UNIVERSITY FOR DEVELOPMENT STUDIES

NITROGEN BIOREMEDIATION BY ARBUSCULAR MYCORRHIZAL FUNGI AND BIOCHAR FOR OPTIMUM REDUCTION IN ENVIRONMENTAL POLLUTION AND SUSTAINABLE PRODUCTION OF GARDEN EGG (*Solanum aethiopicum* L.)

JEAN BOSCO NGARUKIYIMANA

2025

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BY

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UDS/DES/0006/21

[THESIS SUBMITTED TO THE DEPARTMENT OF ENVIRONMENT AND SUSTAINABILITY SCIENCES, FACULTY OF NATURAL RESOURCES AND ENVIRONMENT, UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE GHANA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL MANAGEMENT AND SUSTAINABILITY]

MARCH, 2025

DECLARATION

DECLARATION BY CANDIDATE

I affirm that this thesis represents my original research and has not been submitted, in whole or in part, for any other degree at this University or any other institution. All external sources that contributed to this work have been appropriately cited and acknowledged in the reference section.

Jean Bosco Ngarukiyimana.. 7th May 2025

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DECLARATION BY SUPERVISORS

I hereby confirm that the preparation and presentation of this Thesis were conducted under supervision in full compliance with the guidelines set forth by the University for Development Studies.

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ABSTRACT

Nitrogen (N) loss in agricultural systems is a significant environmental problem, contributing to greenhouse gas emissions and water pollution. This study examined the potential of Arbuscular Mycorrhizal Fungi (AMF) and Biochar as sustainable solutions to mitigate Nitrogen losses and enhance soil health in garden egg (*Solanum aethiopicum* L.) production. The research addressed the combined effects of these amendments on Nitrogen Bioremediation, Nutrient Use Efficiency (NUE), soil properties, and crop productivity, with a focus on nutrient-poor soils in Ghana. The objectives were to (1) assess reduction in leachate volume and model nitrate leaching with AMF and Biochar; (2) evaluate Nitrogen Uptake Efficiency (NUpE) and NUE in garden egg; (3) investigate the impact of AMF and Biochar on soil properties, including pH, organic carbon content, and cation exchange capacity (CEC); and (4) analyze the effects of these treatments on crop productivity across two seasons. A 2 x 2 x 3 factorial study in a Randomized Complete Block Design (RCBD) was used, involving two AMF levels (0, 8 kg/ha), and two Biochar levels (0, 10 tons/ha) and three Nitrogen levels (0, 150, 200 kg ha^{-1}). The study was conducted at the West African Centre for Water, Irrigation, and Sustainable Agriculture (WACWISA), University for Development Studies, Ghana. Nitrate leaching was modeled using an exponential Probability Density Function-based Model, and soil properties, plant growth, and yield were measured with standard methods. The study showed that when Nitrogen was applied at similar rates, the addition of AMF and Biochar significantly reduced nitrate levels in the leachate and decreased the total leachate volume ($P < 0.05$). At the optimal Nitrogen rate of 200 kg N ha^{-1}, the absence of AMF and Biochar resulted in nitrate concentrations and leachate losses from the root zone being 16.77 and 4.99 times higher, respectively, compared to when both were present. The model predicted that, at 200 kg N ha^{-1}, nitrate loss from the root zone would be 60.04% without AMF and Biochar, versus only 4.92% when they were applied. Additionally, regression analysis identified a moderate to strong negative correlation between cumulative field capacity (FC) and Nitrogen leaching (NL), as predicted by the Exponential Probability Density Function-based Model, with a multiple R value of 0.6948. The model's moderate fit ($R^2 = 0.4828$) explained roughly 48% of the variability in nitrate leaching. The negative coefficient for cumulative FC (-0.0244) supported the idea that improved soil water retention effectively reduces nitrate leaching. These findings emphasize the crucial role of soil moisture management in minimizing Nitrogen loss. Results revealed that AMF and Biochar combined significantly reduced leachate volume and Nitrogen leaching, particularly at higher Nitrogen levels (200 kg/ha). The synergistic application of AMF and Biochar also improved NUpE and NUE, with the highest efficiency achieved with AMF and Biochar at 200 kg N/ha. Biochar enhanced soil pH, organic carbon, and CEC, while AMF increased root biomass and colonization. These changes led to higher plant height, fresh fruit yield, and chlorophyll content. The study concluded that integrating AMF and Biochar in Nitrogen-fertilized soils effectively improved Nitrogen bioremediation, soil health, and garden egg yield. It is recommended that farmers adopt these practices to optimize Nitrogen use and minimize environmental impacts. Further research should explore the long-term effects, economic feasibility, and underlying mechanisms of AMF and Biochar applications across agro-ecosystems. Crop-specific studies can determine their broader applicability in sustainable agriculture.

Keywords: Nitrogen Bioremediation; Arbuscular Mycorrhizal Fungi (AMF); Biochar; Nutrient Use Efficiency (NUE); Nitrate Leaching

DEDICATION

This work is dedicated to my Dad, Charlotte Cartwright, Jonathan Cartwright, Sarah Lander, Coventry Anneliese, my Brothers and Sisters.

ACKNOWLEDGEMENTS

The successful completion of this thesis would not have been possible without the support and contributions of many individuals. I am deeply grateful to my supervisors and mentors, Prof. Israel K. Dzomeku and Prof. Abdul-Halim Abubakari, for their unwavering guidance, support, and encouragement throughout my PhD journey.

I owe a special debt of gratitude to my family, especially my father, Mr. Froduard Ugizwenimana, for his endless support and sacrifices. My heartfelt thanks also go to my siblings Ishimwe, Mutoni, Uwumukiza, Lambert, and Angelbert for their steadfast support and encouragement.

To my dear friends Charlotte Cartwright, Jonathan Cartwright, Sarah Lander, and Coventry Anneliese, who have supported me since the beginning of my postgraduate studies, may God bless you abundantly.

I extend my thanks to the West African Centre for Water, Irrigation and Sustainable Agriculture (WACWISA) for their support of my PhD studies. I also appreciate the encouragement and assistance from my friends and colleagues at WACWISA, particularly Didier, Florence, Marie Claire, Jean Damascene, Byiringiro, Pascal and Okoro. A special thank you to Dr. Emmanuel Adjei Amponsah, who graciously accommodated me in his home and provided invaluable support.

Lastly, my gratitude goes to my friends and colleagues at the Department of Environment and Sustainability Sciences, Nyankpala Campus Justice, Tahiru, Takal, Fufa,, Jenita, Emelda and Oluwa-Tobi who made me feel at home and offered tremendous support throughout my studies. May God richly bless you all.

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ABBREVIATIONS AND ACRONYMS

AMF: Arbuscular Mycorrhizal Fungi

Bb: Application of 10 tons ha^{-1} Biochar

Bo: No Biochar application

CEC: Cation Exchange Capacity

CO_2: Carbon Dioxide

CSIR-SARI: Council for Scientific and Industrial Research-Savanna Agricultural Research Institute

ETc: Effective Evapotranspiration

Epan: Pan-evaporation

FC: Field Capacity

IPCC: Intergovernmental Panel on Climate Change

Kc: Crop Coefficient

L: Refers to Carl Linnaeus, the Swedish botanist who developed the system of binomial nomenclature (the formal system for naming species). The "L." is an abbreviation of his last name, Linnaeus

Nav: Quantity of available nitrate in a specific soil layer (kg ha^{-1})

NH_4^+: Ammonium ions

NL: Nitrate Leaching

NLEAP: Nitrate Leaching and Economic Analysis Package

NO_3^-: Nitrate

N_2O: Nitrous Oxide

No: No Nitrogen application

NUE: Nitrogen Use Efficiency

NUpE: Nitrogen Uptake Efficiency

NUtE: Nitrogen Utilization Efficiency

MycoPep: Mycorrhizal biofertilizer (*Glomus intraradices*)

P: Phosphorus

PVC: Polyvinyl Chloride

RCBD: Randomized Complete Block Design

TOC: Total Organic Carbon

UDS: University for Development Studies

USDA: United States Department of Agriculture

WACWISA: West African Centre for Water, Irrigation and Sustainable Agriculture

WATP: Weeks After Transplanting

CHAPTER ONE

INTRODUCTION

1.1 Background of study

One of the most vital macronutrients for growth of plant is Nitrogen (N), which is essential for the production of proteins, nucleic acids, and amino acids, which are important for plant metabolism and development (Epstein & Bloom, 2005; Marschner, 2023; Wang et al., 2025). In agriculture, application of Nitrogen causes environmental pollution despite the importance that it plays in plant growth. Nitrogen fertilizers have been over utilized from the beginning of the Green Revolution for the sake of boosting crop yield as the main goal (Tilman, 1998; Sharma & Bali, 2024). Due to that, the over application of Nitrogen fertilizers has led to environmental pollution through leaching, runoff and Nitrogen volatilization (Bouwman et al., 2002; Galloway et al., 2003; U.S. Environmental Protection Agency, 2025). Nitrate (NO_3^-) leaching is a threat to ground water, which lead to the contamination of drinking water. NO_3^- leaching also causes other potential threat to health of human and aquatic lives (Rivett et al., 2008; Ward et al., 2018). The increase of NO_3^- concentration leads to health impacts as it is shown in Figure 1.1 (Chander et al., 2023). Furthermore, the excessive growth of algae aquatic environment is caused by runoff which is technically called eutrophication, this process reduces the quantity of oxygen in water bodies which endangers aquatic life (Carpenter et al., 1998; Smith & Schindler, (2023). Nitrogen volatilization emits nitrous oxide (N_2O), which is 300 times more potent than carbon dioxide (CO_2) in terms of increase global warming (IPCC, 2014), so sustainable of N management is vital due to various environmental pollution that are caused by Nitrogen fertilizer application (Singh & Ward, 2004; FAO, 2025). Decreasing N pollution is sustainable by the use of bioremediation technique, an environmentally friendly techniques of utilizing organisms for reducing the environmental pollution (Singh & Ward, 2004; Ishii, 2025). Arbuscular mycorrhizal fungi (AMF) are symbiotic soil microorganisms that form associations with plant roots, facilitating nutrient uptake, particularly Nitrogen and Phosphorus, while enhancing plant resilience to environmental stress (Smith & Read, 2008). AMF improve soil structure by stabilizing aggregates and increasing root surface area, which promotes nutrient retention and reduces leaching losses (Bender et al., 2016). Their role in Nitrogen cycling involves enhancing Nitrogen Uptake Efficiency, reducing Nitrogen loss through leaching, and improving plant Nitrogen assimilation (Hodge & Storer, 2015).

Biochar, a carbon-rich material produced through the pyrolysis of organic biomass, is widely recognized for its ability to improve soil fertility and mitigate environmental pollution (Lehmann & Joseph, 2015). It has a high surface area and porosity, which facilitates the adsorption of nutrients, including Nitrogen, thereby reducing leaching losses and enhancing Nitrogen Use Efficiency in agricultural systems (Laird et al., 2010). Biochar also serves as a habitat for beneficial soil microbes, including AMF, promoting synergistic interactions that further enhance soil nutrient dynamics and plant growth (Warnock et al., 2007).

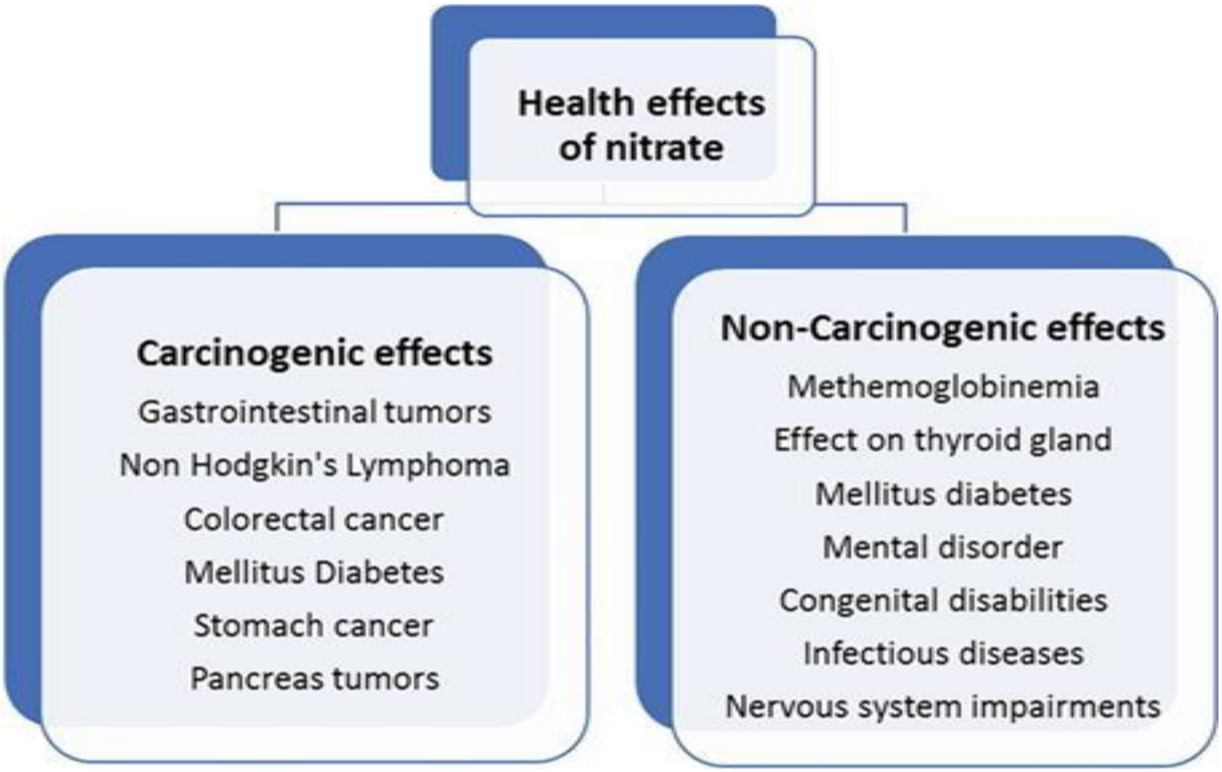

Figure 1.1: Health effect of nitrate (Source: Chander et al., 2023)

This research studied the importance of Arbuscular Mycorrhizal Fungi (AMF) and Biochar in reducing leaching of Nitrogen in garden egg (*Solanum aethiopicum L.*) production in order to promote the use of environmentally friendly agricultural practices. Garden egg is a major crop in terms of food and business purposes in regions of tropics and subtropics. Nitrogen application leads to water pollution which is one of the environmental problems. Additionally, the over use of Nitrogen fertilizer leads to the degradation of organic matter in the soil, augment soil acidity, and disturbs soil microorganisms. (Guo et al., 2010; Tian et al., 2015). Soil degradation is the major cause of reduction of crop productivity, especially in the farmland where the soil degradation is already a problem. (Pimentel & Burgess, 2013).

Lehmann & Joseph, (2015), reported that the Biochar which is a material rich in carbon produced by slow pyrolysis of organic materials, has the capability of positively reducing the pollution of Nitrogen fertilizers. Atkinson et al. (2010) and Verheijen et al. (2010) also found that the structure of Biochar which is porous, has the capability of decreasing nutrients leaching. Additionally, AMF and Biochar are synergically vital for the nutrients cycling and the plants health (Warnock et al., 2007).

Fungi symbiotically connect to the root of plant, which is called AMF. AMF expands their hyphae into the soil which augment the nutrient uptake of the plant (Smith and Read, 2008). AMF increase yield and plant growth by increasing the plant nutrient uptake, especially of the macronutrients such as N and P (Smith & Smith, 2011). Solaiman et al. (2010), reported that the synergistic application of Biochar and AMF is a natural based solution to tackle the agricultural Nitrogen pollution. By employing this natural based solution, an environmentally friendly technique is invented which promise the health of the soil and increase Nitrogen Use Efficiency.

As the promotion of sustainable agricultural practices increases, the capability of plant to efficiently use the nutrients that are available for development and growth, or nutrient use efficiency, has increased in a significant way (Cassman et al., 2002; Andualem et al., 2024). The negative effect of farming practices on the ecosystems can be tackled by the methods that increase nutrient use efficiency and these methods can also augment crop production (Tilman et al., 2002; Wang et al., 2025). Particularly, garden egg needs a nutrition management that increase its growth optimally by employing an environmentally friendly method that rise nutrient uptake efficiency which is a part of nutrient use efficiency (Raun & Johnson, 1999; Akanbi et al., 2024). Smith & Smith, (2011) reported that inoculation of AMF into the soil increases the area of root surface which increase the ability of crop to uptake Nitrogen (Smith & Smith, 2011). Additionally, the Biochar application into the soil enhance nutrient uptake efficiency by improving soil structure which holds much quantity of plant nutrients, and forming a good rhizosphere that favors plant growth. Consequently, employing AMF and Biochar in the agricultural practices that promises a complete environmentally friendly technique that increase nutrient uptake and decrease Nitrogen leaching, and promote a sustainable crop production (Giordano et al., 2021).

The degradation of the soil is the major threat to crop production and sustainability of the environment as well. Thus, it is crucial to maintain and improve health of the soil by maintaining a sustainable agriculture for a long term.

According to Lehmann & Joseph, (2015), Biochar is believed to be a vital treatment for soil degradation because of the capability of Biochar in improving properties of the soil and reducing the pollution of the environment (Lehmann & Joseph, 2015). Beesley et al., (2011); Mukherjee & Zimmerman, (2014), reported that the application the Biochar into the soil indicated the improvement of structure of the soil, increase soil water retention, and decrease the pollutants substances such as heavy metals and pesticides. Furthermore, Lehmann & Joseph, (2015) reported that environmentally friendly methods of managing nutrients is supported by the capabilities of Biochar of holding nutrients which decreases the nutrients leaching.

The synergistic interaction between AMF and Biochar is very important for soil fertility management. Biochar enhances growth and activity of AMF and other useful bacteria in the Mycorrhizosphere (Warnock et al., 2007). Therefore, the AMF enhances the health of plant by improving the bioavailability of the nutrients (Smith & Read, 2008). Mastering the synergistic roles of AMF and Biochar is vital for utilizing the efficient soil fertility management techniques which encourages the environmental protection and agricultural sustainability.

Garden eggs (*Solanum aethiopicum* L.) is an important crop which increase the economy of local community in the regions of tropics and subtropics, it also has a good content in terms of nutrients, for example it is rich in minerals, vitamins and antioxidants) (Adeniji et al., 2007). Though, it is a very difficult for the garden egg crops to grow in the regions where the soil is degraded. (Norman et al., 2011).

The integrated application of AMF and Biochar can increase the growth and the yield of garden egg. Biochar enhances the, biological, and physico-chemical properties of the soil through the increase of soil water retention, improving availability of nutrients and it improves the conditions of the soil for useful soil microorganisms (Lehmann & Joseph, 2015). Alternatively, AMF improves Nutrients Uptake Efficiency and Nutrient s Utilizing Efficiency which leads to the increase of plant growth and crop productivity (Hammer et al., 2015). The synergetic use of AMF and Biochar would be a promising sustainable farming practice which sustainably increases the crop productivity (Jeffery et al., 2011).

1.2. Problem statement

The application of much quantity of much quantities of Nitrogen fertilizers has been a basis in modern agricultural practices since the time of Green revolution, though it has come with pollution of the environment and soil degradation. The greenhouse gas emissions, water pollution and soil degradation have been caused by Nitrogen volatilization, runoff and Nitrogen leaching respectively. The drinking water can be contaminated by runoff and Nitrogen leaching which are the major cause of non-carcinogenic diseases such as methemoglobinemia in infants, thyroid gland effects, diabetes mellitus, mental disorders, congenital disabilities, infectious diseases, and nervous system impairments, nitrate leaching in particular poses a serious threat to the quality of groundwater and carcinogenic diseases such Colorectal cancer, non-Hodgkin's lymphoma, gastrointestinal tumors, and effects on the stomach and thyroid gland.

Furthermore, Nitrogen runoff leads to the increase of eutrophication in water bodies, additionally, nitrous oxide which is a greenhouse gas is emitted by Nitrogen volatilization which contributes to the increase of global warming. Also, the high application inorganic Nitrogen fertilizers lead to soil acidification, decrease of organic matter, and negatively affects soil microorganisms' communities in the soil. These problems particularly hinder the sustainable farming practices in the regions where the soils are already degraded.

Developing a nature-based solutions that can decrease the Nitrogen leaching while keeping producing high crop yields is vital, by taking into consideration the crucial role that Nitrogen fertilizers in growth of the crop. The application of both AMF and Biochar is the best environmentally friendly method which can be employed in bioremediation. The inoculation of AMF is able to enhance nutrients uptake, especially the macronutrients such Nitrogen and Phosphorus. Biochar also has the ability to improve the structure of the soil, nutrient availability, and soil water retention. The AMF and Biochar synergistically improve soil health and crop production.

There is vital importance of applying both AMF and Biochar, but the mechanisms that involve between of AMF and Biochar are still not completely understood. Specifically, garden egg crop

(*Solanum aethiopicum L.*) can be used to optimize the integrated use of AMF and Biochar in the region of tropics and subtropics where crop increase the economy of local communities and crop is used to tackle the major nutritional insecurity. Therefore, this gap has to be filled for reducing Nitrogen leaching, improve the health of the soil and promote to grow garden egg sustainably which will lead to environmental sustainability and food security.

1.3. General objective

To evaluate and model environmentally sustainable Nitrogen management strategies that reduce environmental pollution through Nitrate leaching to enhance soil health by integrating Arbuscular Mycorrhizal Fungi (AMF) and Biochar in the cultivation of garden egg (*Solanum aethiopicum* L.).

1.4. Specific objectives

(i) Determine the reduction of leachate volume from Nitrogen fertilization associated with bioremediation by AMF and Biochar and model environmental risk of nitrate leaching.

(ii) Assess the effect of AMF inoculation and Biochar application on Nitrogen uptake and Use efficiency in the production of garden egg (*Solanum aethiopicum* L.).

(iii) Evaluate the synergistic effect of Biochar with AMF on soil properties

(iv) Determine the growth and yield of garden egg (*Solanum aethiopicum* L.) by integrated AMF and Biochar application.

