Bo No	75.9	F
Mm Bo N150	70.7	E
Mm Bo N200	67.46	D
Mm Bb No	61.42	C
Mm Bb N150	57.48	B
Mm Bb N200	54.25	A

Source: Authors, 2025.

3.7 Chlorophyll content (SPAD Units) in the rainy season

Table 3.13 shows that the three-way interaction between AMF, Biochar, and Nitrogen was extremely significant ($p < 0.05$).

Table 3.1. Analysis of variance (ANOVA)

Variate: Chlorophyll Content (SPAD Units) in the rainy season						
Source of variation		d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum		2	3032.88	1516.44	58.14	
AMF		1	2219.39	2219.39	85.09	<.001
BIOCHAR		1	65.51	65.51	2.51	0.115
NITROGEN	2		790.05	395.03	15.14	<.001
AMF.BIOCHAR		1	4.1	4.1	0.16	0.692
AMF.NITROGEN		2	112.79	56.4	2.16	0.119
BIOCHAR.NITROGEN		2	147.25	73.62	2.82	0.063
AMF.BIOCHAR.NITROGEN		2	432.4	216.2	8.29	<.001
Residual		130	3390.88	26.08		

Source: Authors, 2025.

Chlorophyll content was usually higher with AMF treatments (Table 3.14). The conditions with the highest mean of chlorophyll content (38.55 SPAD units) included 200 kg of Nitrogen per ha, Biochar, and AMF. In general, AMF and Biochar treatments performed better than those without, regardless of the amount of Nitrogen level.

Table 3.14. The results of the Tukey HSD test for Chlorophyll content (SPAD units) during the rainy season.

Treatments	Means (SPAD)	Significant groups

Mo Bo No	19.34	a
Mo Bo N150	25.24	ab
Mo Bo N200	29.44	bc
Mo Bb No	25.39	ab
Mo Bb N150	21.93	a
Mo Bb N200	29.74	bc
Mm Bo No	32.55	cd
Mm Bo N150	32.61	cd
Mm Bo N200	31.41	bc
Mm Bb No	29.59	bc
Mm Bb N150	33.48	cd
Mm Bb N200	38.55	d

Source: Authors, 2025.

3.8 Chlorophyll content (SPAD Units) in dry season

During the dry season, the three-way interaction between AMF, Biochar, and Nitrogen was highly significant ($p < 0.05$) (Table 3.15).

Table 3.15. Analysis of variance (ANOVA).

Variate: Chlorophyll Content (SPAD Units) in the dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2423.35	1211.68	60.74	
AMF	1	867.96	867.96	43.51	<.001
BIOCHAR	1	0.24	0.24	0.01	0.913
NITROGEN	2	671.43	335.72	16.83	<.001
AMF.BIOCHAR	1	32.41	32.41	1.62	0.205
AMF.NITROGEN	2	67.69	33.84	1.7	0.187
BIOCHAR.NITROGEN	2	131.79	65.9	3.3	0.04
AMF.BIOCHAR.NITROGEN	2	372.14	186.07	9.33	<.001
Residual	130	2593.29	19.95		
Total	143	7160.29			

Source: Authors, 2025.

Treatments with AMF indicated greater chlorophyll content, comparable to the rain season (Table 3.16). The highest mean of chlorophyll content (31.31 SPAD units) was found in the treatments that had 200 kg of Nitrogen per hectare, Biochar, and AMF.

Table 3.16. The results of the Tukey HSD test for chlorophyll content (SPAD units) during the dry season.

Treatments	**Means (SPAD)**	**Significant groups**
Mo Bo No	16.97	a
Mo Bo N150	22.15	ab

Mo Bo N200	25.84	bc
Mo Bb No	20.52	ab
Mo Bb N150	17.49	a
Mo Bb N200	24.34	b
Mm Bo No	25.93	bc
Mm Bo N150	25.99	bc
Mm Bo N200	24.93	b
Mm Bb No	22.45	ab
Mm Bb N150	26.17	bc
Mm Bb N200	31.31	c

Source: Authors, 2025.

4. Discussion

Plant height was significantly increased by both AMF and Biochar individually in both seasons (Table 3.1 and Table 3.2). Nitrogen did not have a significant effect on plant height in both rainy and dry seasons. The combined effects of AMF, Biochar, and Nitrogen were not statistically different in both the rain and dry seasons.

The studies have found that AMF enhance plant growth by increasing Nitrogen uptake efficiency, and that Biochar improves soil structure and availability of nutrients (Smith; Read, 2010; Lehmann et al., 2011). Integrated application of AMF and Biochar had synergistic benefits on plant growth. Some researchers have found that although Nitrogen is vital for plant growth, the application of other soil amendments, such as AMF and Biochar, increases plant growth.