1.5. Hypotheses of the study

i. Application of Arbuscular Mycorrhizal Fungi (AMF) and Biochar will significantly reduce leachate volume and nitrate leaching compared to standard Nitrogen fertilization practices.

ii. AMF and Biochar integration will enhance Nitrogen uptake and use efficiency in garden egg (*Solanum aethiopicum* L.).

iii. Biochar application will positively alter soil properties, such as pH, organic matter content, and water retention, and will have synergistic effects when combined with AMF.

iv. The combination of AMF and Biochar will provide greater benefits in terms of plant growth, yield, and Environmental Sustainability compared to their individual applications.

1.6. Significance of the Study

This research is very important to address the severe environmental problems that are caused by the application of Nitrogen fertilizers. The several environmental problems such as nitrate leaching pollutes water bodies which is very dangerous for human health and aquatic life have been reported that they are consequences of using much quantities of Nitrogen fertilizers. The integrated application of AMF and Biochar is new promising approach for tackling these environmental problems through the increase of NUpE and NUE.

The findings were more important for the regions of tropics and subtropics, where the main problems in the farmlands are the soil degradation and nutrient leaching, and garden eggs (*Solanum aethiopicum* L.) in these regions is an important crop. By improving soil health and nutrient availability by employing the integrated application of AMF and Biochar, this research aimed to promote a sustainable agricultural method that not only enhance the productivity of the crop and quality but also lead to a long-term environmental sustainability. Reducing the application inorganic Nitrogen fertilizers while increasing the crop yields is a better sustainable farming practice.

1.7. Scope of the study

The objective of this research was to investigating a nature based solution for reducing Nitrogen pollution in in garden eggs (*Solanum aethiopicum* L.) production using the integration of AMF and Biochar. The aim of this study was also to determine how this environmentally friendly techniques enhance health of the soil, increase NUpE and NUE and reduce Nitrogen loss.

Additionally, this research evaluated the synergistic effects of AMF and Biochar on some soil properties as well as its effect on growth and yield of garden egg. The findings provided the policies that promote sustainable farming practices and reduce the environmental pollution that are caused by inorganic fertilizers application.

1.8. Outline of thesis

CHAPTER ONE of the thesis comprises of Background, Problem statement, Significance of the Study, Objectives, and Hypotheses of the Study, together with the Scope and Organization of the Thesis. The role of Nitrogen in agriculture, the difficulties associated with Nitrogen fertilization, and the possibility for bioremediation using Biochar and Arbuscular Mycorrhizal Fungi (AMF) were all covered in the literature review (CHAPTER TWO), with an emphasis on the beneficial impacts of these two methods on soil health and nutrient uptake. Additionally, it identified gaps in the literature by discussing sustainable Nitrogen management in the production of garden eggs. The research area, experimental design, soil preparation, AMF inoculation, and Nitrogen fertilization techniques were all covered in detail in the Methodology section (CHAPTER THREE), along with the steps involved in data collection and statistical analysis. The impacts of

AMF and Biochar on nitrate leaching, Nitrogen absorption efficiency, soil characteristics, and garden egg yield were reported in the findings chapter (CHAPTER FOUR), along with a comparison of their synergistic effects. The discussion (CHAPTER FIVE) addressed the implications of these findings for Sustainable Agriculture, emphasized possible limits, and evaluated the results in light of the body of current literature. In CHAPTER SIX, the thesis closed with a summary of the main conclusions, policy and practice recommendations, and ideas for more research.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Nitrogen is important for increasing plant growth and boosting crop yield. Though, the overuse of inorganic Nitrogen fertilizers has led to environmental problems such as soil degradation, Nitrogen loss and emissions of greenhouse gases such as nitrous oxides (N_2O). The agricultural sector has two main problems which are the rise of food demand and promoting sustainable farming practices which do not compromise environmental sustainability. Consequently, the nature-based solutions of optimizing Nitrogen without compromising the production of high yield are urgently needed.

The integrated application of AMF and Biochar is an environmentally friendly methods which is very practical in bioremediation of Nitrogen pollution. By employing these methods, health of soil could be improved, Nitrogen leaching could be decreasing, and NUpE could be increased. The integration of AMF and Biochar is a good way of growing crops in sustainable manner. This literature review presents the existing research on the dynamics of Nitrogen in soil and the importance of AMF and Biochar in bioremediation

This review of the literature looks at the current understanding of Nitrogen dynamics in agriculture, the roles that AMF and Biochar play in Bioremediation, and their role in the cultivation of garden eggs (*Solanum aethiopicum* L.) which is an important crop that increase the economy of local community and improve nutritional security in the regions of tropics and subtropics. By reviewing the existing findings, the objective of this literature review was to find the research gap and clearly show the necessity of conducting research on the integrated application of AMF and Biochar in sustainable farming practices.

2.2 Nitrogen in agriculture: importance and challenges

Nitrogen is a vital nutrient for plant growth. Plant needs Nitrogen for production of nucleic acids, proteins and amino acids which are crucial for metabolism (Epstein & Bloom, 2005; Hirel et al., 2020). The Nitrogen dynamics is vital for maintaining fertility of the soil and boosting crop yields. But Tilman, (1998) and Zhang et al. (2020) reported that it is difficulty to regulate Nitrogen in agriculture because of the overuse of inorganic Nitrogen fertilizer for meeting the food demand that keeps increasing. Additionally, Tilman, (1998); Pingali et al. (2012) found that Green Revolution promoted the high application of inorganic fertilizer for the sake of boosting crop productivity. This strategy tremendously increased production of food worldwide, and on the other hand, it had negative effects on the environment due to volatilization, Nitrogen leaching and runoff as reported by Bouwman et al. (2002); GallowFay et al., (2003); Udvardi et al., 2015). The overuse of inorganic Nitrogen fertilizer causes nitrate (NO_3^-) leaching, which pollutes the water bodies and this affects the health of humans and aquatic animals (Rivett et al., 2008; Ward et al., 2018). Several diseases such as bluebaby syndrome and different types of cancer have been found to be caused the increase of NO_3^- concentration in drinking water according to (Chander et al., 2023). Furthermore, runoff of Nitrogen into the aquatic systems causes eutrophication which leads to the death of aquatic animals (Carpenter et al., 1998; Smith & Schindler, (2023)). Also, Nitrogen volatilization emits nitrous oxide (N_2O) which is a greenhouse gas that contributes in the increase of global warming (IPCC, 2014). These environmental problems highlight the necessity of a nature-based solution for reducing Nitrogen leaching without compromising high production of crop yields.

2.3 Rationale for selecting an Exponential Probability Density Function-based Model to predict nitrate leaching

The exponential probability density function-based model Corrêa et al., 2005) an easy and effective model to predict NO_3^- leaching by using available NO_3^- (Nav), surplus of water (R), and field capacity (FC). This model is appropriate for this research due to these following reasons:

2.3.1 Nonlinearity of nitrate leaching

Nitrate leaching is mostly dependent on water movement into the soil and soil characteristics because of its basic nonlinearity (Di & Cameron, 2002; Zhao et al., 2016). The exponential form of this model reflects how nitrate leaching reduces as soil FC approaches, emphasizing the reducing effects of water flow on NO_3^- leaching.

2.3.2 Simplicity and computational efficiency

The parameters of the model are simply available, such as available NO_3^- (Nav), surplus of water (R), and field capacity (FC) compare to other very sophisticated complex models such as SWAT or HYDRUS, which need too many parameters. Therefore, this is the reason why the exponential Probability Density Function-based Model is more reliable for a large-scale prediction while still generating reliable results.

2.3.3 Focus on key factors

The model certainly focusses on the interactions between parameters such as Nav, R and FC and these three factors are the key factors which normally affect the loss of NO_3^- in the soils. The predictions of NO_3^- leaching can be generated with greatest precision by adding very few factors and some targeted method.

2.4 Bioremediation and sustainable agriculture

Bioremediation is a crucial technique of removing the substances that are pollutant in the environment by using the microorganisms, thus bioremediation can be a better natural based solution of decreasing pollution that are caused by Nitrogen in agricultural soils (Singh & Ward, 2004; Ishii, 2025). Arbuscular Mycorrhizal Fungi (AMF) and Biochar are two organic materials are able to augment Nutrient Usage Efficiency and improve health which can lead to sustainable farming practices (AMF) (Lehmann & Joseph, 2015).

2.4.1 Arbuscular Mycorrhizal Fungi (AMF): Role and mechanisms

AMF are the fungi that symbiotically connect to the roots of plant in a mutualistic way for spreading their thread like called hyphae into the soil and improve the surface area that are available for nutrient uptake (Smith and Read, 2008). This symbiotic relationship improves the uptake of macronutrients, especially N and P that are very vital for plant growth and crop productivity (Smith & Smith, 2011). AMF are very important in improving soil health and reducing the degradation of the soil due to their ability of improving the soil structure, soil aggregation and their ability of increasing soil water holding capacity (Rillig et al., 2016).

Additionally, AMF increase the ability of plant to resist the pests, diseases and abiotic stress which enhance a sustainable agricultural production (Barea et al., 2002; Nimmo, 2009).

2.4.2 Biochar: Production, properties, and applications

Lehmann & Joseph, (2015) found that the Biochar which is material rich in carbon has the capability of enhance the health of the soil and reduce the pollution of Nitrogen fertilizers in agricultural soils. Biochar with its porous structure, improves the availability of soil nutrients, decreases nutrients loss and enhance soil water retention (Atkinson et al., 2010; Verheijen et al., 2010). Additionally, Biochar is a host for AMF, thus improve soil nutrient dynamics and increase the health of the plant (Warnock et al., 2007).

Several studies found that Biochar application into the soil also augment Soil Organic Matter, soil pH, and reduce the heavy metals and other pollutants of the soil (Beesley et al., 2011; Mukherjee & Lal, 2013). This is a perfect soil nutrient management practice which an environmentally friendly.

2.4.3 Synergistic effects of AMF and Biochar on soil health and nutrient uptake

The application of both AMF and Biochar into the soil is a useful farming practices that can sustainably improve Nitrogen management in agriculture. Warnock et al. (2007) found that Biochar in soil is a good environment that can enhance AMF colonization. Integrated application of AMF and Biochar enhance Nutrient Uptake Efficiency especially N, by augmenting root surface area and increase the availability of nutrient in the soil (Solaiman et al., 2010). Several studies emphasized that the synergy effect of AMF and Biochar significantly enhance health of the soil, plant growth and crop yield which is a promising practical approach for a sustainable farming (Giordano et al., 2021).

2.5 Sustainable Nitrogen management in garden egg production

Garden egg (*Solanum aethiopicum* L.) is an important crop in regions of tropics and subtropics, it is also very vital for nutrition security and it boost the economy of local communities in these regions. A sustainable nutrients management is needed for an optimum plant growth and crop yield (Adeniji et al., 2007; Han et al., 2021). Historically, synthetic Nitrogen fertilizers have been the key for boosting the garden egg; but this has caused the environmental pollution due to nitrate leaching, soil degradation and reduction of Nutrient Use Efficiency (Norman et al., 2011). Additionally, many researchers have found that synthetic Nitrogen fertilizers have been causing polluting the environment.

2.5.1 Nutrient requirements and fertilization practices for garden egg

Different types of nutrients are required for garden egg production according to different factors, such as type of soil, climate, and farming practices. Nitrogen is vital nutrient for garden egg production because it increases both development of fruit and plant growth (Run and Johnson, 1999). Application of 207 kg N ha^{-1} and organic manure was recommended for optimum production of garden egg in Ghana (Adjei et al., 2023). The excessive Nitrogen uptake leads to nutritional imbalances which affect the quality of fruit, and also make plant to be vulnerable to pests and diseases (Norman et al., 2011). Thus, it is important to apply the recommended rate of Nitrogen for ensuring balanced nutrient uptake in order to maintain health and growth of the plant. Consequently, it is required to use a sustainable Nitrogen fertilization method for increasing a sustainable production of garden egg.

2.5.2 Impact of AMF and Biochar on garden egg growth and yield

In last decade, the researchers found that the integrated application of AMF and Biochar is beneficial in farming of garden egg. Hammer et al., (2015) found that application of AMF into the soil augment root biomass and increase the availability of nutrient in the soil, which improves the Nutrient Uptake Efficiency in garden egg production. Though, the application of Biochar boost crop yield by improvement of soil structure, increase of availability of nutrient in the soil and soil water retention (Lehmann & Joseph, 2015). The integrated application of AMF and Biochar increase the growth of garden egg and yield (2011), and this maintain a sustainable production of garden egg by decreasing Nitrogen leaching.

2.6 Previous studies on AMF, Biochar, and Nitrogen management: Identifying research gaps

2.6.1 Existing research on AMF and Nitrogen Use Efficiency (NUE)

Arbuscular Mycorrhizal Fungi (AMF) have been widely studied for their role in improving plant Nitrogen Uptake Efficiency (NUE) and soil fertility. Many studies have demonstrated that AMF increase root absorption of Nitrogen by increasing root surface area and facilitating the exchange of nutrients between the fungus and the plant (Smith & Read, 2008; Hodge, A., & Storer, 2015). AMF symbiosis has been reported to improve Nitrogen Uptake in many crops, such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and legumes (Deng Yin et al., 2009; Tian et al ., 2023). Additionally, AMF have been shown to decrease Nitrogen leaching by stabilizing soil aggregates and influencing the soil microbial community (Rillig & Mummey, 2006; Wang et al., 2021). However, despite these well-documented benefits, limited research has examined the specific effects of AMF on Nitrogen Uptake and Utilization in garden egg (*Solanum aethiopicum* L.), creating a gap in understanding how AMF can be effectively applied to optimize Nitrogen use in this crop.

2.6.2 Biochar and its role in soil fertility and Nitrogen retention

Biochar has been extensively studied for its ability to enhance soil properties, increase water retention, and reduce Nitrogen leaching (Lehmann & Joseph, 2015). The high surface area and porosity of Biochar allow it to retain Nitrogen in the soil, thereby improving Nitrogen Use Efficiency (NUE) and reducing environmental Nitrogen losses (Zheng et al., 2013). Studies have shown that Biochar application can boost crop yields and enhance soil microbial activity, particularly in degraded soils (Atkinson et al., 2010; Beesley et al., 2011). However, despite these advantages, Biochar's effectiveness in Nitrogen retention varies significantly depending on type of organic materials used in Biochar production, pyrolysis conditions, and soil properties (Mukherjee & Lal, 2013). Moreover, there is a lack of research on the long-term impacts of Biochar application in tropical soils, especially in garden egg production systems. This gap necessitates further investigation into the specific Biochar types and application rates that optimize NUE in garden egg cultivation.

2.6.3 Interaction between AMF and Biochar in Nitrogen bioremediation

Many studies have indicated that AMF and Biochar may have synergistic effects on soil nutrient dynamics and plant growth (Warnock et al., 2007; Solaiman et al., 2010). The porous structure of Biochar provides a favorable habitat for AMF spores, enhancing fungal colonization and activity (Lehmann & Joseph, 2015). This interaction has been linked to improved Nitrogen uptake and increased resistance to environmental stress (Giordano et al., 2021). Despite these findings, most existing studies have focused on temperate crops such as wheat, maize, and soybean, with limited research on their combined effects in tropical vegetable crops like garden egg. Furthermore, the mechanisms through which Biochar enhances AMF colonization and Nitrogen bioremediation remain underexplored, particularly in relation to different soil types and climate conditions.

2.6.4 The need for integrated Nitrogen management in garden egg cultivation

Garden egg is a Nitrogen-demanding crop that requires efficient nutrient management to maximize yield and quality (Adeniji et al., 2007). Conventional Nitrogen fertilization practices in garden egg farming often lead to low Nitrogen Use Efficiency and high nitrate leaching (Norman et al., 2011). Despite the potential benefits of AMF and Biochar in improving Nitrogen retention and uptake, there is a significant gap in research regarding their combined effects in garden egg production. Few studies have investigated the optimal application rates and timing for integrating AMF and Biochar to enhance Nitrogen efficiency while minimizing environmental impacts. Addressing these gaps is essential to develop sustainable Nitrogen management strategies that balance productivity and environmental conservation in garden egg farming.

2.7 Gaps in the literature and justification for the present research

From the review of existing literature, it is evident that while AMF and Biochar have individually shown potential in Nitrogen management, their combined effects remain underexplored in tropical vegetable production systems. Key research gaps that this study seeks to address include:

1. **Limited studies on AMF and Nitrogen uptake in garden egg**: Although AMF have been widely studied in cereals and legumes, their role in Nitrogen acquisition in garden egg remains poorly understood.

2. **Uncertainty regarding optimal Biochar application for Nitrogen retention**: Most Biochar research has focused on improving soil fertility, with insufficient data on the best Biochar types and application rates for Nitrogen Use Efficiency in garden egg.

3. **Lack of studies on the synergistic effects of AMF and Biochar in Nitrogen bioremediation**: While AMF and Biochar interactions have been studied in a few temperate crops, their combined effects on Nitrogen Uptake and environmental sustainability in garden egg cultivation remain largely unexplored.

4. **Limited research on the environmental benefits of integrating AMF and Biochar in Nitrogen management**: Understanding how these bioremediation approaches reduce nitrate leaching and improve Nitrogen efficiency is crucial for sustainable agriculture.

This research aims to fill these gaps by investigating the integrated application of AMF and Biochar in garden egg production, contributing new insights to sustainable Nitrogen management and Environmental conservation.

2.8. Summary of review

This literature review highlighted the critical role of Nitrogen in agriculture, emphasizing its importance for plant growth and development, and the challenges posed by excessive use of synthetic fertilizers. The Environmental consequences of Nitrogen overuse, such as nitrate leaching, greenhouse gas emissions, and water pollution, underscored the need for sustainable Nitrogen management practices. Bioremediation, particularly through the use of Arbuscular Mycorrhizal Fungi (AMF) and Biochar, offers promising strategies for mitigating these environmental impacts. AMF enhances nutrient uptake and soil health, while Biochar improves soil structure, nutrient retention, and water-holding capacity. The synergistic effects of AMF and Biochar present a novel approach to sustainable Nitrogen management, particularly in the cultivation of garden egg (*Solanum aethiopicum* L.). However, the literature reveals gaps in understanding the combined effects of AMF and Biochar on garden egg production, highlighting the need for further research. This study aimed to address some of these gaps, providing insights into the potential for integrating AMF and Biochar in Sustainable Agriculture, particularly in the context of garden egg cultivation.

CHAPTER THREE

METHODOLOGY

3.0. Introduction

This chapter presented the overview of the methodology used to achieve the four objectives of this research. The research was carried out at the farm of West African Centre of Excellence for Water, Irrigation, and Sustainable Agriculture (WACWISA). WACWISA is in the region of tropical savannah. Treatments, Open field pot experiment and Field experiment, Data collection, Nitrogen fertilization, Experimental design and Statistical analyses were used. The Methodology for every objective was clearly presented.

3.1 Research Site Description

The study was conducted in the research farm of West African Centre for Water, Irrigation, and Sustainable Agriculture (WACWISA) (Figure 3.1). WACWISA is situated in Tamale in the North region of Ghana at an altitude of approximately 180 m above sea level (Ghana Meteorological Agency, 2018). The region has one rain season which starts from May up to October, with a dry season which starts from November until April. Annually the average of rainfall is 1100 mm (Owusu and Waylen, 2009). Annually, the average temperature is within 24°C and 35°C (Ghana Meteorological Agency, 2018; Yamba et al., 2023). The soil texture of the farm is sandy loam and the soil is slightly acidic (soil pH of 5.5 to 6.9). The garden egg was the test crop used in this study and it is suitable for climate and soil characteristics of the region.

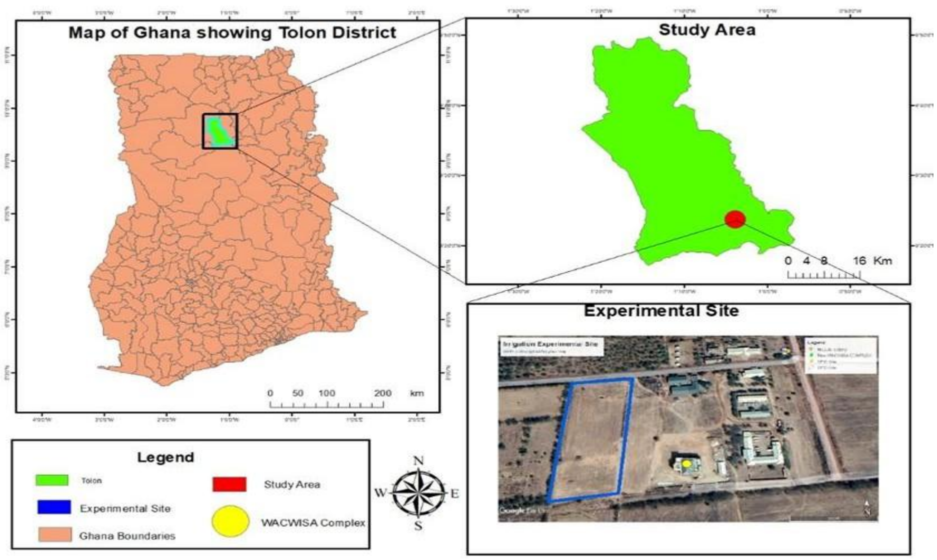

Figure 3.1: Map of the study site

3.2 Determining the reduction of leachate volume from Nitrogen fertilization associated with bioremediation by AMF and Biochar and model environmental risk of nitrate leaching.

3.2.1 Experimental design and treatments

The experimental design was an asymmetrical 2 x 2 x 3 factorial study in a Randomized Complete Block Design (RCBD) with three replications (Figures 3.3 and Figure 3.4) for determining the effect of Arbuscular Mycorrhizal Fungi (AMF), Biochar, and Nitrogen (N).

For AMF, there were two levels:

1. **Mo**: No AMF application
2. **Mm**: Application of 8 kg ha^{-1} MycoPep (*Glomus intraradices*)

For Biochar, there were also two levels:

1. **Bo**: No Biochar application

2. **Bb**: Application of 10 tons ha⁻¹ Biochar and

For Nitrogen, there were three levels:

1. **No**: No Nitrogen application
2. **N150**: 150 N ha^{-1}
3. N200**: 200 N ha^{-1}**

Twelve different treatments were created by all possible combinations of the levels of three factors: two levels of AMF, two levels of Biochar, and three levels of Nitrogen (Figure 3.2).

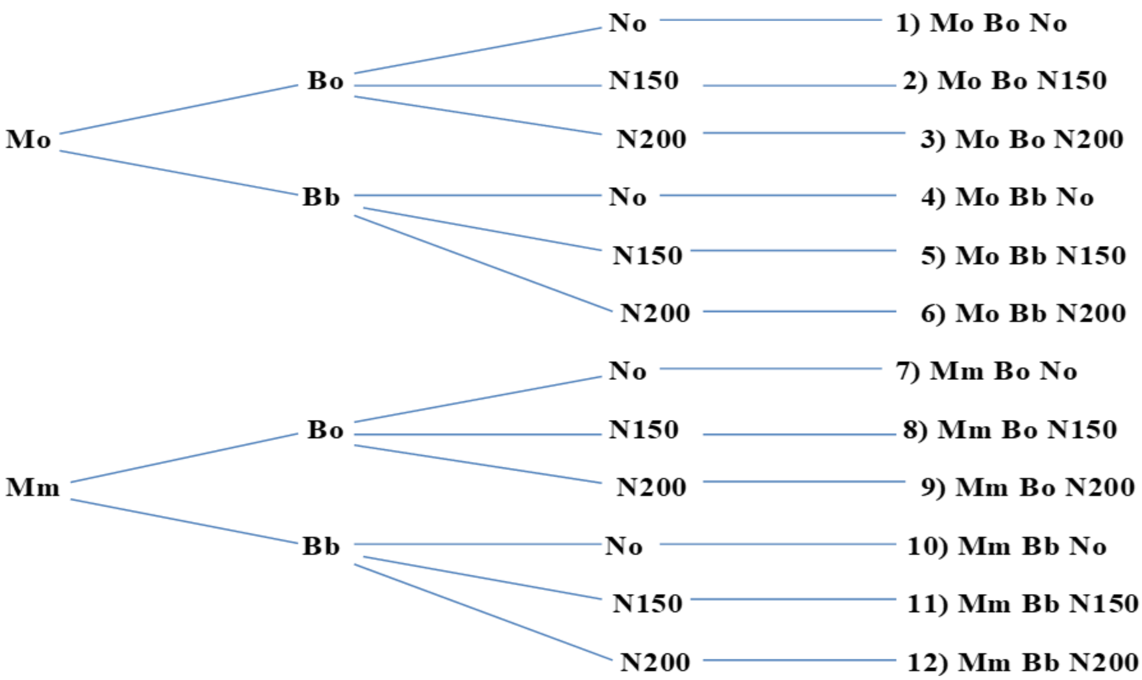

Figure 3.2. Factorial treatment structure

Key: **Mo**: 0 kg.ha^{-1} of AMF, **Mm**: 8 kg.ha^{-1} of AMF, **Bo:** 0 kg.ha^{-1} of Biochar, **Bb:** 10 ton.ha^{-1} of Biochar, **No:** 0 kg.ha^{-1} of N, N**150**: 150 kg.ha^{-1} of N and **N200**: 200 kg.ha^{-1} of N

Within each block, all 12 treatments were randomized using random numbers, sequences, and ranks as described by Gomez & Gomez (1984).

Plots of one meter by one meter were placed in rows with a 50 centimeters gap between each plot in the field/plot experiment (Figure 3.4 & Figure 3.5). Every row had an extra 50 centimeters of space at its ends. Four plants were cultivated in each plot, with a 70 cm × 70 cm spacing between each plant. In order to reduce interference and make administration procedures easier, this design preserved a uniform distance between plots and plants, allowing for the methodical evaluation of treatments inside each plot.

As though Plastic buckets measuring 35 cm in diameter by 35 cm in height were used as pots in the pot experiment (Figure 3.3 & Figure 3.5). Every pot featured a base with a cover that included a drainage hole with a diameter of 15 mm (Figure 3.3). To help with leachate collection, a PVC drainage outlet (15 mm in diameter by 35 mm in length) was attached to this cap. To help with drainage, a 200 g coating of washed sand was added to the base of each pot after filter paper to stop soil loss (Figure 3.3). Twenty kg of a soil and sand mixture were used to fill each pot. Additionally, each pot included one plant.

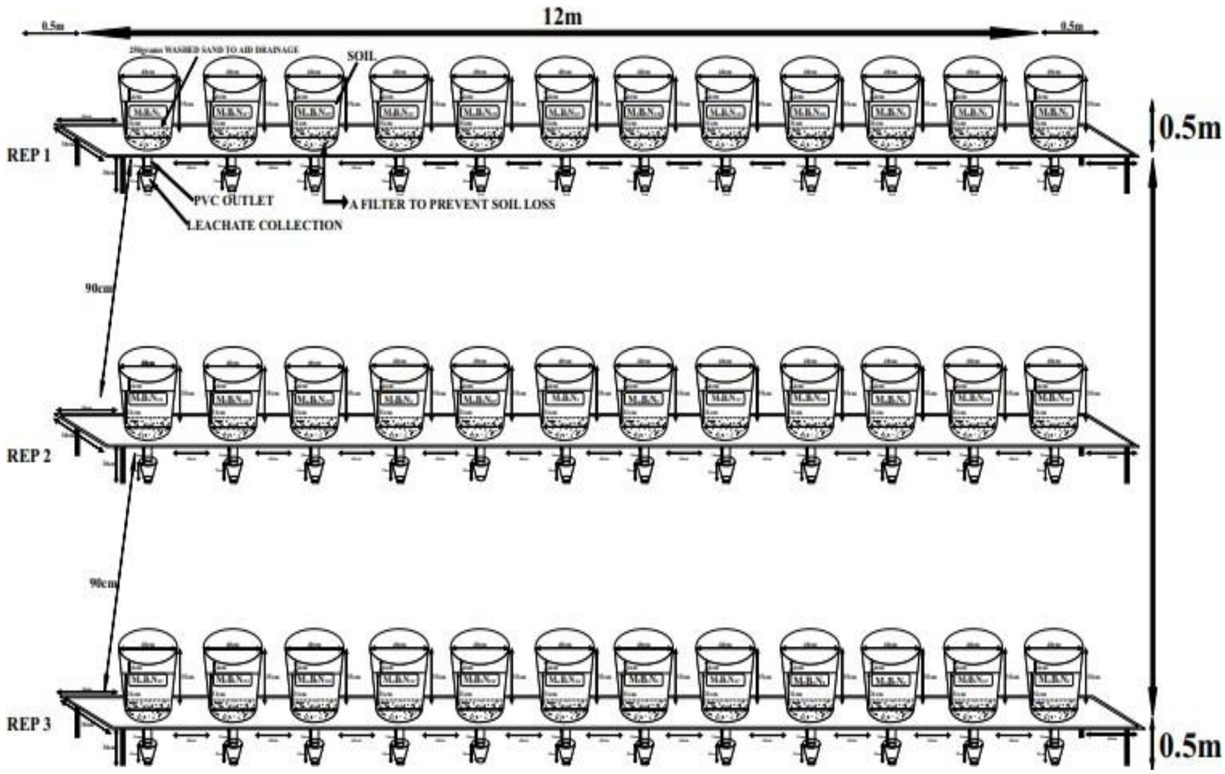

Figure 3.3. A Schematic diagram of the pot experiment that shows the leaching system

Figure 3.4: Transplanting and AMF inoculation in Pot and Field experiments.

Figure 3.5. Pot and field experiments at the harvesting stage.

3.2.2. Nitrogen fertilization

Using urea (which contains 46% N), Nitrogen was supplied at three different levels: 0, 150, and 200 kg N ha^{-1}. Four portions of the fertilizer were applied: four, eight, twelve, and sixteen-weeks after transplanting (WATP).

3.2.3. Source of inoculant and Biochar

Agromonti Limited in Accra, Ghana distributes MycoPep (Vascular Arbuscular Fungi *Glomus intraradices*), a biofertilizer made by Peptech Bioscience Ltd in New Delhi, India. Through a low-temperature pyrolysis procedure, rice husks from the Avnash Rice Processing Factory in Nyankpala, Ghana, were converted into Biochar (Figure 3.6).

Figure 3.6. Production of rice husk Biochar using the pyrolysis method.

3.2.4. Data collection

Table 3.1 details the analysis performed on Biochar and soil samples prior to incubation, including measurements of pH, Cation Exchange Capacity (CEC), Total Organic Carbon (TOC), Total Nitrogen (N), Ammonium ions (NH_4^+), Nitrate ions (NO_3^-), and Available Phosphorus (P). As indicated in Table 3.2, each measurement was carried out in triplicate, and the average value was noted. Over the course of the study, weekly measurements were made of important soil chemical characteristics, such as pH, accessible nitrate in the field experiment's soil, and nitrate concentration in the pot experiment's leachates. Until the experiment's conclusion, leachates from the pot experiment and field capacity from the field experiment were also observed once a week.

Table 3. 1. Methods of measuring preliminary soil physico-chemical characteristics and soil chemical parameters used in this study

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.	Horiba. (2012). Instruction Manual for LaquaTwin Nitrate Meter Model B-743. Horiba Scientific.
Available P (mg kg^{-1})	Extracted using the Bray-1 and measured using spectrophotometry.	Holliday & Gartner, 2007.
Soil texture	Determined using the hydrometer method	Gee & Bauder, 1986.

Table 3.2. 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
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	

3.2.5. Data for modeling

For Nyankpala, which is located at latitude 9°25'45" N and longitude 0°58'42" W, at an elevation of 183 meters above sea level, monthly rainfall and pan-evaporation data (long-term averages) from 1970 to 2023 were provided by the Meteorological Division of the Council for Scientific and Industrial Research-Savanna Agricultural Research Institute (CSIR-SARI). Equation 1, Table 3.3) utilized a crop factor of 1.15 to convert pan-evaporation (Epan) measurements to effective evapotranspiration (ETc) (USDA, 2016, Corrêa et al., 2005). Based on weekly soil sample data obtained over a four-month period, from the first to the sixteenth WATP Epan (mm/day) was measured from an evaporation pan utilizing a Class A evaporation pan at the Meteorological Division of CSIR-SARI. This allowed for the modeling of NO_3^- leaching.

ETc = Epan x Kc .... Equation 1

Where:

ETc = Effective evapotranspiration (mm day^{-1})

Epan = Pan evaporation (mm day^{-1})

Kc = Crop coefficient

R (mm $Month^{-1}$) = Rainfall (Monthly) - ETc (mm $Month^{-1}$)....................................... Equation 2

Where

R = Surplus water for leaching

ETc = Effective evapotranspiration (mm $Month^{-1}$)

Table 3.3. Long-term climate information for Nyankpala, Ghana (1973-2023)

Month	Rainfall	ETo/day)	ETo/month	Effective evapotranspiration	Surplus water for leaching
			mm		
January	1.79	4.92	147.6	169.74	-167.95
February	10.81	5.18	155.4	178.71	-167.90
March	34.9	5.5	165	189.75	-154.85
April	77.01	5.17	155.1	178.365	-101.36
May	107.74	4.8	144	165.6	-57.86
June	137.93	4.12	123.6	142.14	-4.21
July	215.97	3.69	110.7	127.305	88.67
August	183.02	3.37	101.1	116.265	66.76
September	174.85	3.49	104.7	120.405	54.45
October	89.98	3.97	119.1	136.965	-46.99
November	6.49	4.32	129.6	149.04	-142.55
December	2.88	4.3	129	148.35	-145.47

3.2.6 Leaching Model for Nitrate (NO3-)

The Nitrate Leaching and Economic Analysis Package (NLEAP), developed by Shaffer et al. (1991), is a quick and site-specific model for estimating the possibility of nitrate (NO_3^-) leaching. The exponential probability density function (pdf) has been recognized by White et al. (2022) as having tremendous potential as a management tool. The leached NO_3^- over specified time periods or cumulative drainage, which may be calculated from meteorological data by subtracting Effective evapotranspiration (ETc) from precipitation (Equation 2), is predicted by this equation using an exponential function. The parameters of exponential pdf model are easily available which make the model to be a better model for predicting Nitrate leaching (White et al., 2022). The anions such as NO_3^- is homogeneously distributed in the soil pores (Edis, 1998) which hypothesizes that this model could be accurate in predicting the concentration of NO_3^- in the soil (White et al., 2022).

The following exponential pdf model was employing for determining the NO_3^- which was expected to leach (NL) from the soi:

$$NL = Nav\,[1 - \exp\,(\frac{-R}{FC})] \text{ .... Equation 3}$$

Where :

NL : NO_3^- expected to leach (kg/ha),

Nav : quantity of available NO_3^- in a specific soil layer (kg ha^{-1})

R : surplus water available for leaching (mm)

FC : Soil Field Capacity (mm)

Nav values (kg ha^{-1}) were calculated by using the concentrations of NO_3^- (mg kg^{-1} soil) measured during 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 weeks after transplanting (WATP).

The values were converted from milligrams kg-1 to milligrams ha-1 and milligrams L-1 while accounting for bulk density, soil field capacity (FC), and soil layer depth of 20 cm. Weekly measurements of the leachate volume were made up to the experiment's conclusion. Among several nitrate leaching models, this exponential probability density function-based model was selected because of its computational efficiency and simplicity. This exponential probability density function-based model uses easily accessible factors such as nitrate concentrations, excess water, and field capacity, in contrast to more complex mechanistic models (e.g., HYDRUS or SWAT), which require significant input data and are computationally costly. This still yields dependable results but makes it more feasible for field-scale forecasts.

3.3 Assessing the effect of AMF inoculation and Biochar application on Nitrogen uptake and Use efficiency in the production of garden egg (*Solanum aethiopicum* L.).

3.3.1 Experimental Design and Treatments

Two separate growth seasons were used for objective two's open-field pot experiments: the rain season (June to October 2023) and the dry season (December 2023 to April 2024). Section 3.2.1 provided specifics on the treatments and experimental design.

3.3.2 Pot experiment

One garden per pot was employed in the same pot experiment as detailed in Section 3.2.2. To guarantee that each pot received the same amount of exposure to the environment, they were placed in a grid pattern.

3.3.3 Source of inoculant and Biochar

In this experiment, the same AMF inoculant and Biochar were used, as explained in Section 3.2.3.

3.3.4 Nitrogen fertilization

The process detailed in Section 3.2.2 was followed while applying Nitrogen fertilizer.

3.3.5 Data collection

A overview of the data collection techniques used for this study's second aim is given in Table 3.4, which also includes information on the units of measurement, the methodologies, the parameters measured, and pertinent references. At the conclusion of the experiment, specific measurements were made of the Nitrogen use efficiency (NUE), Nitrogen utilization efficiency (NUtE), and Nitrogen uptake efficiency (NUpE).

Table 3.4. Data collection methods and procedures

Parameter	Method	Unit	Reference
Nitrogen Uptake Efficiency (NUpE)	Kilogram (above ground N) at harvest per Kilogram available N (from soil plus fertilizer)	Kg kg-1 (Unitless)	Moll et al., 1982; Fageria et al., 2009)
Nitrogen Utilization Efficiency (NUE)	Kilogram (Fruit dry mass) per kilogram (above-ground N) at harvest	Kg kg-1 (Unitless)	(Moll, 1982)
Nitrogen Use Efficiency (NUE)	Kilogram (Fruit dry mass) at harvest per kilogram available N (from soil plus fertilizer) = NUpE X NUtE	Kg kg-1 (Unitless)	Fageria, 2014

3.4 Evaluating the impact of Biochar on soil properties and any synergistic effect with AMF.

3.4.1 Experimental site and design

Experimental Site and Design are outlined in Section 3.2.1.

3.4.2 Pot experiment

The same pots were used as it is indicated in Section 3.2.2 and one garden egg plant was transplanted in every pot and a soil-sand combination (3:1) were used in every pot. The pots were positioned in a way that guaranteed regular exposure to weather elements like sunlight and precipitation.

3.4.3 Biochar production and application

In this experiment, Biochar is the same as the one generated in Section 3.2.3.

3.4.4 Data collection

Soil pH, Cation Exchange Capacity (CEC), Total Organic Carbon (TOC), root dry biomass, and root colonization were among the parameters that were examined. Weekly analyses of soil pH, CEC, and TOC were conducted to track the effects of treatments over time. At the conclusion of the experiment, root dry biomass and root colonization were evaluated, as indicated in Table 3.5.

Table 3.5. Data collection methods and procedures

Data Collected	Method	Reference
Soil pH	Measured using a pH meter in a 1:2.5 soil-water suspension	Thomas, 1996
Total Soil Organic Carbon	Dry combustion using an elemental analyzer	Nelson & Sommers, 1996.
Cation Exchange Capacity (CEC)	Measured using the ammonium acetate method	Rhoades (1982)
Root Biomass	Roots were washed, dried at 70°C for 48 hours, and weighed	Standard Plant Biomass Measurement
Root Colonization	Cleared with 10% KOH, stained with trypan blue, examined under a microscope	Phillips and Hayman (1970) Gridline Intersect Method

3.5 Determine the growth and yield of garden egg (*Solanum aethiopicum* L.) by integrated AMF and Biochar application.

3.5.1 Experimental design and treatments

This objective used a 2 × 2 × 3 asymmetrical factorial study in a RCBD with three replications, and the experimental design and treatments were in line with the previous objectives. The Kotobi+ garden egg variety was chosen because to its exceptional output potential and environment adaptability.

3.5.2 Planting and management

Each pot held one plant. Standard agronomic techniques, such as routine hand weeding, watering, and insect control, were used to manage the plants. To keep the soil moisture content at ideal levels, manual watering was used, with modifications made in response to the weather.

3.5.3 Data collection

Following transplantation, monthly measurements of plant height and chlorophyll content (spad unit) were made (WAT). At WATP 10, 12, 14, and 16, fruit production and non-marketable yield were reported. Chlorophyll content was measured using the proper techniques, as indicated in Table 3.6, along with yield and yield components.

Table 3.6 Methods 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.	Upadhyaya et al., 2011; Khaliq et al., 2017
Total Fruit Yield	Weighed using a digital scale. Harvested fruits are collected and their fresh weight is recorded.	Agegnehu et al., 2016; Lehmann et al., 2011
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.	Majumdar et al., 2007; Savvas et al., 2009
Chlorophyll Content	Measured using a SPAD chlorophyll meter (Soil Plant Analysis Development). SPAD readings were taken from the topmost fully expanded leaves.	Uddling et al., 2007; Monje and Bugbee, 1992

3.6 Statistical data analysis

Microsoft Excel 2016 (64-Bit Edition) was the first tool used to organize and manage the data for this study. It was a useful tool for data entry, layout, and preliminary computations, including summary metrics and descriptive statistics. The data were arranged in excel and imported into GenStat 12 edition for statistical analysis. The GenStat 12 edition was suitable for handling agricultural data. The experiment was conducted by employing an asymmetrical 2x2x3 factorial study in a Randomized Complete Block Design (RCBD) with 3 replications. The general Analysis of Variance was done for assessing the significance of the treatments effect on the variables. The separation of means was done by employing the Tukey's Honestly Significant

Difference (HSD) test at a 5% significance level. Additionally, Nitrate expected to leach was calculated using an exponential probability density function based model by using the parameters such as field capacity (FC), excess water (R), and available nitrate (Nav). The regression analysis was also performed in order to find how FC predicts NL.

3.7 Justification for methodological approaches

The methods used in this study were selected based on how well they address the specific objective. It was correct to use an asymmetrical factorial study in RCBD due to the facts that the agricultural research like this which use many factors needs such factorial study for studying the interactions of factors. The Nitrogen fertilizers were applied in four splits for maximizing Nitrogen Use Efficiency during growth of the plant and reducing the Nitrogen loss. Mukherjee & Zimmerman, (2014) reported that Biochar application into the soil enhance soil quality, augment the availability of nutrient in soil, and decrease greenhouse gas emission. Several studies have shown that application of AMF into the soil increase nutrient uptake, particularly Nitrogen which very vital for plant growth. The variety of garden egg, Kotobi+ was selected to be used in this experiment due to its high yield, pests and disease tolerance and its ability of adapting to the local environmental condition.

3.8 Limitations and assumptions

This research had several limitations and Assumptions. One of the limitations was the seasonal variation of the weather between the rain and dry seasons, but to tackle or reduce this problem, the experiments were highly monitored, and watered whenever it was necessary for maintaining the growth conditions constant. The other limitation was the biological systems, especially how plant reacts to inoculant like AMF and application of Biochar which was unpredictable but to tackle this problem, the advanced data analyses were performed for removing the variability in the studies.

Furthermore, the present study used the assumption that impact of AMF and Biochar observed in pot experiment would be the same in the field experiment, Pot experiment finds may note effectively represent the field experiment findings.

3.9 Summary

This chapter described the thorough approaches used to accomplish the study's four objectives. The research was methodologically sound thanks to the implementation of an asymmetrical 2 x 2 x 3 factorial study in an RCBD, as well as thorough pot and plot trials, Nitrogen fertilization regimens, and data gathering techniques. The selection of statistical analysis methods adds credence to the findings' validity and lays a solid framework for the presentation of the results and discussion of their consequences in the following chapters.

CHAPTER FOUR

4.0. RESULTS

4.1 Determining the reduction of leachate volume from Nitrogen fertilization associated with bioremediation by AMF and Biochar and modeling environmental risk of nitrate leaching.

4.1.1. Physical and chemical properties of soil and Biochar used

4.1.1.1. Soil properties

A moderate acidity in the soil is often good for growing a variety of crops, including garden eggs (Table 4.1). Moderate acidic soils may make vital nutrients more accessible to plants (Marschner, 2012). Moderate nutrient-holding capacity, as indicated by a CEC of 22.00, is advantageous for preserving important elements including potassium, calcium, and magnesium that are vital for plant growth (Brady & Weil, 2002; Marschner, 2012). Generally, there was not a lot of organic matter in the soil used in this study, as indicated by the low total organic carbon content. Organic matter is essential because it enhances soil structure, nutrient availability, and water retention for the health of the soil (Lehmann & Joseph, 2015). If Nitrogen fertilizers are not used, the lower-than-average total Nitrogen content may restrict plant growth and yield (Havlin et al., 2014). The moderate quantities of ammonium gave plants easy access to applied Nitrogen (Fageria, 2009). Given the low nitrate level, there may be a constraint on the immediate availability of Nitrogen for plant absorption (Brady & Weil, 2002). The amount of phosphorus that was readily available was sufficient to sustain the growth and development of plants, especially the growth of their roots and the transmission of energy within them (Marschner, 2012). Sandy loam soil has low water holding capacity and low availability of nutrient in soil. Additionally, sandy loam is well aerated and has good infiltration rate, thus sandy loam soil needs to be irrigated frequently and it also needs enough fertilizers inputs for maintaining nutrient requirement (Brady & Weil, 2002; Lehmann & Joseph, 2015).