The three-way interaction (AMF x Biochar x Nitrogen) was highly significant ($p < 0.05$) (Appendix 21). The treatments with the lowest mean crop yields were Mo Bo No, Mo Bo N150, and Mo Bb N150, indicating that crop yields were not as great as they may have been in the absence of AMF and Biochar (Table 3.6). Though the treatments with the highest crop yields, such as Mm Bo N150 and Mm Bb N200, emphasized the benefit of AMF and Biochar application.

These findings were supported by previous studies (Smith & Read, 2011) that found the beneficial effects of AMF on plant growth and crop yield by improving soil structure and increasing nutrient uptake efficiency. Several studies have found that Biochar enhances soil fertility, microbial activity, and water holding capacity, which are critical factors for plant growth (Lehmann et al., 2011; Jeffery et al., 2011).

During the dry season, the three-way interaction (AMF x Biochar x Nitrogen) was also highly significant ($p < 0.05$) (Table 3.7). The importance of this interaction showed that, even in situations where water is scarce, applying AMF, Biochar, and Nitrogen together can increase fruit yield. The treatments with the lowest mean yields during the dry season were Mo Bo No, Mo Bb N150, and Mm Bo N200; these treatments had less yield compared to treatments like Mm Bo N150 and Mm Bb N200 (Table 3.8). These results indicate that AMF and Biochar were more helpful in dry conditions, probably because of their functions in boosting plant stress tolerance and retaining soil moisture (Lehmann et al., 2011; Warnock et al., 2007).

Seasonal differences in the effects of treatments on yield indicated the necessity for specialized soil management techniques. By enhancing soil health and plant resilience, two factors critical to sustainable agriculture, particularly in areas with variable climates, integrating AMF and Biochar can greatly increase crop yield. Tables 3.9 and 3.11 indicate that the combined effect of AMF, Biochar, and Nitrogen was not only additive but also synergistic due to the strong three-way interaction between these factors. Both the rain and dry seasons showed this interaction, indicating that the combined treatments had different effects than those predicted from the individual treatments alone (Table 3.10 and Table 3.12).

Non-marketable yield was significantly reduced as a result of the three-way interaction between AMF, Biochar, and Nitrogen (Table 3.10 and Table 3.12). This result was probably brought about by the synergistic effects of better soil structure, increased nutrient uptake, and optimum Nitrogen usage (Liang et al., 2023). The greater abiotic stress during the dry season can be the reason for the higher non-marketable yield in the dry season when compared to the wet season (Table 3.10 and Table 3.12). Drought-stressed plants frequently use more energy to survive than to grow, which increases their yields that are not suitable for the market (Cramer et al., 2020).

The significant effect of AMF on chlorophyll content indicated a highly beneficial influence of AMF on chlorophyll content during the rainy season. This conclusion was consistent with research that showed that AMF improves nutrient uptake, especially of Nitrogen and phosphorus, which increases plant production of chlorophyll (Smith; Read, 2010). Investigate the long-term impacts of AMF and Biochar on soil health and crop productivity. This includes assessing changes in soil microbial communities, nutrient cycling, and soil structure over multiple growing seasons.

Determine the optimal application rates and combinations of AMF, Biochar, and Nitrogen for different crops and soil types. This will help in developing precise recommendations for farmers to maximize benefits while minimizing costs. Study the effects of AMF and Biochar under various abiotic stress conditions, such as drought, salinity, and extreme temperatures. Understanding how these amendments help plants cope with stress can improve resilience in changing climates.

Conduct a cost-benefit analysis to evaluate the economic feasibility of using AMF and Biochar in agricultural practices. This should consider the costs of amendments, potential yield increases, and long-term benefits to soil health. Explore the underlying mechanisms of how AMF and Biochar interact with nitrogen and other soil nutrients. This includes studying root morphology, nutrient uptake pathways, and the role of microbial interactions in enhancing plant growth.

Extend research to other crops to determine if the observed benefits of AMF and Biochar are consistent across different plant species. This will help in developing broader agricultural recommendations. Implement large-scale field trials to validate the results obtained from controlled experiments. Field trials in different geographic regions and under varying farming practices will provide more robust data for practical applications.

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7. Authors' Contributions

The study was designed by *Jean Bosco Ngarukiyimana*, who also conducted the experiments, analyzed the data, and wrote the manuscript. *Israel K. Dzomeku* and *Abdul-Halim Abubakari* provided advice on the manuscript revision, data interpretation, and research design. Hamudu Rukangantambara helped with the improvement of the manuscript, critical review, and data analysis. The final version of the manuscript was read and approved by all authors.

8. Conflicts of Interest

Not applicable.

9. Ethics Approval

Not applicable.

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