4.1.1.2. Rice husk Biochar properties

Biochar has a high pH, due to that Biochar can decrease soil acidity and increase soil nutrient retention (Lehmann et al., 2011). Biochar has high Cation Exchange Capacity (CEC) which is great potential for Biochar in increasing soil nutrient retention when Biochar is applied in the soil (Lehmann & Joseph, 2009). Biochar also has high soil organic carbon which indicate the ability of Biochar of increasing the soil structure and soil water holding capacity (Lehmann et al., 2011). Biochar has high Nitrogen content which make Biochar to be a crucial source of Nitrogen, Biochar can release slowly the Nitrogen and these can lead to a better plant growth (Chintala et al., 2014). Biochar with its low ammonium content can be a reliable source of ammonium (Lehmann & Joseph, 2009). Additionally, the nitrate content in the Biochar is low which indicate that the key role of Biochar is to improve the soil fertility for a long term but it cannot be the quick source of nitrate (Clough et al., 2013). The available phosphorus in the Biochar is very high, thus the application of Biochar into the soil can improve root development and increase the health of the plant generally (Yuan & Xu, 2011).

Table 4.1. 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
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	

4.1.2. Nitrate concentration in leachate

The factors of AMF, Biochar, and Nitrogen showed a highly significant three-way interaction ($p<0.05$). This indicated a complicated interplay whereby these three factors combined effects greatly affected the leaching of nitrate (Appendix 1).

The concentration of NO_3^- in the leachate collected from the soil columns was significantly lower in treatments combining Biochar and/or Arbuscular Mycorrhizal Fungi (e.g., Mo Bb No, Mo Bb N150, Mo Bb N200, Mm Bo No, Mm Bo N150, Mm Bo N200, Mm Bb No, Mm Bb N150, and Mm Bb N200) than in treatments lacking AMF and Biochar during the 16-week experiment (Figure 4.1).

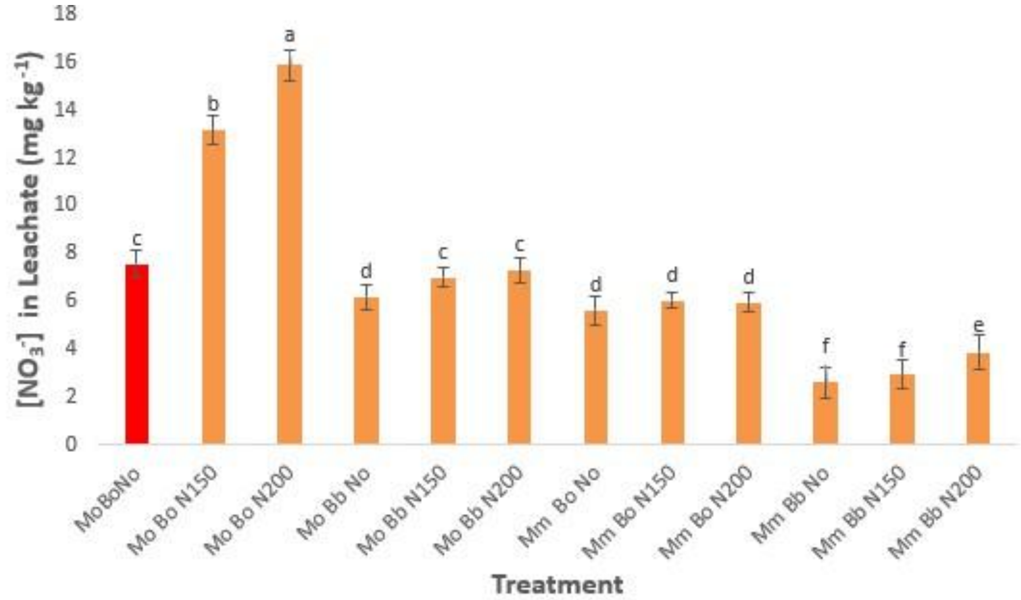

Figure 4.1. Nitrate concentration in the leachate as affected by treatments. Bars represent Standard deviation

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%

Significant three-way interactions between AMF, Biochar, and Nitrogen indicated that these factors combined effects on soil pH were intricate and interdependent (Appendix 2).

Significant differences in treatment means were found using the Tukey analysis, as indicated by the different letters (a, b, c, d, and e) in Figure 4.2.

In terms of soil pH, treatments such as "Mo Bo No," "Mo Bo N150," "Mo Bo N200," "Mo Bb No," "Mm Bo No," "Mm Bo N150," and "Mm Bo N200" did not differ significantly from one another (Figure 4.2). This indicated that the effects of both interventions on soil pH were comparable. The pH levels of the groups labeled "b," "c," "d," and "e" varied significantly. In particular, there were significant differences between treatments "Mo Bb N150" (c), "Mo Bb N200" (d), "Mm Bb N150" (b), and "Mm Bb N200" (e). Particularly, "Mm Bb 200" had the highest soil pH (8.54) (Figure 4.2).

The significant impacts of Nitrogen and Biochar on soil pH were supported by the Tukey results, which also supported the ANOVA findings (Figure 4.2). Particularly under the "Mm" condition, treatments containing Biochar and optimal Nitrogen levels had the greatest soil pH, indicating that the combination of Biochar and optimal Nitrogen levels significantly raised soil pH (Figure 4.2).

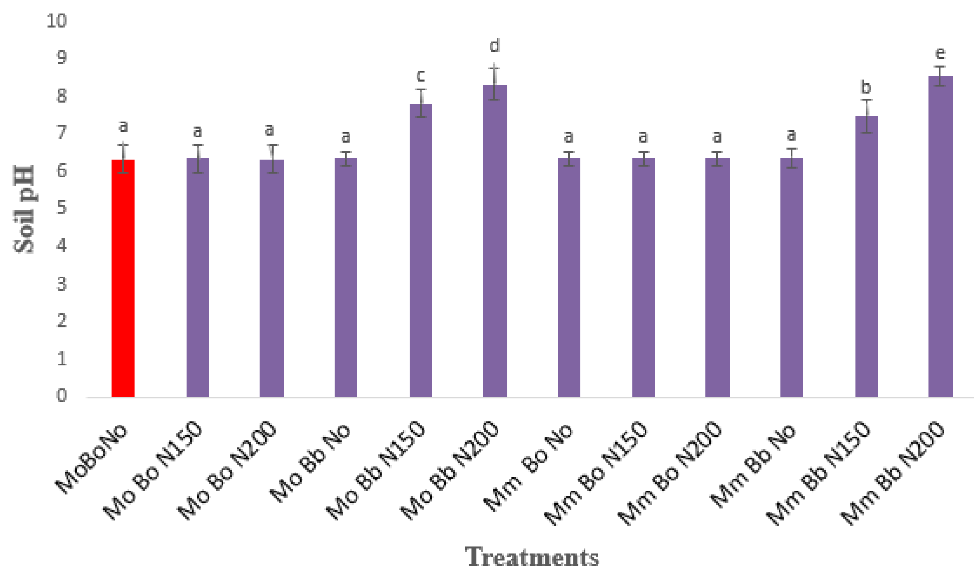

Figure 4.2. The soil pH value of the treatments. Bars represent Standard deviation

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

The strong three-way interaction among AMF, Biochar, and Nitrogen indicates that these factors combined effects on leachate volume were complicated rather than additive (Appendix 3).

Based on leachate volume, the Tukey results classified treatment combinations (Figure 4.3). From "a" (lowest) to "j" (highest), they were ranked. The treatments with the lowest leachate quantities were Mm Bb N200 (45.5 ml) and Mm Bb N150 (49.2 ml), both of which differed significantly from the other treatments (Figure 4.3). This showed that, either as a result of better soil structure or nutrient retention, the combination of AMF (Mm), Biochar (Bb), and optimum Nitrogen (N200) or decreased Nitrogen (N150) resulted in minimum leachate (Figure 4.3).

The leachate volumes of Mm Bo N200 (80.5 ml), Mm Bo N150 (86.3 ml), and Mm Bo No (95.7 ml) were greater than those of the Mm Bb treatments, but they are still much smaller than those of the Mo treatments (Figure 4.3). This showed that there was more leachate when AMF and Biochar with no Nitrogen were absent.

The leachate volume of Mo Bo No (235.6 ml) was the highest, while Mo Bo N150 (229.4 ml) and Mo Bo N200 (226.9 ml) were the next closest (Figure 4.3). These treatments differed greatly from the rest, indicating that the highest leachate was caused by either inadequate nutrient retention or changed soil structure in the case of AMF-free and Nitrogen-free Biochar.

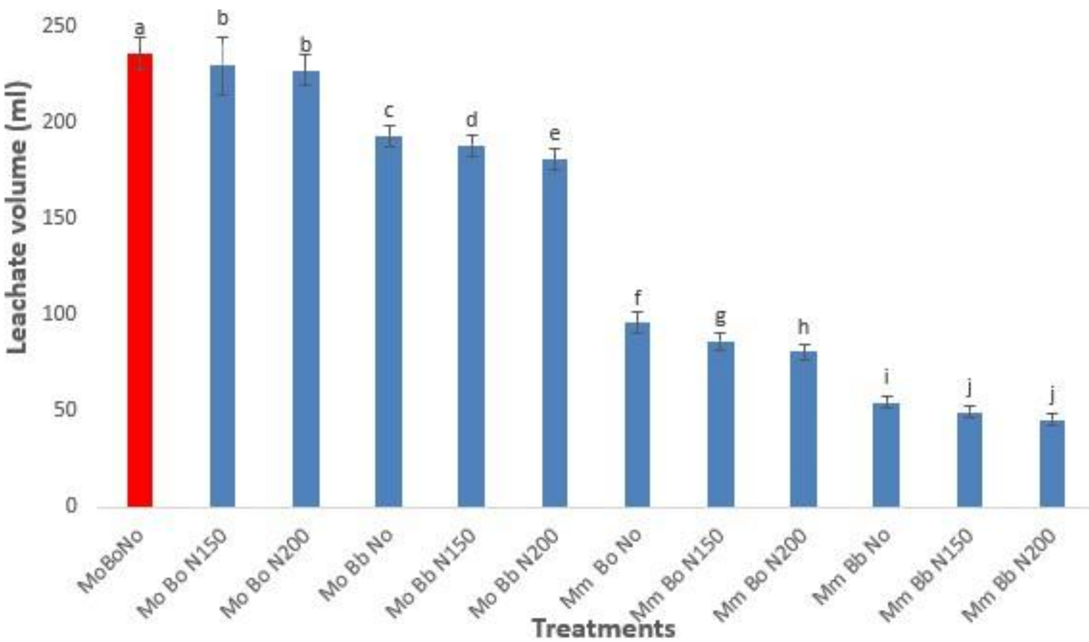

Figure 4.3. Volume of leachate collected from the soil columns per treatment.

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

4.1.3. Field capacity (FC)

The interaction between AMF and Biochar was statistically significant ($P<0.05$), as shown in Appendix 4. This indicated that AMF and Biochar had distinct combination effects than when they were used separately (Table 4.2). The interaction between AMF and Nitrogen was also very significant ($P<0.05$) (Appendix 4). This indicates that the amount of Nitrogen had an impact on the way the AMF affected field capacity (Table 4.3). The interaction between Nitrogen and Biochar was not significant ($P>0.05$). This indicated that there is no significant difference between the combined effects of Nitrogen and Biochar and their individual effects. AMF x Biochar x Nitrogen showed no significant three-way interaction ($P>0.05$) (Appendix 4). This indicated that there was no significant combined influence of AMF, Biochar, and Nitrogen on field capacity in a three-way interaction.

Table 4.2. Field capacity (mm)

AMF	Biochar (10 ton ha^{-1})	No Biochar (0 ton ha^{-1})
Mm	111.31^{d}	53.06^{c}
Mo	39.88^{b}	6.05^{a}
LSD	0.627	
p-value	<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Table 4.3. Field capacity (mm)

AMF	NITROGEN		
	0	150	200
Mm	83.36^{c}	82.42^{c}	80.78^{b}
Mo	23.06^{a}	22.95^{a}	22.9^{a}
LSD	0.768		
p-value	<.001		

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

However, Tukey post-hoc test for the three-way interaction model was performed as an exploratory analysis to examine the potential trends or interactions that might be biologically meaningful, even if they were not statistically significant (Figure 4.4). Group 'a' was created by the MoBoNo, MoBoN150, and MoBoN200 treatments, with very low means of 6.04-6.06 (Figure

4.4). This indicated that there were no significant variations between these treatments. Groups 'e' and 'f' were formed by the MmBbNo, MmBbN150, and MmBbN200 treatments; these groups' highest averages ranged from 109.85 to 112.88, which was significantly different from all other groups (Figure 4.4).

Generally, there was no significant relationship between Nitrogen and Biochar (Appendix 4). This indicates that, on average, the combined impacts of Nitrogen and Biochar did not differ significantly from the sum of their individual effects across all levels of the other factors. Likewise, there was no overall significant combined influence of all three factors on field capacity, as evidenced by the non-significant three-way interaction (Appendix 4).

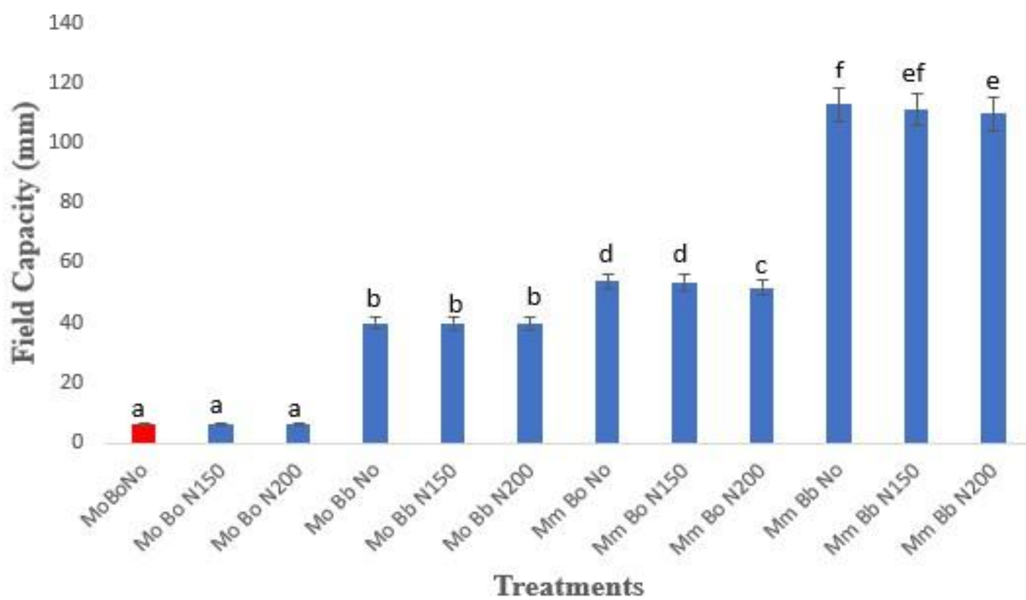

Figure 4.4. Volume of leachate collected from the soil columns. Bars represent Standard deviation

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

4.1.4. NO3- expected to leach (NL)

According to the exponential Probability Density Function-based Model, the estimated leaching of nitrate (NO_3^-) from soils treated with Mo Bo N200 and Mm Bb N200 ranged between 4.92% and 60.04% of the available NO3- for leaching at the highest rates of Nitrogen (N) application (Table 4.4). The enhanced water retention at field capacity was ascribed to the decreased nitrate leaching (NL) seen in the Mm Bb N150 and Mm Bb N200 treatments (Table 4.4). Table 4.4 showed that soils treated with Mm Bb N150 and Mo Bo N200 were expected to have NO_3^- concentrations of 4.86% to 59.94% respectively. In contrast, the probability of NO_3^- leaching was almost 17 times higher in soils lacking Arbuscular Mycorrhizal Fungi (AMF) and Biochar than in those with their presence at the same optimal N treatment rate (200 kg N ha^{-1}). Similarly, the risk of NO_3^- leaching was around 20 times higher in soils lacking AMF and Biochar compared to those with their presence at the lower N application rate (150 kg N ha^{-1}) (Table 4.4).

Table 4.4. NO_3^- expected to leach from Available NO_3^-

Treatments	No	Cumulative FC	NL	NL	Expected Loss
	kg ha^{-1}	mm	kg ha^{-1}	mg kg^{-1}	%
Mo Bo No	36.76	97.01	22.02	11.01	59.91
Mo Bo N150	103.39	96.93	61.97	30.985	59.94
Mo Bo N200	105.47	96.67	63.33	31.665	60.04
Mo Bb No	25.72	640.94	3.32	1.66	12.92
Mo Bb N150	92.31	637.77	11.98	5.99	12.98
Mo Bb N200	100.69	636.19	13.10	6.55	13.01
Mm Bo No	11.96	861.55	1.17	0.585	9.78
Mm Bo N150	75.18	857.85	7.38	3.69	9.82
Mm Bo N200	83.63	827.61	8.50	4.25	10.16
Mm Bb No	17.63	1806.45	0.84	0.42	4.79
Mm Bb N150	65.16	1779.78	3.17	1.585	4.86
Mm Bb N200	76.75	1757.53	3.78	1.89	4.92

4.1.4.1. Regression analysis between cumulative Field Capacity (FC) and Nitrate expected to Leach (NL) predicted by exponential Probability Density Function based model

The link between Cumulative Field Capacity (FC) and Nitrogen Expected to Leach (NL), as anticipated by an Exponential Probability Density Function-based Model (Tables 4.5 and 4.6, and Figure 4.5), was investigated using regression analysis.

Table 4.5. The regression analysis output showing the relationship between Cumulative Field Capacity (FC) and Nitrogen Expected to Leach (NL) predicted by Exponential Probability Density Function based Model

Regression Statistics	
Multiple R	0.694816664
R Square	0.482770196
Adjusted R Square	0.431047216
Standard Error	16.80975826
Observations	12

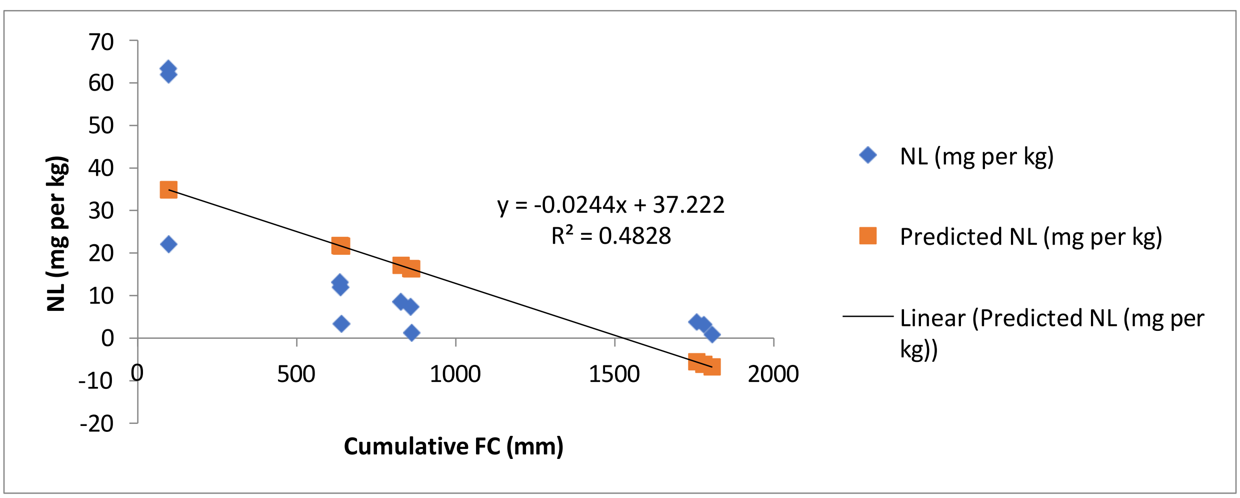

Figure 4.5. Regression cumulative FC vs NL

Table 4.6. The regression coefficients in the regression model

	Coefficients	Standard Error	t Stat	P-value
Intercept	37.22205096	8.283135	4.493715	0.001154
Cumulative FC (mm)	-0.024375771	0.007979	-3.05512	0.012145

4.2. Assessing the effect of AMF inoculation and Biochar application on Nitrogen uptake and Use efficiency in the production of garden egg (*Solanum aethiopicum* L.).

4.2.1. Nitrogen Uptake Efficiency

The three-way interaction between AMF, Biochar, and Nitrogen was found to be statistically significant ($p < 0.05$) by the Analysis of Variance (ANOVA) (Appendix 5). With a mean of 0.08922, significantly greater than all other combinations, the treatment combination of Mm x Bb x N200 (AMF and Biochar at the optimum Nitrogen level) showed the greatest NUpE (Figure 4.6).

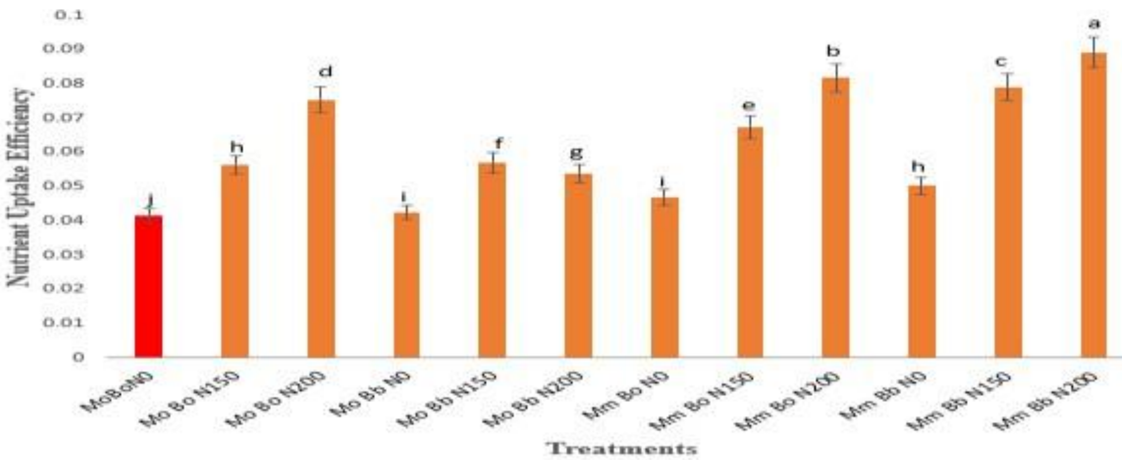

Figure 4.6. Nitrogen Uptake Efficiency as affected by treatments. Bars represent Standard Deviation (Rain season: June 2023 to October 2023).

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%

The three-way interaction (AMF x Biochar x Nitrogen) was statistically significant ($p < 0.05$) in the dry season, much like it was in the rain season (Appendix 6). Although the results were lower than those during the rain season, indicating seasonal change, the treatment with the highest NUpE was once more found to be Mm x Bb x N200 (Figure 4.7).

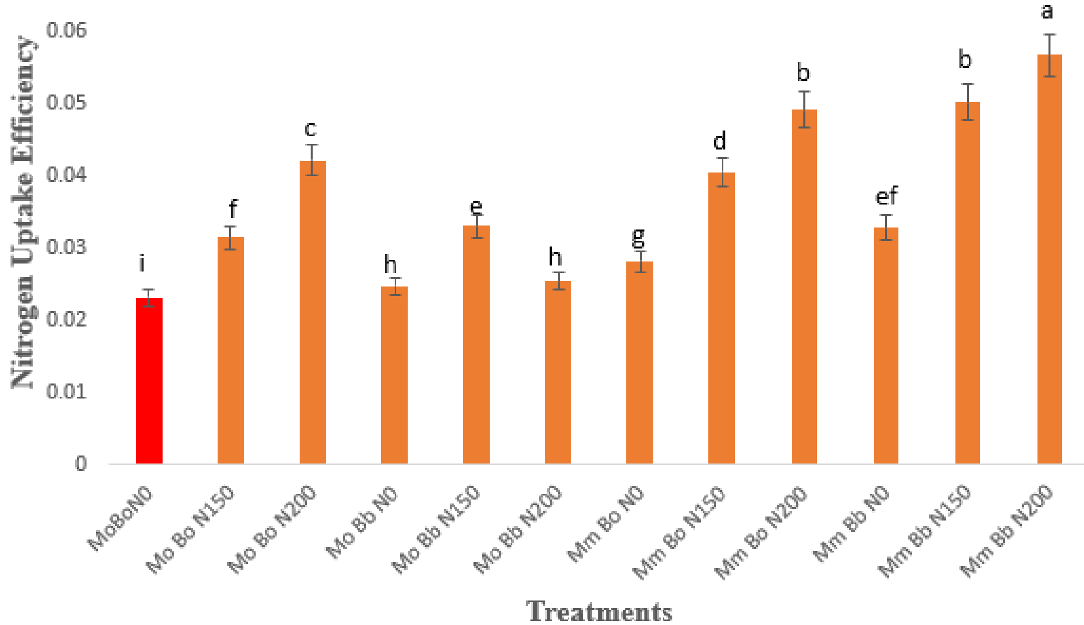

Figure 4.7. Nitrogen Uptake Efficiency (Dry season: December 2023 to April 2024)

Key: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

4.2.2. Nitrogen Use Efficiency (NUE)

The AMF × Nitrogen and Biochar × Nitrogen interactions were highly significant ($p < 0.05$), but the three-way interaction (AMF × Biochar × Nitrogen) was not significant ($p > 0.05$), indicating that the three factors did not have a combined effect on NUE (Appendix 7).

Table 4.7 indicates that for all Nitrogen levels, NUE increased significantly after AMF application. The combination of 8 kg ha^{-1} AMF and 200 kg ha^{-1} Nitrogen had the highest NUE (61.85 g g^{-1}), whereas the absence of both AMF and Nitrogen had the lowest NUE (24.71 g g^{-1}). The highest value (56.95 g g^{-1}) was recorded at 10 t ha^{-1} Biochar and 200 kg ha^{-1} Nitrogen, indicating that Biochar also enhanced NUE, as Table 4.8 shows.

Table 4.7. Nitrate Use Efficiency ($g.g^{-1}$)_Rain season

AMF	NITROGEN		
	0	150	200
Mm	35.45^{b}	52.35^{d}	61.85^{e}
Mo	24.71^{a}	36.12^{b}	41.71^{c}
LSD		1.458	
p-value		<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Table 4.8. Nitrate Use Efficiency ($g.g^{-1}$)_Rain season

Biochar	NITROGEN		
	0	150	200
Bb	33.24^{b}	49.74^{e}	56.95^{f}
Bo	26.92^{a}	38.72^{c}	46.61^{d}
LSD		1.458	
p-value		<.001	

Key: Bo: 0 ton ha^{-1} Biochar**; Bb:** 10 ton ha^{-1}**.** Mean values with Superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Though, Tukey post-hoc tests for the three-way interaction model were conducted as an exploratory analysis to explore possible trends or interactions that could be biologically meaningful, despite the lack of statistical significance (Figure 4.8). The treatment Mm x Bb x N200 (AMF and Biochar at the optimal Nitrogen level) had the highest NUE, with a mean of 67.19 (Figure 4.8).

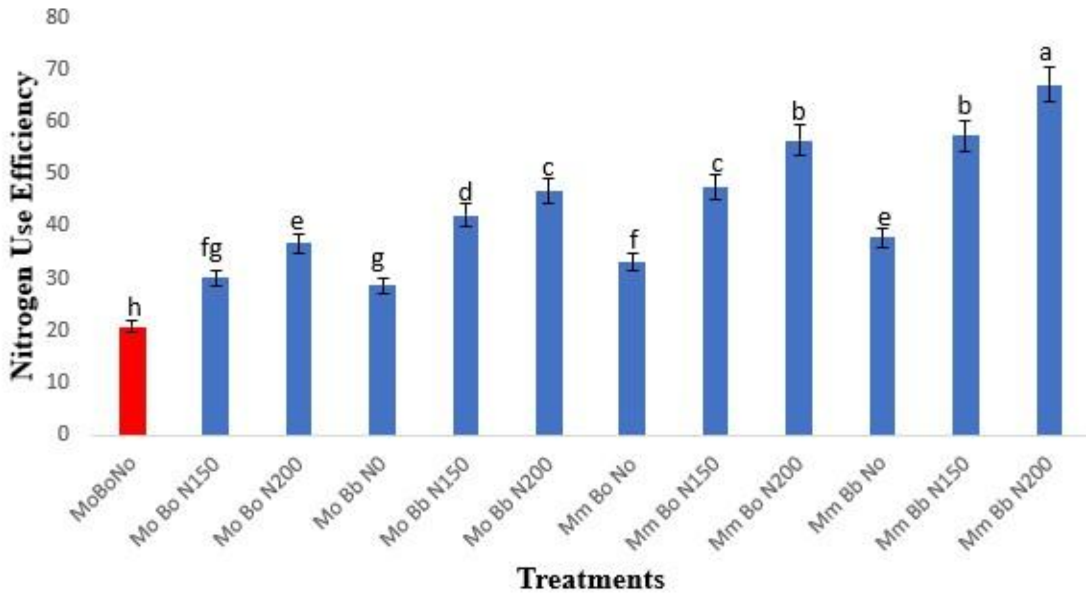

Figure 4.8. Nitrogen Use Efficiency (Rain season: June 2023 to October 2023)

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Appendix 8 indicates that the AMF × Nitrogen and Biochar × Nitrogen interactions were significant ($p < 0.05$) during the dry season, whereas the three-way interaction was significant ($p > 0.05$).

AMF significantly increased NUE, as shown in Table 4.9, with the highest mean (54.94 g g^{-1}) occurring at 8 kg ha^{-1} AMF and 200 kg ha^{-1} Nitrogen. At 0 kg ha^{-1} AMF and 0 kg ha^{-1} Nitrogen, the lowest NUE (20.04 g g^{-1}) was recorded. The application of Biochar also increased NUE (Table 4.10), with the highest value (49.81 g g^{-1}) occurring at 10 t ha^{-1} Biochar and 200 kg ha^{-1} Nitrogen.

Table 4.9. Nitrate Use Efficiency ($g.g^{-1}$)_Dry season

AMF	NITROGEN		
	0	150	200
Mm	31.48^{c}	46.51^{e}	54.94^{f}
Mo	20.04^{a}	29.29^{b}	33.80^{d}
LSD		1.314	
p-value		<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Table 4.10. Nitrate Use Efficiency ($g.g^{-1}$)_Dry season

Biochar	NITROGEN		
	0	150	200
Bb	29.01^{b}	43.45^{e}	49.81^{f}
Bo	22.50^{a}	32.36^{c}	38.93^{d}
LSD		1.314	
p-value		<.001	

Key: Bo: 0 ton ha^{-1} Biochar**; Bb:** 10 ton ha^{-1}**.** Mean values with Superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

However, Tukey post-hoc tests for the three-way interaction model were also conducted as an exploratory analysis to identify potential trends or interactions that might have biological significance, even if they were not statistically significant. The Tukey's Test revealed that the highest NUE was once again in Mm x Bb x N200 (AMF and Biochar at the optimum Nitrogen level), with a mean of 61.26, demonstrating consistency across the seasons (Figure 4.9).

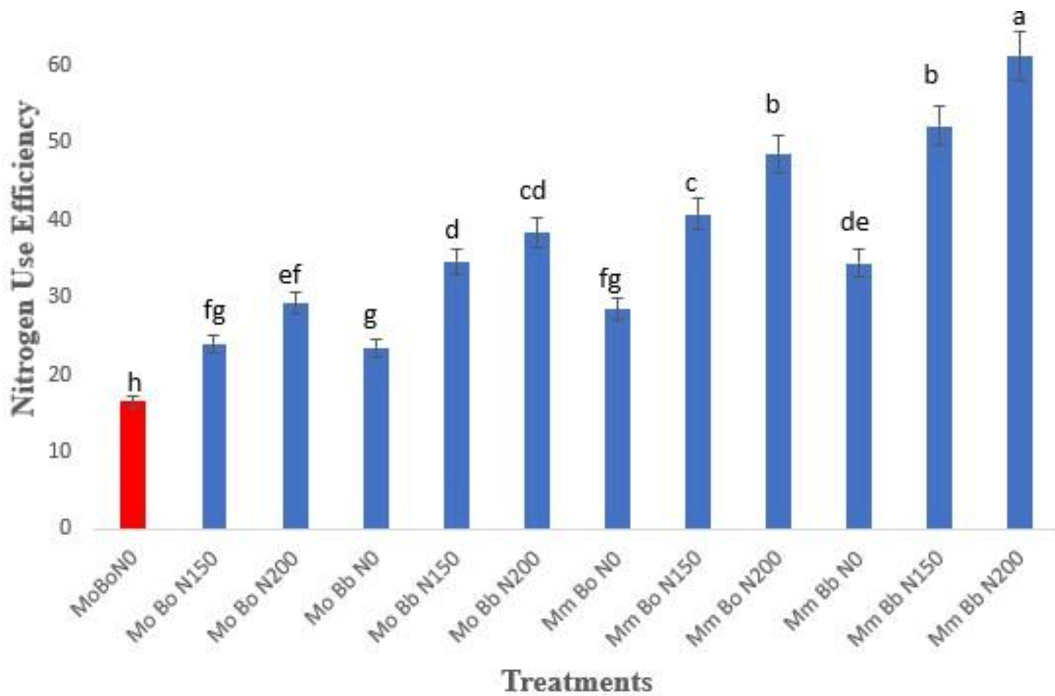

Figure 4.9. Nitrogen Use Efficiency of garden egg (Dry season: December 2023 to April 2024). Bars represent Standard Deviation.

Key: Mo: 0 kg ha^{-1} AMF; **Mm:** 8 kg ha^{-1} AMF; **Bo:** 0 ton ha^{-1} Biochar; **Bb:** 10 ton ha^{-1} Biochar; **No:** 0 kg N ha^{-1}; **N150:** 150 kg N ha^{-1}; **N200:** 200 kg N ha^{-1}. Mean values with distinct letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

4.2.3. Regression analysis (Rain season)

The relationship between Nitrogen use efficiency (NUE) and Nitrogen uptake efficiency (NUpE) was displayed in the regression analysis output (Table 4.11). Differential R (0.8006) According to Table 4.11, there was a high positive correlation (r=0.8006) between NUpE and NUE. NupE accounts for about 64.1% of the variability in NUE, according to R Square (0.6410) (Figure 4.10, Table 4.11). Strong model fit was also demonstrated by corrected R Square (0.6304), which was corrected for the number of predictors. According to Table 4.11, NUpE accounts for almost 63.0% of the variability in NUE.

Table 4.11. The regression analysis output showing the relationship between Nitrogen Uptake Efficiency (NUpE) and Nitrogen Use Efficiency (NUE)

	Regression Statistics
Multiple R	0.80059823
R Square	0.640957525
Adjusted R Square	0.630397453
Standard Error	8.082798893
Observations	36

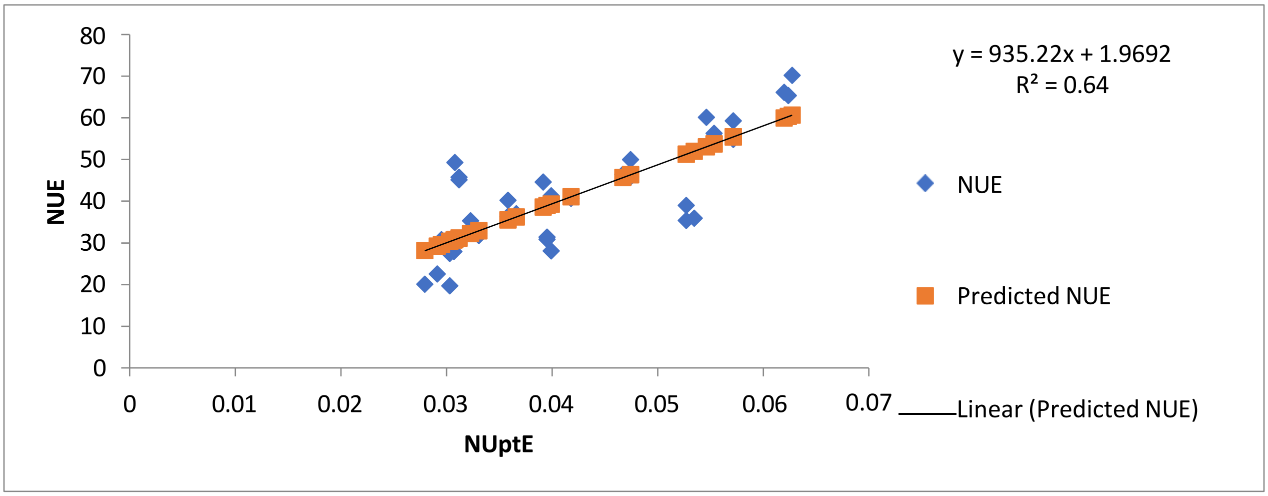

Figure 4.10. Regression NUpE vs NUE

With a high R Square value of 0.6410, the model showed a strong and significant association between NUpE and NUE and indicates that it accounts for a large amount of the variance in NUE (Figure 4.10, Table 4.11). The model is confirmed to be statistically significant by the incredibly low p-value (4.58E-09) for the entire model (Significance F) (Table 4.12).

A significant positive influence on NUE is indicated by the NUpE coefficient (935.2157) (Table 4.12). In particular, NUE dramatically rises as NUpE does, which is consistent with the theory that effective Nitrogen uptake might improve plants' total Nitrogen usage efficiency. This predictor is very significant, as confirmed by the huge t-statistic and the extremely low p-value for NUpE (Table 4.12).

Table 4.12. The coefficients in the regression model

	Coefficients	Standard Error	t Stat	P-value
Intercept	1.969221486	5.315691615	0.370454	0.713342
NUptE	935.2157295	120.0412842	7.790784	4.58E-09

4.2.4. Regression analysis (Dry season)

During the dry season, the regression analysis between Nitrogen Use Efficiency (NUE) and Nitrogen Uptake Efficiency (NUpE) revealed how these variables interacted with various environmental factors. A very significant positive link between NUpE and NUE was revealed by the correlation coefficient Multiple R (0.8600) (Table 4.13). NUpE accounted for almost 73.95% of the variability in NUE, according to R Square (0.7395), demonstrating the model's good explanatory ability (Figure 4.11, Table 4.13). The robustness of the association is further supported by the modified R Square (0.7319), which takes the number of predictors into account while still demonstrating a very good model fit (Table 4.13).

Table 4.13. The regression analysis output showing the relationship between Nitrogen Uptake Efficiency (NUpE) and Nitrogen Use Efficiency (NUE)

	Regression Statistics
Multiple R	0.859964
R Square	0.739538
Adjusted R Square	0.731878
Standard Error	6.611704
Observations	36

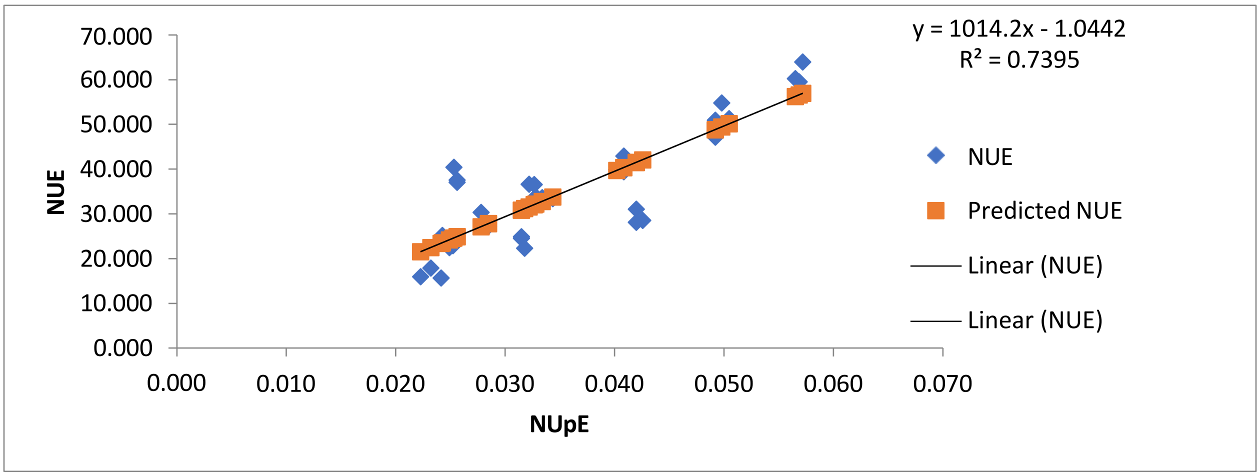

Figure 4.11. Regression NUpE vs NUE

According to the NUpE Coefficient (1014.2059), NUE increases by roughly 1014.21 units for every unit increase in NUpE (Figure 4.12). This association was highly significant, as evidenced by the low p-value (1.82818E-11) (Table 4.14).

Table 4.14. The regression coefficient in the regression model

	Coefficients	Standard Error	t Stat	P-value
Intercept	-1.04417	3.92896	-0.26576	0.792027
NUpE	1014.21	103.2234	9.825346	1.83E-11

4.3. Evaluating the impact of Biochar on soil properties and any synergistic effect with AMF.

4.3.1. Soil pH (Rain Season)

Only main effects were statistically significant ($P < 0.05$), but all the two way interactions and three way interaction were not statistically significant ($P > 0.05$) (Appendix 9). Nevertheless, Tukey post-hoc tests for the three-way interaction model were performed as an exploratory analysis to detect potential trends or interactions that could have biological significance, despite the lack of statistical significance (Table 4.15).

Table 4.15. The results of the Tukey HSD test for soil pH during the rain season

Treatments	Means	Significant groups
Mo Bo No	6.2	cd
Mo Bo N150	6.1	ab
Mo Bo N200	6.1	a
Mo Bb No	6.4	fg
Mo Bb N150	6.3	de
Mo Bb N200	6.3	d
Mm Bo No	6.3	de
Mm Bo N150	6.2	bc
Mm Bo N200	6.1	ab
Mm Bb No	6.5	g
Mm Bb N150	6.4	ef
Mm Bb N200	6.3	de

4.3.2. Soil pH (Dry Season)

The main effects of AMF, Biochar, and Nitrogen continued to have a significant impact on soil pH during the dry season (Appendix 10). Also, there were no statistical significance in two way interactions and three way interaction (Appendix 10). The findings throughout the seasons indicated how consistently these treatments affected the pH of the soil.

Nonetheless, Tukey post-hoc tests for the three-way interaction model were carried out as an exploratory analysis to identify potential trends or interactions that might possess biological significance, even in the absence of statistical significance (Figure 4.16).

Table 4.16. The results of the Tukey HSD test for soil pH during the dry season

Treatments	Means	Significant groups
Mo Bo No	5.5	d
Mo Bo N150	5.2	bc
Mo Bo N200	5.0	a
Mo Bb No	6.0	g
Mo Bb N150	5.7	ef
Mo Bb N200	5.6	de
Mm Bo No	5.6	de
Mm Bo N150	5.3	c
Mm Bo N200	5.1	ab
Mm Bb No	6.1	g
Mm Bb N150	5.8	f
Mm Bb N200	5.7	ef

4.3.3. Soil Organic Carbon (Rain Season)

Only the main effects were statistically significant ($P < 0.05$), while all two-way and three-way interactions were not statistically significant ($P > 0.05$) (Appendix 11).

Tukey post-hoc tests for the three-way interaction model were nonetheless performed as an exploratory analysis to identify potential trends or interactions that might hold biological significance, despite the absence of statistical significance (Table 4.17).

Table 4.17. The results of the Tukey HSD test for soil organic carbon during the rain season

Treatments	Means (mg kg^{-1})	Significant groups
Mo Bo No	15.28	a
Mo Bo N150	17.28	b
Mo Bo N200	19.29	c
Mo Bb No	59.17	e
Mo Bb N150	61.18	f
Mo Bb N200	63.19	g
Mm Bo No	17.29	b
Mm Bo N150	19.3	c
Mm Bo N200	21.31	d
Mm Bb No	61.21	f
Mm Bb N150	63.22	g
Mm Bb N200	65.23	h

4.3.4. Soil organic carbon (Dry Season)

During the dry season, AMF, Biochar, and Nitrogen showed significant impacts on Soil Organic Carbon (SOS) , with $P<0.05$, similar to the rain season (Appendix 12).

Tukey post-hoc tests for the three way interaction model was conducted with a purpose of exploratory analysis and the trend was consistent with the rain season (Table 4.18).

Table 4.18. The results of the Tukey HSD test for soil organic carbon during the dry season

Treatments	Means (mg kg^{-1})	Significant groups
Mo Bo No	12.64	a
Mo Bo N150	14.36	b
Mo Bo N200	16.1	c
Mo Bb No	50.81	e
Mo Bb N150	52.55	f
Mo Bb N200	54.3	g
Mm Bo No	14.36	b
Mm Bo N150	16.11	c
Mm Bo N200	17.87	d
Mm Bb No	52.58	f
Mm Bb N150	54.33	g
Mm Bb N200	56.07	h

4.3.5. Cation exchange capacity (Rain Season)

Only the main effect (Nitrogen, Biochar, and AMF)) had a high significant impact on CEC ($P < 0.05$), and all two way interactions and three way interaction were not statistically significant ($P > 0.05$) (Appendix 13).

Tukey post-hoc tests for the three-way interaction model were performed as an exploratory analysis to identify potential trends or interactions that could hold biological significance, even though they were not statistically significant (Table 4.19). For instance, group 'a"s "Mo Bo No" (22.13) had the lowest mean CEC, which was significantly lower than that of the other groups. There were significant differences in CEC across each Nitrogen level and treatments involving Biochar (Bb), Lower Nitrogen Level (N150), and Optimal Nitrogen level (N200) (Table 4.19).

Table 4.19. The results of the Tukey HSD test for cation exchange capacity (CEC) during the rain season

Treatments	Means Cmol (+) kg^{-1}	Significant groups
Mo Bo No	22.5	a
Mo Bo N150	22.75	ab
Mo Bo N200	23	abc
Mo Bb No	54.91	d
Mo Bb N150	55.16	de
Mo Bb N200	55.41	def
Mm Bo No	23.25	abc
Mm Bo N150	23.5	bc
Mm Bo N200	23.75	c
Mm Bb No	55.66	def
Mm Bb N150	55.91	ef
Mm Bb N200	55.98	f

4.3.6. Cation exchange capacity (Dry Season)

CEC was highly impacted by AMF ($P<0.05$), Biochar ($P<0.05$), and Nitrogen ($P<0.05$) levels. The factors of AMF, Biochar, and Nitrogen did not significantly interact with one another ($P>0.05$) (Appendix 14).

Tukey post-hoc tests for the three-way interaction model were conducted as an exploratory analysis to uncover potential trends or interactions that might have biological significance, despite not being statistically significant (Table 4.20).

Table 4.20. The results of the Tukey HSD test for cation exchange capacity (CEC) during the dry season

Treatments	Means (Cmol (+) kg^{-1})	Significant groups
Mo Bo No	19.8	a
Mo Bo N150	20.03	ab
Mo Bo N200	20.26	abc
Mo Bb No	49.34	d
Mo Bb N150	49.53	de
Mo Bb N200	49.75	def
Mm Bo No	20.43	abc
Mm Bo N150	20.66	bc
Mm Bo N200	20.89	c
Mm Bb No	49.98	def
Mm Bb N150	50.21	ef
Mm Bb N200	50.27	f

4.3.7. Root dry biomass (Rain Season)

Appendix 15 shows that AMF, Biochar, and Nitrogen significantly affected root dry biomass individually ($p < 0.05$). AMF, Biochar, and Nitrogen had a three-way interaction that was not significant ($p > 0.05$), indicating that there was no combined effect that went beyond each individual factor. Additionally, none of the interactions (AMFxBiochar, AMFxNitrogen, BiocharxNitrogen) were significant.

However, Tukey post-hoc tests for the three-way interaction model were carried out as an exploratory analysis to reveal potential trends or interactions that could have biological significance, even though they were not statistically significant (Table 4.21).

Table 4.21. The results of the Tukey HSD test for root dry mass during the rain season

Treatments	Means (g)	Significant groups
Mo Bo No	6.5	a
Mo Bo N150	7.34	ab
Mo Bo N200	7.91	bc
Mo Bb No	7.91	bc
Mo Bb N150	8.67	cd
Mo Bb N200	9.03	de
Mm Bo No	9.59	ef
Mm Bo N150	9.79	ef
Mm Bo N200	10.31	fg
Mm Bb No	10.31	fg
Mm Bb N150	11.19	gh
Mm Bb N200	11.75	h

4.3.8. Root dry biomass (Dry season)

AMF, Biochar, and Nitrogen had highly significant effects ($p < 0.05$) on root dry biomass, similar to the rain season (Appendix 16). There was no combination effect, since the two way interactions remained non-significant ($p > 0.05$). Additionally, no significant three way interaction (AMF x Biochar x Nitrogen) was found($p>0.05$) (Appendix 16).

However, Tukey post-hoc tests for the three-way interaction model were performed as an exploratory analysis to uncover potential trends or interactions that might hold biological significance, even if they were not statistically significant (Table 4.22).

Table 4.22. The results of the Tukey HSD test for root dry biomass during the dry season

Treatments	Means (g)	Significant groups
Mo Bo No	5.31	a
Mo Bo N150	5.99	ab
Mo Bo N200	6.46	bc
Mo Bb No	6.46	bc
Mo Bb N150	7.08	cd
Mo Bb N200	7.38	de
Mm Bo No	7.83	ef
Mm Bo N150	7.99	ef
Mm Bo N200	8.42	fg
Mm Bb No	8.42	fg
Mm Bb N150	9.14	gh
Mm Bb N200	9.59	h

4.3.9. Root colonization (Rain season)

Two way interactions (AMF x Nitrogen and AMF x Biochar) were significantly different (Appendix 17, Table 4.23, Table 4.24). Three way interaction (AMF x Biochar x Nitrogen) were not statistically different (p>0.05) (Appendix 17).

Table 4.23. Root colonization (%)_Rain season

AMF	NITROGEN		
	0	150	200
Mm	65^{f}	60^{e}	55^{d}
Mo	12.5^{c}	10^{b}	7.5^{a}
LSD		0.62	
p-value		<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Table 4.24. Root colonization (%)_Rain season

AMF	Biochar (10 ton ha^{-1})	No Biochar (0 ton ha^{-1})
Mm	65^{d}	55^{c}
Mo	12.33^{b}	7.67^{a}
LSD	0.51	
p-value	<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Nonetheless, Tukey post-hoc tests for the three-way interaction model were conducted as an exploratory analysis to identify potential trends or interactions that could hold biological significance, even if they were not statistically significant (Table 4.25).

Table 4.25. The results of the Tukey HSD test for root colonization during the rain season

Treatments	Means (%)	Significant groups
Mo Bo No	10	c
Mo Bo N150	8	b
Mo Bo N200	5	a
Mo Bb No	15	e
Mo Bb N150	12	d
Mo Bb N200	10	c
Mm Bo No	60	h
Mm Bo N150	55	g
Mm Bo N200	50	f
Mm Bb No	70	j
Mm Bb N150	65	i
Mm Bb N200	60	h

4.3.10. Root colonization (Dry season)

Significant interactions were found between AMFxNitrogen and AMFXBiochar ($P<0.05$) (Appendix 18, Table 4.26, Table 4.27), indicating synergistic effects in these treatments. In line with the findings from the rain season, the three-way interaction (AMF x Biochar x Nitrogen) was not significant ($p > 0.05$).

Table 4.26. Root colonization (%)_Dry season

AMF	NITROGEN		
	0	150	200
Mm	53.33^{f}	49.17^{e}	45.17^{d}
Mo	10.83^{c}	8.67^{b}	6.83^{a}
LSD		0.65	
p-value		<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Table 4.27. Root colonization (%)_Dry season

AMF	Biochar (10 ton ha^{-1})	No Biochar (0 ton ha^{-1})
Mm	53.11^{d}	45.33^{c}
Mo	10.78^{b}	6.78^{a}
LSD	0.53	
p-value	<.001	

Key: Mo: 0 kg ha^{-1} AMF**; Mm:** 8 kg ha^{-1} AMF**;** Mean values with superscripted letters indicate significant differences as determined by Tukey simultaneous tests for mean differences at a confidence level of 95%.

Tukey post-hoc tests for the three-way interaction model were nevertheless conducted as an exploratory analysis to detect potential trends or interactions that might hold biological significance, even if they were not statistically significant (Table 4.28).

Table 4.28. The results of the Tukey HSD test for root colonization during the dry season

Treatments	Means (%)	Significant groups
Mo Bo No	8.67	c
Mo Bo N150	7	b
Mo Bo N200	4.67	a
Mo Bb No	13	e
Mo Bb N150	10.33	d
Mo Bb N200	9	cd
Mm Bo No	49.33	h
Mm Bo N150	45.33	g
Mm Bo N200	41.33	f
Mm Bb No	57.33	j
Mm Bb N150	53	i
Mm Bb N200	49	h

4.4. Determine the growth and yield of garden egg (*Solanum aethiopicum* L.) by integrated AMF and Biochar application.

4.4.1. Plant Height in Rain Season

According to Appendix 19, the effect of AMF was highly significant, meaning that it had a large impact on plant height during the rain season. Additionally, Appendix 19 indicates that the impact of Biochar on plant height during the rain 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 (Appendix 19). 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.

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 4.29). 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 4.29).

Table 4.29. The results of the Tukey HSD test for plant height during the rain 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

4.4.2. Plant Height in Dry Season

AMF had a significant impact on plant height ($P<0.05$), indicating that AMF influenced the plant height throughout the dry season (Appendix 20). Biochar was also highly significant ($P<0.05$). Similar to the findings during the rain season, none of the interactions were significant (Appendix 20).

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 4.30). Mm Bb N200 (35.03 cm) differed significantly from MoBoNo (17.13 cm) in terms of mean plant height (Table 4.30). Treatments with AMF (Mm) and Biochar (Bb) tended to grow taller plants, just like during the rain season.

Table 4.30. 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

4.4.3. Fresh fruit yield in rain season

Appendix 21 shows that there was a strong interaction effect among AMFxBiocharxNitrogen that was highly significant ($p < 0.05$) on fresh fruit.

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 4.31). 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 4.31. The results of the Tukey HSD test for fruit fresh yield during the rain 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

4.4.4. Fresh fruit yield in dry season

The three-way interaction (AMF x Biochar x Nitrogen) remained extremely significant ($p < 0.05$) throughout the dry season (Appendix 22).

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 4.32).

Table 4.32. 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

4.4.5. Non-marketable yield (Rain season)

Appendix 23 shows that the three-way interaction between AMF, Biochar, and Nitrogen was extremely significant ($p < 0.05$). Appendix 23 shows that the two-way interactions were likewise quite significant. A Tukey HSD (Honestly Significant Difference) test was performed to compare the means of different treatment combinations for non-marketable yield during the rain season. The findings are shown in Table 4.33. 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 4.33). 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 rain season.

Table 4.33. The results of the Tukey HSD test for Non-marketable yield during the rain season

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

4.4.6. 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 (Appendix 24).

The mean of non-marketable yield for each combination of treatments was shown in Table 4.34. 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 4.34). 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 4.34. 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

4.4.7. Chlorophyll content (SPAD Units) in Rain Season

Appendix 25 shows that the three-way interaction between AMF, Biochar, and Nitrogen was extremely significant ($p < 0.05$).

Chlorophyll content was usually higher with AMF treatments (Table 4.35). 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 4.35. The results of the Tukey HSD test for Chlorophyll content (SPAD units) during the rain 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

4.4.8. Chlorophyll content (SPAD Units) in dry season

During the dry season, the three-way interaction between AMF, Biochar, and Nitrogen was highly significant ($p < 0.05$) (Appendix 26).

Treatments with AMF indicated greater chlorophyll content, comparable to the rain season (Table 4.36). 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 4.36. 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

CHAPTER FIVE

5. DISCUSSION

5.1. Determine the reduction of leachate volume from Nitrogen fertilization associated with bioremediation by AMF and Biochar and model environmental risk of nitrate leaching.

5.1.1. Nitrate concentration in leachate

The combination of AMF, Biochar, and Nitrogen produced a system where Nitrate leaching was highly reduced, possibly as a result of better nutrient retention and uptake, as indicated by the significant AMF x Biochar x Nitrogen interaction (Appendix 1) (Biederman & Harpole, 2013). The complex interplay between AMF, Biochar, and Nitrogen shown by the three-way interaction indicated the necessity of integrated management approaches in order to maximize Nitrogen utilization and reduce adverse environmental consequences of nitrate leaching.

Due to the fact that AMF increased the nutrient uptake of plants by symbiotically interconnecting with plant roots which increased the surface area of the plant roots, AMF is absolutely the main cause of decrease of concentration of nitrate in the leachate (Cavagnaro et al., 2015). (Figure 4.1).

The pots which received AMF inoculants indicated the reduction of Nitrate concentration in the leachates compare to the pots that did not receive the AMF inoculants. Increase of Nitrate uptake, improvement of microbial activity and decrease of leachate volume which were associated with AMF application in the soil might be the mechanisms that are leading to the decrease of nitrate leaching (Asghari et al., 2011; Corkidi et al., 2011; Asghari et al., 2012).

The inoculation of AMF had significant effects on the quantity of the leachates (Figure 4.3, Appendix 3), this indicated that the significant reduction in nitrate leaching in this research could be caused by the high nitrate retention by AMF or the high nitrate uptake by the AMF-treated plants.

Furthermore, since applying Biochar also raises the pH in the soil column, it may also aid in the decrease of NO_3^-concentration in the leachate (Figure 4.2, Appendix 2). As seen in Figure 4.2, as compared to treatment No Bo N200, the pH values in treatments Mo Bb N150, Mo Bb N200, Mm Bb N150, and Mm Bb N200 increased by 1.5, 2.01, 1.17, and 2.22, respectively. This may indicate that adding Biochar increased pH-dependent negative charges and raised the soil's Cation Exchange Capacity (CEC). Several research have found that the application of Biochar into the soil enhance its CEC, which lead to the increase the soil retention of ammonium (NH_4^+) (Dempster et al., 2012). Furthermore, the other studies indicated that the nitrification rate which can be suppressed could be the cause of reduction in NO_3^- leaching via leachate due to the Biochar application (Dempster et al., 2012).

Furthermore, Biochar has the ability to decrease the leachate volume (Figure 4.3, Appendix 3). This might be caused by the improved soil water holding capacity and structure of the soil, which also reduce the nitrification rate (conversion of NH_4^+ to NO_3^- by nitrifiers). The factors that influence the nitrifiers such as soil moisture, availability of soil organic carbon and enough oxygen affect the speed of process of nitrification. Soil aeration also has a major impact on the process of nitrification due to the fact that all nitrifiers are metabolically active in aerobic conditions (Robertson & Groffman, 2015). Additionally, controlling oxygen in microenvironment of the soil, and soil water content affects the metabolic activities of soil microorganisms responsible for nitrification process. When soil water content increase from wilting point to field capacity, the microbial activities of nitrifiers reach the optimum (Liu et al., 2014; Liu et al., 2018). Besides, the high soil water content can lead to the decrease of oxygen in the soil and that inhibit the microbial activities of nitrifiers (Stark & Firestone,(1995). Thus, application of Biochar in the soil increase soil water content, the increase of soil water content inhibits nitrification rate which lead to the increase the uptake of NH_4^+ instead of NO_3^- (Uchimiya et al., 2010). Application of Biochar in the soil modifies soil chemical properties such as Soil pH, electrical conductivity (EC), Cation Exchange Capacity (CEC) which lead to the increase of nutrient uptake efficiency (Amonette & Joseph, 2012). Wang et al. (2015) found that the application of Biochar inhibits the conversion of NH_4^+ into NO_3^- in two acidic soils. Wang et al. (2015), reported that peanut shells Biochar decreased the nitrification rate and the phenolic components were found to be the main cause of the reduction of nutrification rate.

Furthermore, several studies indicated that the Biochar could be able to absorb NO_3^- from the soil due to the chemical properties of the material used to produce Biochar (Zheng et al., 2013), and one of such property is the anion exchange capacity (AEC) of Biochar. It is important to keep in mind that in this present study, urea was used as the source of Nitrogen, the urea converts to NH_3 which also converts to (NH_4^+), which is also converted to NO_3^- by nitrification of the NO_2^- due to nitrification process. Because NO_3^- is very mobile, it can be readily leached below the root zone of plants. Since NH_4^+ is mainly adsorbed onto negatively charged soil colloids, the amount of NH_4^+ lost from the soil is significantly less than that of NO_3^- (Asghari et al., 2012).

5.1.2. NO3- expected to leach (NL)

Leachate concentrations less than 10 mg NO_3^- kg^{-1} are thought to pose little concern to groundwater contamination, according to Joshua et al. (1998). Nevertheless, soils treated without Arbuscular Mycorrhizal Fungi (AMF) and/or Biochar constituted a risk of groundwater contamination regardless of the amount of Nitrogen applied, as the projected NO3-leachate concentrations surpass 10 mg NO3- kg^{-1} (Table 4.2). Since a large amount of applied and mineralized Nitrogen is taken by plants, the presence of AMF and Biochar can significantly reduce NO_3^- leaching (Shepherd, 1996; Edis, 1998). However, the contributions from each factor (amount of accessible nitrate in a particular soil layer, excess water for leaching, and field capacity of the soil layer) may alter under different rainfall regimes (Shepherd, 1996; Smith et al., 1998).

5.1.2.1. Regression Analysis between cumulative Field Capacity (FC) and Nitrate expected to Leach (NL) predicted by Exponential Probability Density Function based model

A moderate to high negative correlation between cumulative FC and NL was shown by the Multiple R (0.6948), a correlation coefficient (Table 4.3). Cumulative FC explains roughly 48.28% of the variability in NL, according to the R Square (0.4828), indicating a moderate fit for the model (Table 4.3, Figure 4.5). The model's moderate explanatory power was further supported by the corrected R Square (0.4310), which was corrected for the number of predictors and still demonstrated a significant amount of the variation explained (Table 4.3).

According to the Cumulative FC Coefficient (-0.0244), the NL falls by roughly 0.0244 units for every unit rise in Cumulative FC (Table 4.4). Table 4.4 shows that Cumulative FC was a significant predictor of NL with a p-value of 0.0121.

A moderate negative correlation between cumulative FC and NL was shown by the R Square value of 0.4828 (Table 4.3). This indicated that although cumulative FC was a strong predictor of NL, other factors may have been equally relevant. This aligned with research conducted by Di and Cameron, 2002) who reported the complexity of Nitrogen leaching mechanisms impacted by several elements, such as soil composition, precipitation, and farming methods.

Higher FC was negatively correlated to decreased Nitrate leaching, according to the negative coefficient for cumulative FC (-0.0244) (Table 4.4). The studies indicated that soils with high FC hold much water which reduces leachate volumes and reduces nitrate leaching. Dinnes et al. (2002) found that increase of soil water holding capacity decreases leaching of nitrate.

Increase of soil water holding capacity was statistically significant for reducing leaching of nitrate as it was indicated by the significant P-value of the cumulative FC coefficient (Table 4.4). These findings were consistent with the findings of Shepherd & Webb, (1999) who indicated the role of soil water retention in decreasing leaching.

The findings of the regression analysis indicated how FC is for reducing leaching of nitrate. Employing nature based method such as AMF and Biochar is a promising method of reducing nitrate leaching, enhance Nitrogen Use Efficiency, and reducing the environmental pollution caused by nitrate. Additionally, these findings were also in line with the findings of Lehman et al. (2011), who reported that Biochar improve soil structure and water holding capacity.

5.2. Assessing the effect of AMF inoculation and Biochar application on Nitrogen uptake and Use efficiency in the production of garden egg (*Solanum aethiopicum* L.)

5.2.1. Nitrogen Uptake Efficiency (NUpE)

Integrated application of AMF and Biochar synergistically affect growth of plant and nutrient uptake (Biederman and Harpole, 2013). This present study indicated this synergy in increase of NUpE in both rain and dry season (Appendices 5 and 6, Figures 4.6 and 4.7).

5.2.2. Nitrogen Use Efficiency (NUE)

Nitrogen Use Efficiency (NUE) can be increased by promoting the AMF and Biochar, increase availability of nutrient in soil and beneficial soil microorganisms (Rillig et al., 2010). In the present study, the synergistic effect was indicated by the integrated application of AMF and Biochar which increase the NUE in both rain and dry seasons (Table 4.7, Table 4.8, Table 4.9 and Table 4. 10).

5.2.3. Regression analysis (Rain season)

In this study, Nitrogen Uptake efficiency indicated that it increases Nitrogen Use Efficiency. Fageria and Baligar (2005) found that increasing nutrient uptake efficiency also led to nutrient utilization efficiency which is vital in farming practices. Govindasamy et al. (2023) found that nutrient Uptake efficiency directly affect Nutrient Use Efficiency in crop production which is consistent with the significant correlation (R = 0.8006) as it was found in this study.

Biederman and Harpole, (2013) found that Arbuscular Mycorrhizal Fungi (AMF) and Biochar can enhance Nutrient Uptake and Utilization Efficiency, which is similar to the significant synergistic effect of AMF and Biochar Application as it was indicated in this present study. The findings of Lehmann et al. (2011), which described how Biochar improved physico-chemical properties of the soil, and this leads to the increase of both nutrient uptake efficiency (NUpE and nutrient use efficiency (NUE) of plants, this was supported by the R Square (R^2 = 0.6410) obtained in the regression analysis of this present study (Figure 4.10, Table 4.11).

The low p-value for the NUpE coefficient indicated the importance of Nitrogen uptake efficiency in predicting NUE. This was supported by the findings of Raun and Johnson's (1999), which emphasized the need for NUpE for increasing crop yield. Additionally, the strong t-statistic (7.7908) of the NUpE coefficient emphasize its significance and is supported by the findings of Finzi et al. (2007).

5.2.4. Regression analysis (Dry season)

There was a moderate to strong positive correlation between NUpE and NUE during the dry season (R Square = 0.7395) because nutrient uptake efficiency increased in dry conditions where water and fertilizer supply are more limited. Marschner's (1995) reported that, in dry conditions, plants would rely more on nutrient uptake efficiency mechanisms for maintaining plant growth and crop yield, which would be explained by the higher correlation observed in this present study. Similarly, to the rain season, the integrated application of Biochar and AMF significantly increased the NUpE and NUE during the dry season.

Lehmann et al. (2011) found that Biochar improves soil water holding capacity and availability of nutrients, which might be particularly important in dry lands. Biederman and Harpole (2013) found the evidence to support the significant positive impact on NUE: Biochar increased Nutrient Uptake Efficiency with inoculants.

Furthermore, the effect of NUpE on NUE was significant ($P < 0.05$) significant. This was supported by Raun and Johnson (1999), who emphasized that improving crop yield, especially in dry conditions, requires a high NUpE. The high t-statistic (9.8253) of the NUpE coefficient in Table 4.12 provided additional evidence of its relevance. This is was supported by the study of Finzi et al. (2007).

5.3. Evaluating the impact of Biochar on soil properties and any synergistic effect with AMF.

5.3.1. Soil pH (Rain Season)

This present study found that Arbuscular Mycorrhizal Fungi (AMF), Biochar, and Nitrogen individually had a significant effect on soil pH during the rain season (Appendix 9).

These significant findings were also reported in previous studies indicating that AMF can affect soil pH by releasing metabolites and organic acids that affect soil chemical properties (Entry et al., 2002). Additionally, other studies indicated that Biochar has the ability to increase soil pH, especially in acidic soils, due to its alkaline nature (Lehmann et al., 2011). Guo et al. (2010) reported that inorganic Nitrogen fertilizers cause soil acidification.

The three-way interaction (AMF x Biochar x Nitrogen) was not significant, and this indicated that each factor affected soil pH independently.

5.3.2. Soil pH (Dry Season)

Appendix 10 indicated that during the dry season, AMF, Biochar, and Nitrogen had individual significant effects on soil pH. Similar to the rain season, the three-way interaction (AMF x Biochar x Nitrogen) was not significant. This seasonal consistency indicated how these factors change soil pH.

The three-way interactions (AMF x Biochar x Nitrogen) in the ANOVA were not significant which indicated that integrating these factors did not change soil pH. The findings were supported by Biederman and Harpole, (2013) who also did not find any significant interactions between the factors for the pH.

5.3.4. Soil organic carbon (Rain Season)

The results of the ANOVA indicated the high significance of the main effect of AMF, Biochar, and Nitrogen on Soil Organic Carbon (SOC) during the rain season Appendix 11). This indicated that each of these factors alone had a significant effect on soil organic carbon.

Significant interactions between AMF, Biochar, and Nitrogen were not observed. This indicated that, beyond their individual contributions, the combined effects of these factors did not significantly change soil organic carbon (Appendix 11).

These findings showed that differences in SOC were significantly and independently impacted by the three factors, AMF, Biochar, and Nitrogen. These factors did not appear to significantly modify soil organic carbon beyond their individual impacts, as indicated by the lack of significant interactions among them (Appendix 11).

But exploratory analysis was conducted using a three way interaction model and significant differences between several treatment combinations were found using the Tukey HSD test (Table 4.17). Among all the treatment groups, treatment groups, Mo Bo No treatment had the lowest mean of SOC (15.28). Higher Nitrogen levels (N150, N200) and Biochar (Bb) treatments generally showed higher SOC, with significant differences observed between the Nitrogen levels (Table 4.17). This showed that soil organic carbon was positively impacted by both Nitrogen levels and Biochar, and that the benefits of these treatments were stronger when combined.

5.3.5. Soil organic carbon (Dry Season)

AMF, Biochar, and Nitrogen also individually showed significant impacts during the dry season ($P < 0.05$). As with the rain season, no significant interactions were found between these factors (Table 4.18).

Also exploratory analysis were conducted using the three way interaction model and thee Tukey HSD test revealed a trend that was consistent with the rain season. Mo Bo No treatment had the lowest mean (12.64), whereas treatments with Biochar and optimal Nitrogen levels showed high SOC (Table 4.18). The significant effects of Nitrogen and Biochar on soil organic carbon were indicated by the significant differences between treatment combinations (Table 4.18). These findings showed that soil organic carbon was significantly affected by AMF, Biochar, and Nitrogen individually, but not by their combinations. This indicated that rather than being antagonistic or synergistic, these factors effects might be additive.

Furthermore, several studies have shown that Biochar can improve soil fertility and reduce carbon loss through mineralization (Lehmann et al., 2011; Zhang et al., 2018). Biochar has pores where soil microorganisms live and this increases the fertility of the soil sequesters carbon (Glaser et al., 2002).

Inorganic Nitrogen fertilizer increases biomass from plant development and increase soil organic carbon from residues from roots and shoots (Ladha et al., 2011). The positive effects of Nitrogen on soil organic carbon have been shown in many agroecosystems (Zhou et al., 2014).

AMF increase soil organic carbon by improving growth of plant and increasing the carbon incorporated into the soil by root exudates and fungal biomass (Rillig et al., 2001).

5.3.6. Cation exchange capacity (Rain and dry seasons)

The Analysis of Variance (ANOVA) indicated that AMF, Biochar, and Nitrogen had individual significant effects in both the rain and dry seasons, but two way and three way interactions were not significant($P>0.05$) (Appendix 13 and 14). This indicated that every factor affected CEC independently, the sum of each factor`s individual effects was equal to the sum of the factors` combined effects.

Though, Tukey post-hoc tests for the three-way interaction model were conducted as an exploratory analysis to detect potential trends or interactions that might hold biological significance and Tukey HSD was able to detect significant differences between specific combinations of treatments, despite all interactions in the ANOVA were not significant (Table

4.19 and Table 4.20). The reason for this is that Tukey HSD has a great ability of detecting the differences between the means of treatment.

The main effects AMF, Biochar, and Nitrogen independently were significant during both rain and dry seasons According to the ANOVA test.

Biochar has shown to significantly increase CEC by providing carbon consistently, improving structure of the soil, and enhancing microbial activity (Glaser et al., 2002; Lehmann & Joseph, 2009). Major et al. (2010) reported that application Biochar in different types of soils increase CEC.

Nitrogen fertilizer enhance soil fertility and may increase CEC by increasing the amount of organic matter in the soil and improve microbial activity (Bationo et al., 2012). Zhao et al. (2017) found that Nitrogen application in the soil increases CEC.

AMF increased CEC and increased Nitrogen uptake by fungal biomass and root exudates (Smith & Read, 2008). High increase of CEC has been linked to the inoculation of AMF in soils.

5.3.7. Root dry biomass (Rain and dry seasons)

During both the rain and dry seasons, the ANOVA consistently indicated that the main effects AMF, Biochar, and Nitrogen were significant (Appendix 15 and 16). This emphasize how vital these factors are to increase root growth. Several studies support the effect of AMF on plant growth (Jeffries et al., 2003) and the effect of Biochar on the plant growth (Sohi et al., 2010).

While the main effects Nitrogen, Biochar, and AMF were significant individually, their two and three-way interactions were not significant (Appendix 17 and 18). This indicated that there were no synergistic interactions This was consistent with the findings of Lehmann and Joseph, (2009), who found that while fertilizers and Biochar can both increase plant growth on their own, their interactions may not always be significant.

By conducting exploratory analysis by using the Tukey's HSD test, significant differences in Root Dry Biomass were found within several treatments (Tables 4.21 and 4.22). This indicated that there were potential trends or interactions that might have biological significance, even if thee way were not statistically significant. The interactions might not be covered by the broad interaction terms but are significant in specific treatment pairs.

The findings indicated clear seasonal trends, with AMF, Biochar, and Nitrogen indicating significant effects in both Rain and dry seasons. Augé, (2001) indicated the importance of seasonal variations in the effectiveness of Arbuscular Mycorrhizal Fungi, which may help to explain these differences.

5.3.8. Root colonization (Rain and dry season)

Appendix 17 and Appendix 18 are indicating the significant effects of Nitrogen, Biochar, and AMF on root colonization. These findings were supported by several studies that found how AMF improve root colonization and plant health (Smith and Read, 2008). Many researchers reported the role of Nitrogen in plant growth (Galloway et al., 2008) and the role of Biochar on soil structure and availability of nutrients (Lehmann et al., 2011). Significant interactions between AMF x

Biochar and AMF x Nitrogen were found (Appendix 17, Appendix 18, Table 23, Table 24, Table 26 and Table 27), indicating that both combinations had a synergistic effect on root colonization. This was supported by the findings of Rillig et al. (2010) who discovered that integrated application of AMF and Biochar improve plant-soil microorganisms' interactions. The three way interaction was not significant which indicated that the impact of the three factors was not greater than the sum of their two interaction or the main effects.

Tukey Post Hoc analysis was conducted as an exploratory analysis and the specific treatment means varied between the rain and dry seasons (Table 4.25 and Table 4.28). This was supported by the findings of Augé (2001) who found how showing how the seasons affected treatment efficacy and root colonization, as well as how beneficial mycorrhizal symbiosis was.

Tables 4.25 and 4.28 of the Tukey's HSD test revealed significant differences between treatment combinations, even in if there was non-significant three-way interaction in the ANOVA. This shown that, even in cases when the overall interaction term is not significant, particular combinations of AMF, Biochar, and Nitrogen can have a significant impact on root colonization. This indicated that specific treatment combinations can have significant effects on root colonization even though overall interaction effects can be small.

5.4. Determining the growth and yield of garden egg (*Solanum aethiopicum* L.) by integrated AMF and Biochar application.

5.4.1. Plant height (Rain and dry season)

Plant height was significantly increased by both AMF and Biochar individually in both seasons (Appendix 19 and Appendix 20). Although Nitrogen did not have a significant effect on plant height both in rain and dry seasons. The combined effects of AMF, Biochar, and Nitrogen were not statistically different in both rain and dry season).

The studies have found that AMF enhance plant growth by increasing Nitrogen uptake efficiency, and that Biochar improved soil structure and availability of nutrient (Smith and Read, 2008, Lehmann and Joseph, 2015). Integrated application of AMF and Biochar has synergistic benefits on plant growth (Solaiman and Anawar, 2015). Some researchers have found that although Nitrogen is vital for plant growth, the application of other soil amendments such as AMF and Biochar, increase the plant growth.

5.4.2. Fresh fruit yield (Rain season)

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 4.31). 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, 2008; Barea et al., 2005) that found the beneficial effects of AMF on plant growth and crop yield by improving soil structure and increasing nutrients uptake efficiency. Several research has found that Biochar enhance soil fertility, microbial activity, and water holding capacity which are critical factors for plant growth (Lehmann & Joseph, 2015; Jeffery et al., 2011).

5.4.3. Fresh fruit yield (Dry season)

During dry season, the three-way interaction (AMF x Biochar x Nitrogen) was also highly significant ($p < 0.05$) (Appendix 22). 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 compare to treatments like Mm Bo N150 and Mm Bb N200 (Table 4.32). 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 & Joseph, 2015; 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.

5.4.4. Non-marketable yield (Rain and dry seasons)

Appendix 23 and Appendix 24 indicate that the combined effect of AMF, Biochar, and Nitrogen were 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 (Tables 4.33 and 4.34).

Non-marketable yield was significantly reduced as a result of the three-way interaction between AMF, Biochar, and Nitrogen (Table 4.33 and Table 4.34). This result was probably brought about by the synergistic effects of better soil structure, increased nutrient uptake, and optimum Nitrogen usage (Zhang et al., 2020).

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 4.33 and Table 4.34). Drought-stressed plants frequently use more energy to surviving than to growing, which increases their yields that are not suitable for market (Cramer et al., 2020).

5.4.5. Chlorophyll content (SPAD Units) in the rain season

The significant effect of AMF on chlorophyll content indicated a high beneficial influence of AMF on chlorophyll content during the rain 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, 2008).

Nitrogen also shown a significant impact. Since Nitrogen is an essential part of chlorophyll molecules, its function in the synthesis of chlorophyll is well understood (Taiz & Zeiger, 2010).

The interaction between Biochar and AMF and Nitrogen indicated a high significant effect ($p<0.05$).

Lehmann et al. (2011) reported that Biochar has the ability to enhance microbial activity, improve soil structure, and increase water holding capacity. The amount of chlorophyll may be indirectly affected by these actions. Integrated application of AMF, Biochar, and Nitrogen led to higher chlorophyll content than applying them separately, as it was indicated by the significant interactions between these factors (Appendix 25, Table 4.35). Studies have found that the combined application of AMF and Biochar enhance the health of soil and increase nutrient retention, which increase plant growth and also increase the chlorophyll content (Warnock et al., 2007).

The Tukey HSD test indicated that treatments with AMF tended to have higher chlorophyll contents, especially when it was applied with optimum rate of Nitrogen. This indicated that integrated application of AMF and Nitrogen together is especially advantageous for increasing chlorophyll content during the rain season.

5.4.6. Chlorophyll content (SPAD Units) in the dry season

AMF significantly increased the chlorophyll content during the dry season, even though the effect appeared to be less than it was during the rain season ($p < 0.05$) (Appendix 26). This could be caused by the water stress during the dry season and generally reduced availability of nutrients, which led to lower chlorophyll content that AMF may grow (Smith & Read, 2008).

Nitrogen also had a significant effect during the dry season (P< 0.05). and this emphasized the crucial role of Nitrogen in chlorophyll synthesis (Taiz & Zeiger, 2010).

Contrary to the rain season, Biochar did not significantly change the chlorophyll content during the dry season (p>0.05). This could have been caused by the dry conditions which decreased nutrient availability and microbial activity and this affected the effectiveness of Biochar (Lehmann et al., 2011).

The three way interaction (AMF x Biochar x Nitrogen) and the two way interaction (Biochar x Nitrogen), these indicated that these factors could increase the chlorophyll content even in less than optimal conditions (Table 4.36). Atkinson et al. (2010) found that Biochar could increase the availability of nutrient and water holding capacity wich reduce the negative effects of abiotic factor such as drought.

The Tukey HSD results indicated the trends that were similar to those observed during the rain season, with treatments containing AMF and higher Nitrogen rates generally showing high chlorophyll content (Table 4.36). This finding supported the idea that these amendments are vital for maintaining chlorophyll content in a range of environmental conditions.

CHAPTER SIX

INTRODUCTION, SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FROM THIS STUDY AND RECOMMENDATIONS FOR FURTHER STUDIES

6.1 Introduction

The Summary of the study, Conclusions, Recommendations from this research, and recommendations for further research were provided in this chapter six.

6.2 Summary

In summary, integrated application of AMF and Biochar is an appropriate Nitrogen management method which has the potential to enhance environmentally friendly farming practices. The reduction in nitrate leaching, the increase in soil water holding capacity, the increase in Nitrogen uptake efficiency and Nitrogen Use Efficiency, and the positive impacts on plant growth and crop yield, all indicate the successful implementation of these environmentally friendly method in improving sustainable agriculture and environmental sustainability. The study recommends the further research on application of AMF and Biochar for improving Nitrogen management and reducing environmental pollution.

Although some ANOVA results indicated that interactions between factors were not statistically significant ($P>0.05$), and post-hoc tests (Tukey's HSD) were conducted for three way interaction model as an exploratory analysis to identify any potential trends or understated differences that may not have been detected by the ANOVA. The purpose of this approach was to provide deeper insight into the interaction effects that might be biologically relevant or deserve further studies. Thus, Post-hoc tests in this context were not used to make definitive conclusions but rather to identify trends for future studies, because sometimes unexpected patterns can appear that may be biologically or practically significant despite statistical insignificance.

6.3 Conclusions

Less quantity of leachate volume was found to indicate that the integrated application of AMF and Biochar significantly increase soil water holding capacity. The treatments (Mm Bb N150 and Mm Bb N200) that combined AMF and Biochar had the lowest quantity of leachate volumes due to the improvement of soil structure, and increase of soil water holding capacity. This increased Field Capacity of the soil, which reduced nitrate leaching and leachate volume. The integrated application of AMF and Biochar improved soil structure and enhanced water management for maintaining a better soil conditions and decrease runoff of nutrients. The research findings indicated that the integrated application of AMF and Biochar significantly reduced nitrate leaching.

The treatments that had the least concentration of nitrate in the leachate were Mm Bb N150 and Mm Bb N200, which combined AMF and Biochar. This significant reduction in concentration of nitrate leaching is crucial for reducing the environmental pollution from inorganic Nitrogen fertilizers. These treatments reduce the risk potential of nitrate pollution in groundwater by enhancing the ability of soil in retaining Nitrogen, which reduced nitrate leaching and its negative effects in the environment. The important benefits of integrated application of AMF and Biochar were illustrated by the nitrate that expected to leach, which was calculated by employing an Exponential Probability Density Function-based model. Soils treated with these amendments

(AMF and Biochar) indicated significantly lower concentration of nitrate leaching than in the soil with absence of AMF and Biochar.

The model predicted that in the absence of AMF and Biochar, nitrate leaching could be up to 17–20 times higher, indicating the potential risk of Nitrogen of polluting the environment due to the current farming practices. These findings indicated that AMF and Biochar could decrease the concentration of nitrate leaching and reduce environmental pollution caused by inorganic fertilizers that are used in farming practices.

It was found that application of Biochar increased the pH of the soil, which decrease the concentration of nitrate leaching. The treatment with AMF and Biochar indicated high soil pH levels led to reduction of nitrification rates and increase of cation exchange capacity (CEC). One of the most important method of reducing nitrate leaching is through the process of decreased or inhibit nitrification, which is the conversion of ammonium (NH_4^+) to nitrate (NO_3^-). Biochar is vital amendment that can be used to optimize Nitrogen Use Efficiency and decreasing the environmental pollution caused by inorganic Nitrogen fertilizers due to ability of Biochar of changing soil pH and reactions of Nitrogen.

The findings of the study indicated that the integrated application of AMF and Biochar increased the Nitrogen Uptake Efficiency (NUpE) and Nitrogen Use Efficiency (NUE) significantly in garden egg (*Solanum aethiopicum* L.). The optimal application rate of Nitrogen with AMF and Biochar (Mm x Bb x N200) had the highest NUpE, especially during the rain season. During the dry season, NUpE reduced, but (Mm x Bb x N200) remained to be the treatment that had the highest NUpE. This indicate how AMF and Biochar is able to tackle the environmental pollution and increases NUpE.

The regression analysis indicated a high positive correlation between NUpE and NUE. NUpE could significantly predict the variability in NUE. A regression analysis showed that increasing NUpE directly increased NUE, hence supporting the nutrient dynamics in soil and plants. Both seasons consistently had the highest NUE with the integrated application of AMF and Biochar, indicating how strongly AMF and Biochar improve Nitrogen Use Efficiency. The research provided valuable insights into the effects of applying AMF, Biochar, and Nitrogen into the soil. Both AMF and Biochar had significant effects on root colonization, root dry biomass, pH, SOC, and CEC of the soil.

(e.g: Biochar increased the soil pH and SOC significantly, whereas AMF increased root colonization significantly). Based on unique effects of each treatment on soil properties, these amendments (AMF and Biochar) have the potential for improving soil fertility and health.

The integrated application of AMF and Biochar had a positive effect on plant height, fresh fruit yield, and chlorophyll content. The plants that received the highest Nitrogen rate with AMF and Biochar (Mm Bb N200) had the highest plant height and yielded much fruits. The three way interaction was significant for fruit yield and chlorophyll content which indicated how these amendments improve crop yield and plant health. Furthermore, the reduction in unmarketable fruit yield emphasized the benefits of AMF and Biochar in increasing the yield and quality of garden eggs.

Recommendations from this study

i. Integrated application of AMF and Biochar: Recommend the integrated application of AMF and Biochar to decrease Nitrogen leaching and improve Nitrogen Use Efficiency. This environmentally friendly method significantly reduced leachate volume and nitrate concentrations by reducing nitrate pollution, improving environmental sustainability.

ii. The integrated application of AMF and Biochar with the optimal Nitrogen rate (200 kg. ha^{-1}) to increase Nitrogen retention and reduce Nitrogen leaching.

iii. Integrated application of AMF inoculants and Biochar in soil fertility management can help to improve the environmentally friendly farming practices and this can promote to use little quantity of inorganic Nitrogen fertilizers.

iv. Integrated application of AMF and Biochar can increase plant growth and crop yield in challenging climatic conditions.

v. Encourage local production of AMF to enhance accessibility and affordability, and promoting policies that incentivize farmers to adopt these sustainable practices.

vi. Provide subsidies or incentives for farmers to adopt these sustainable practices

vii. Support research and development on AMF and Biochar technologies

viii. Establish regulations to ensure the responsible use of fertilizers, minimizing Nitrogen pollution and promoting environmental sustainability.

Recommendations for further research

i. Conduct research to assess the long-term feasibility of integrated application of AMF and Biochar on soil physico-chemical properties, nitrate leaching, and nutrient cycling over various growing seasons and climate conditions.

ii. Study the effects of different types forms of Biochar (e.g. Biochar made of wood, bamboo, or animal manure,…etc) on soil health and Nitrogen dynamics.

iii. Study how soil microorganisms, especially nitrifiers and denitrifiers, relate to AMF and Biochar.

iv. Study the effects of AMF and Biochar on plant nutrition and bioremediation of Nitrogen in different crops and different agroecosystems to scale their applicability.

v. Improve nitrate leaching models to incorporate other variables such as climate, soil moisture, and activities of soil microorganisms to better predict the risks of nitrate leaching in different farming practices.

vi. Study the trade-offs between AMF and Biochar in large-scale farming in terms of the economy and the environment. Cost-benefit studies could provide vital information that will help the farmers and policy makers to decide about using these environmentally friendly methods.

vii. Study how AMF and Biochar affect other vital nutrients such as Phosphorus and Potassium uptake and utilization efficiencies for having a full understanding of their roles in nutrient management.

viii. Study the effects of different rates of Biochar and particle sizes of Biochar with AMF for providing more precise application recommendation rates.

ix. Study how AMF and Biochar affect greenhouse gas emissions, especially nitrous oxide (N_2O) for finding out more about how they can help to reduce climate change.

x. Study the effects of AMF and Biochar on soil microbial communities and how these impacts plant health, nutrient cycling, and soil fertility.

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APPENDICES

Appendix 1. Analysis of variance (ANOVA)_Nitrate in Leachate (mg kg^{-1})

Variate: Nitrate in Leachate					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.012	0.006	0	
AMF	1	3632.247	3632.247	2828.35	<.001
BIOCHAR	1	2359.915	2359.915	1837.61	<.001
NITROGEN	2	752.572	376.286	293.01	<.001
AMF.BIOCHAR	1	252.3	252.3	196.46	<.001
AMF.NITROGEN	2	394.138	197.069	153.45	<.001
BIOCHAR.NITROGEN	2	256.34	128.17	99.8	<.001
AMF.BIOCHAR.NITROGEN	2	395.292	197.646	153.9	<.001
Residual	562	721.737	1.284		
Total	575	8764.553			

Appendix 2: Analysis of variance (ANOVA)_Soil pH

Variate: Soil pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.2582	0.1291	1.32	
AMF	1	0.01436	0.01436	0.15	0.702
BIOCHAR	1	189.3427	189.3427	1938.96	<.001
NITROGEN	2	105.5392	52.76961	540.39	<.001
AMF.BIOCHAR	1	0.0785	0.0785	0.8	0.37
AMF.NITROGEN	2	1.99371	0.99686	10.21	<.001
BIOCHAR.NITROGEN	2	105.376	52.68797	539.55	<.001
AMF.BIOCHAR.NITROGEN	2	1.61903	0.80952	8.29	<.001
Residual	562	54.88025	0.09765		
Total	575	459.1019			

Appendix 3: Analysis of variance (ANOVA)_ Leachate volume (ml)

Variate: Leachate volume (ml)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	93.12	46.56	1.02	
AMF	1	2836579	2836579	62063.09	<.001
BIOCHAR	1	238288.4	238288.4	5213.64	<.001
NITROGEN	2	12077.06	6038.53	132.12	<.001
AMF.BIOCHAR	1	1117.93	1117.93	24.46	<.001
AMF.NITROGEN	2	75.82	37.91	0.83	0.437
BIOCHAR.NITROGEN	2	174.46	87.23	1.91	0.149
AMF.BIOCHAR.NITROGEN	2	614.59	307.3	6.72	0.001
Residual	562	25686.08	45.7		
Total	575	3114706			

Appendix 4. Analysis of Variance (ANOVA)

Variate: Field Capacity (mm)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	16.974	8.487	1.15	
AMF	1	505016	505016	68707.03	<.001
BIOCHAR	1	305249.7	305249.7	41528.98	<.001
NITROGEN	2	182.005	91.002	12.38	<.001
AMF.BIOCHAR	1	21471.61	21471.61	2921.2	<.001
AMF.NITROGEN	2	145.555	72.778	9.9	<.001
BIOCHAR.NITROGEN	2	17.906	8.953	1.22	0.297
AMF.BIOCHAR.NITROGEN	2	9.245	4.623	0.63	0.534
Residual	562	4130.858	7.35		
Total	575	836239.8			

Appendix 5: Analysis of Variance_ Nitrogen Uptake Efficiency (NUpE) in rain Season

Variate: NUpE					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AMF	1	1.94E-03	1.94E-03	4283.79	<.001
BIOCHAR	1	1.43E-06	1.43E-06	3.17	0.088
NITROGEN	2	5.48E-03	2.74E-03	6047.48	<.001
AMF.BIOCHAR	1	4.49E-04	4.49E-04	990.82	<.001
AMF.NITROGEN	2	3.25E-04	1.63E-04	358.8	<.001
BIOCHAR.NITROGEN	2	2.75E-04	1.37E-04	303.13	<.001
AMF.BIOCHAR.NITROGEN	2	2.76E-04	1.38E-04	304.56	<.001
Residual	24	1.09E-05	4.53E-07		
Total	35	8.75E+00			

Appendix 6: Analysis of Variance_ Nitrogen Uptake Efficiency (NUpE) in Dry season

Variate: NUpE					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	9.62E-07	4.81E-07	1.89	
AMF	1	1.51E-03	1.51E-03	5916.6	<.001
BIOCHAR	1	1.76E-05	1.76E-05	69.04	<.001
NITROGEN	2	1.67E-03	8.33E-04	3264.17	<.001
AMF.BIOCHAR	1	3.15E-04	3.15E-04	1235.63	<.001
AMF.NITROGEN	2	2.39E-04	1.19E-04	468.02	<.001
BIOCHAR.NITROGEN	2	1.66E-04	8.28E-05	324.48	<.001
AMF.BIOCHAR.NITROGEN	2	1.85E-04	9.24E-05	362.44	<.001
Residual	22	5.61E-06	2.55E-07		
Total	35	4.10E-03			

Appendix 7. Analysis of variance_Nitrogen Use Efficiency (NUE) in rain season

Variate: NUE					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	76.749	38.374	28.31	
AMF	1	2218.9	2218.9	1636.75	<.001
BIOCHAR	1	765.786	765.786	564.87	<.001
NITROGEN	2	2911.772	1455.886	1073.92	<.001
AMF.BIOCHAR	1	5.3	5.3	3.91	0.061
AMF.NITROGEN	2	133.552	66.776	49.26	<.001
BIOCHAR.NITROGEN	2	38.698	19.349	14.27	<.001
AMF.BIOCHAR.NITROGEN	2	6.085	3.043	2.24	0.13
Residual	22	29.825	1.356		

Appendix 8: Analysis of variance_Nitrogen Use Efficiency (NUE) in dry season

Variate: NUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AMF	1	2480.497	2480.497	760.1	<.001
BIOCHAR	1	810.771	810.771	248.44	<.001
NITROGEN	2	2143.359	1071.679	328.39	<.001
AMF.BIOCHAR	1	2.739	2.739	0.84	0.369
AMF.NITROGEN	2	142.869	71.434	21.89	<.001
BIOCHAR.NITROGEN	2	40.065	20.033	6.14	0.007
AMF.BIOCHAR.NITROGEN	2	7.774	3.887	1.19	0.321
Residual	24	78.322	3.263		
Total	35	5706.396			

Appendix 9: Analysis of variance (ANOVA)

Variate: Soil pH in rain season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.048606	0.024303	7.86	
AMF	1	0.099751	0.099751	32.26	<.001
BIOCHAR	1	1.290117	1.290117	417.29	<.001
NITROGEN	2	0.689068	0.344534	111.44	<.001
AMF.BIOCHAR	1	0.000156	0.000156	0.05	0.822
AMF.NITROGEN	2	0.000501	0.000251	0.08	0.922
BIOCHAR.NITROGEN	2	0.008235	0.004117	1.33	0.268
AMF.BIOCHAR.NITROGEN	2	0.000312	0.000156	0.05	0.951
Residual	130	0.401919	0.003092		
Total	143	2.538666			

Appendix 10. Analysis of variance (ANOVA)

Variate: Soil pH in dry season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
AMF	1	0.31174	0.31174	18.59	<.001
BIOCHAR	1	10.61674	10.61674	633.15	<.001
NITROGEN	2	4.99014	2.49507	148.8	<.001
AMF.BIOCHAR	1	0.0034	0.0034	0.2	0.653
AMF.NITROGEN	2	0.00014	0.00007	0	0.996
BIOCHAR.NITROGEN	2	0.07681	0.0384	2.29	0.105
AMF.BIOCHAR.NITROGEN	2	0.00014	0.00007	0	0.996
Residual	130	2.17986	0.01677		
Total	143	18.3966			

Appendix 11. Analysis of variance (ANOVA)

Variate: Soil Organic Carbon (rain season)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AMF	1	1.4E+02	1.48E+02	1.1E+05	<.001
BIOCHAR	1	6.9E+04	6.94E+04	5.2E+07	<.001
NITROGEN	2	3.8E+02	1.93E+02	1.4E+05	<.001
AMF.BIOCHAR	1	4.7E-03	4.78E-03	3.64	0.059
AMF.NITROGEN	2	4.5E-04	2.26E-04	0.17	0.842
BIOCHAR.NITROGEN	2	1.5E-04	7.71E-05	0.06	0.943
AMF.BIOCHAR.NITROGEN	2	1.7E-04	8.82E-05	0.07	0.93
Residual	130	1.7E-01	1.31E-03		
Total	143	6.9E+04			

Appendix 12. Analysis of variance (ANOVA)

Variate: Soil Organic Carbon in dry season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AMF	1	1.12E+02	1.12E+02	12362.07	<.001
BIOCHAR	1	5.25E+04	5.25E+04	5.81E+06	<.001
NITROGEN	2	2.92E+02	1.46E+02	16137.01	<.001
AMF.BIOCHAR	1	7.98E-03	7.98E-03	0.88	0.349
AMF.NITROGEN	2	2.36E-03	1.18E-03	0.13	0.878
BIOCHAR.NITROGEN	2	7.78E-04	3.89E-04	0.04	0.958
AMF.BIOCHAR.NITROGEN	2	3.78E-03	1.89E-03	0.21	0.811
Residual	130	1.18E+00	9.04E-03		
Total	143	5.29E+04			

Table 13. Analysis of variance (ANOVA)

Variate: Cation Exchange Capacity (CEC) in Rain season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.421	0.7105	1.79	
AMF	1	18.9513	18.9513	47.64	<.001
BIOCHAR	1	42724.81	42724.81	1.07E+05	<.001
NITROGEN	2	5.3198	2.6599	6.69	0.002
AMF.BIOCHAR	1	0.0034	0.0034	0.01	0.927
AMF.NITROGEN	2	0.0604	0.0302	0.08	0.927
BIOCHAR.NITROGEN	2	0.102	0.051	0.13	0.88
AMF.BIOCHAR.NITROGEN	2	0.0604	0.0302	0.08	0.927
Residual	130	51.7132	0.3978		
Total	143	42802.44			

Appendix 14. Analysis of variance (ANOVA)

Variate: Cation Exchange Capacity in Dry season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.042	0.521	1.79	
AMF	1	13.8975	13.8975	47.64	<.001
BIOCHAR	1	31331.17	31331.17	1.07E+05	<.001
NITROGEN	2	3.9011	1.9506	6.69	0.002
AMF.BIOCHAR	1	0.0025	0.0025	0.01	0.927
AMF.NITROGEN	2	0.0443	0.0222	0.08	0.927
BIOCHAR.NITROGEN	2	0.0748	0.0374	0.13	0.88
AMF.BIOCHAR.NITROGEN	2	0.0443	0.0222	0.08	0.927
Residual	130	37.9226	0.2917		
Total	143	31388.1			

Appendix 15. Analysis of variance (ANOVA)

Variate: Root Dry Biomass in Rain season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.87865	3.93932	43.16	
AMF	1	47.17964	47.17964	516.94	<.001
BIOCHAR	1	21.4417	21.4417	234.93	<.001
NITROGEN	2	14.65899	7.32949	80.31	<.001
AMF.BIOCHAR	1	0.02374	0.02374	0.26	0.615
AMF.NITROGEN	2	0.00095	0.00048	0.01	0.995
BIOCHAR.NITROGEN	2	0.0024	0.0012	0.01	0.987
AMF.BIOCHAR.NITROGEN	2	0.12602	0.06301	0.69	0.512
Residual	22	2.00786	0.09127		
Total	35	93.31995			

Appendix 16. Analysis of variance (ANOVA)

Variate: Root Dry Biomass in dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5.25184	2.62592	43.16	
AMF	1	31.44952	31.44952	516.94	<.001
BIOCHAR	1	14.29284	14.29284	234.93	<.001
NITROGEN	2	9.77155	4.88577	80.31	<.001
AMF.BIOCHAR	1	0.01582	0.01582	0.26	0.615
AMF.NITROGEN	2	0.00064	0.00032	0.01	0.995
BIOCHAR.NITROGEN	2	0.0016	0.0008	0.01	0.987
AMF.BIOCHAR.NITROGEN	2	0.08401	0.042	0.69	0.512
Residual	22	1.33842	0.06084		
Total	35	62.20623			

Appendix 17. Analysis of variance (ANOVA)

Variate: Root colonization in rain season

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	54	27	99	
AMF	1	22500	22500	82500	<.001
BIOCHAR	1	484	484	1774.67	<.001
NITROGEN	2	337.5	168.75	618.75	<.001
AMF.BIOCHAR	1	64	64	234.67	<.001
AMF.NITROGEN	2	37.5	18.75	68.75	<.001
BIOCHAR.NITROGEN	2	0.5	0.25	0.92	0.415
AMF.BIOCHAR.NITROGEN	2	0.5	0.25	0.92	0.415
Residual	22	6	0.2727		
Total	35	23484			

Appendix 18. Analysis of variance (ANOVA)

Variate: Root colonization dry season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	38	19	62.7	
AMF	1	14721.78	14721.78	48581.87	<.001
BIOCHAR	1	312.1111	312.1111	1029.97	<.001
NITROGEN	2	222.1667	111.0833	366.58	<.001
AMF.BIOCHAR	1	32.1111	32.1111	105.97	<.001
AMF.NITROGEN	2	26.0556	13.0278	42.99	<.001
BIOCHAR.NITROGEN	2	0.7222	0.3611	1.19	0.323
AMF.BIOCHAR.NITROGEN	2	0.3889	0.1944	0.64	0.536
Residual	22	6.6667	0.303		

Appendix 19: Analysis of variance (ANOVA)

Variate: Plant height in rain 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			

Appendix 20: 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			

Appendix 21 Analysis of variance (ANOVA)

Variate: Fresh fruit yield in rain season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	5716441	2858221	135.62	
REP.*Units* stratum					
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			

Appendix 22. 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			

Appendix 23. Analysis of variance (ANOVA)

Variate: Non-marketable Yield (g per plant) in rain 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			

Appendix 24. 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			

Appendix 26: Analysis of variance (ANOVA)

Variate: Chlorophyll Content (SPAD Units) in 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			

Appendix 27: MYCOPEP THECNICAL DATA SHEET

PBL

TECHNICAL DATA SHEET

MYCO-PEP C **PRODUCT CODE –BF 005**

PRODUCT PROPERTIES

Active Ingredient: Vesicular Arbuscular Mycorrhizal fungi *Glomus intraradices*

Finished Product – Bentonite based granular formulation of VAM (Mycorrhiza).

Infective Propagules – NLT 70 IP/gm

Functional Uses – Bio-Fertilizer

STD PACKING – 4.00 Kg

PRODUCT BENEFITS

- It improves the root canopy and plant growth.
- It increase the uptake and mobilization of water and nutrients like phosphorus, nitrogen, potassium, iron, manganese, magnesium, copper, zinc, boron, sulphur and molybdenum etc. from soil to root.
- It is useful in overcoming plant stress conditions viz. drought, disease and nutrients deficiency.
- It improves the soil texture by decomposing organic matter present in soil.
- It is useful in all the Agricultural, Floricultural and Horticultural crops.

METHOD OF APPLICATION & DOSAGE

- Mix 1 kg of granular formulation with 25-50 kg of well decomposed FYM and broadcast in one acre field.
- It can be soil drenched along with required volume of water.
- 50 gm of granular formulation may be applied per ditch before transplanting the horticultural crops
- To get better result do not mix with chemical fungicides and agrochemicals for storage and field application.

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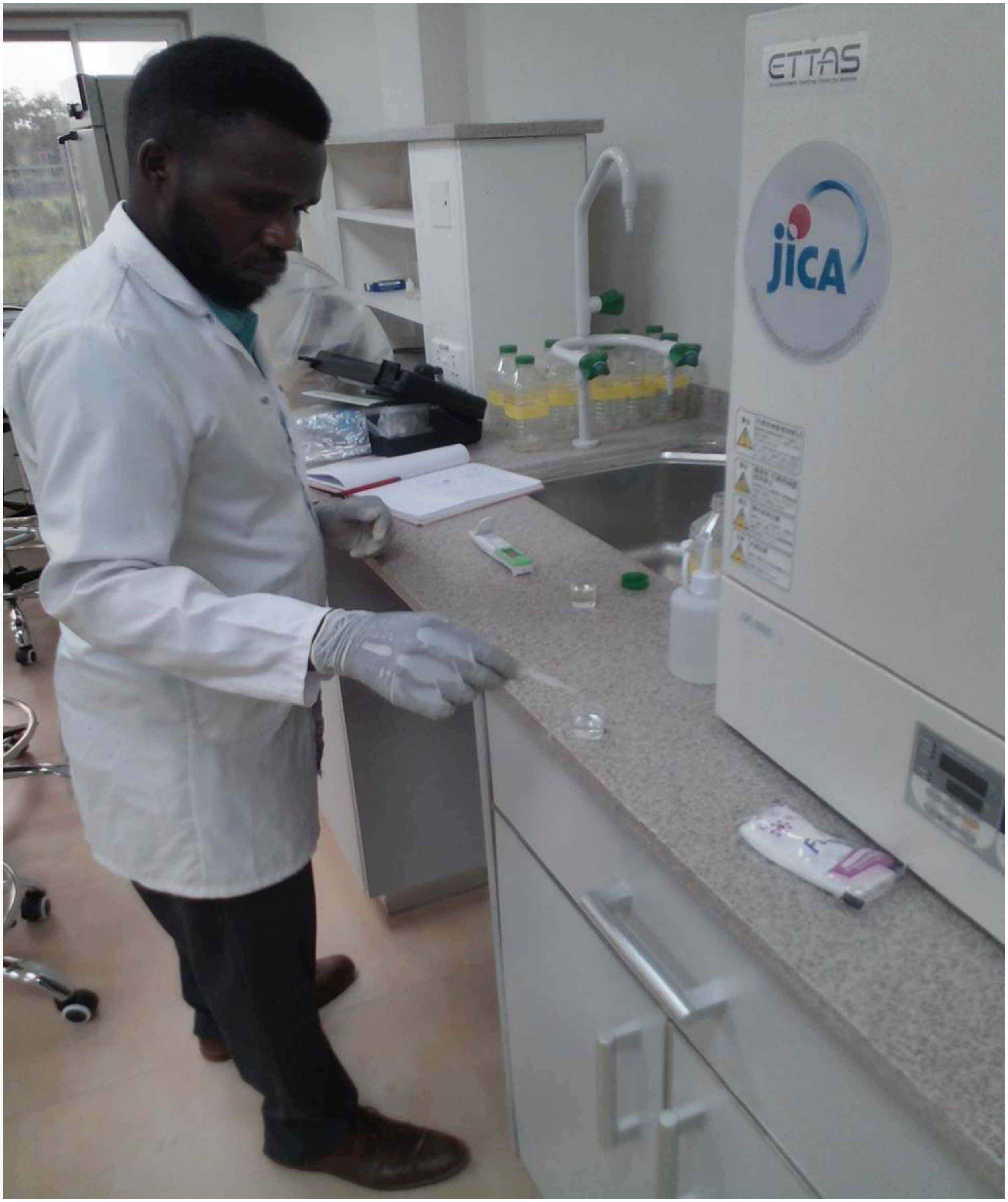
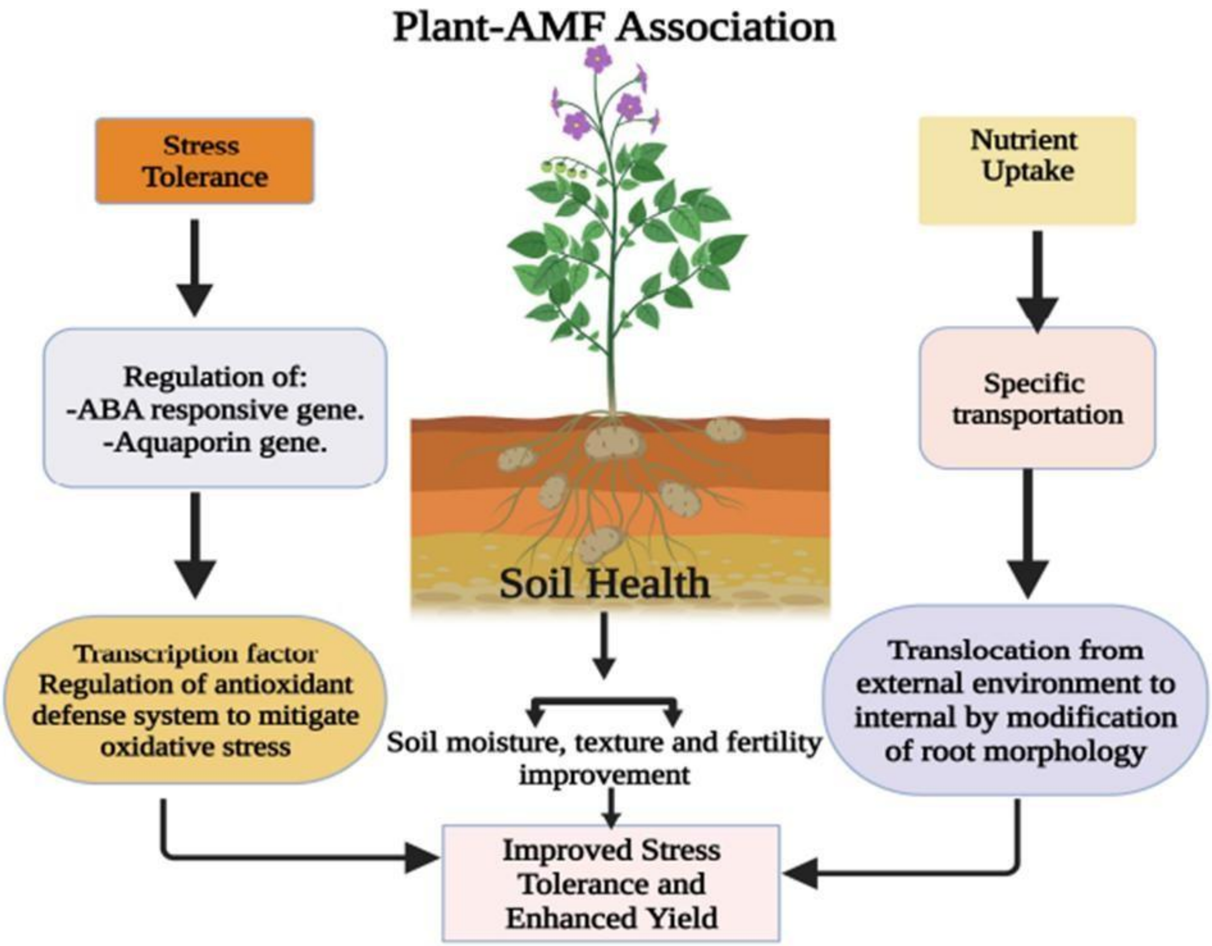
Appendix 28: Measuring Nitrate concentration in leachates with HORIBA LaquaTwin Nitrate



Appendix 29: Benefit of AMF (Wahab eta al., 2023)



Appendix 30: Graphical depiction of the effect of mycorrhizal association on plant and root ecosystem Khaliq et al., 2022).