

BINDURA UNIVERSITY OF SCIENCE EDUCATION

FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

DEPARTMENT OF CROP SCIENCE

Effects of Arbuscular Mycorrhizal Fungal and Rhizobia on plant growth of cowpeas CB2.



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A research project submitted in part fulfillment of the requirements for the Bachelor of
Agricultural Science Honors Degree in Crop Science

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APPROVAL FORM

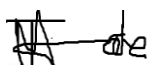
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Effects of Arbuscular Mycorrhizal Fungal and Rhizobia on plant growth cowpeas CB2

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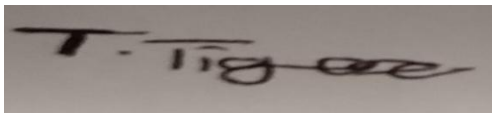
DECLARATION

I declare that this research project is my original work and has not been submitted to any other institution or published. All sources of information used in this study have been acknowledged and referenced. The data presented in this project was collected, analysed and interpreted in accordance with accepted academic and research standards.

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A photograph of a handwritten signature in dark ink on a light-colored surface. The signature appears to be 'T. Tigere' written in a cursive, slightly slanted style.

Date: 26 June 2026

DEDICATION

I want to dedicate this work to my family and friends whose unwavering support, encouragement, prayers and sacrifice have made this achievement possible. Special appreciation goes to my lectures and supervisor for their guidance and valuable contributions during the course of this study. May this work serve as a source of knowledge and inspiration to future researchers and students in the field of Crop Science and plant production.

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I would like to express my sincere gratitude to my supervisor for the guidance and support provided throughout the course of this research. Their expertise and encouragement greatly contributed to the successful completion of this study. I also want to extend my appreciation to the lectures and staff of the Department of Crop Science at Bindura University of Science Education for the knowledge, assistance and resources they provided during my academic journey. Also I would want to thank the Soil Productivity Research Laboratory in Marondera for their guidance and support during the period I did my dissertation especially Mr Mativhunye .

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ABSTRACT

Cowpea (*Vigna unguiculata*) is an important grain legume widely grown in sub Saharan Africa for its nutritional and economic value. However, low soil fertility particularly nitrogen and phosphorus deficiency limits the productivity of the crop. This study evaluated the effects of Arbuscular Mycorrhizal Fungal and Rhizobia on the growth and development of cowpea. The experiment was conducted using eight treatments, growth parameters included plant height, root length and mass, nodule weight and number and accumulated biomass were assessed at different growth stages. The results indicated that inoculated treatments significantly improved cowpea growth compared to control. The study concludes that co-inoculation of AMF and Rhizobia has a significant potential to enhance cowpea growth and could be recommended as an effective biofertilizer strategy for sustainable crop production.

Keywords

Rhizobia and Arbuscular Mycorrhizal Fungal.

TABLE OF CONTENTS

DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENT	v
ABSTRACT.....	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES	ix
LIST OF APPENDICES	Error! Bookmark not defined.
LIST OF ABBREVIATIONS.....	x
CHAPTER ONE	1
1.1 Background of the study	1
1.2 Problem Statement	4
1.3 Justification	5
1.4 Objectives.....	6
1.4.1 Main objectives.....	6
1.4.2 Specific objectives	6
1.5 Hypothesis.....	6
CHAPTER TWO.....	8
LITERATURE REVIEW	8
2.1 Cowpea Origins.....	8
2.2 Cowpea Climatic requirements	9
2.3 Cowpea production in Zimbabwe	9
2.4 Biofertilizers.....	11
CHAPTER THREE	14
METHODOLOGY AND EXPERIMENTAL DESIGN	14
3.1 Site Description	14
3.2 Experimental Design.....	14
Table 3.1 Experimental Design Summary	15
3.3 Isolation and Quantification of mycorrhizal	15
3.4 Agricultural Practices.....	16
3.4.1 PH Testing	16
3.4.2 Germination Test	16

3.4.4 Management	17
3.4.5 Data Collection	18
3.4.6 Data Analysis.....	19
CHAPTER 4	20
RESULTS	20
4.1 effects of AMF and Rhizobia on plant height at 2 weeks interval from 2 weeks up to 8 weeks after planting	20
4.2 effects of AMF and Rhizobia on root length at 8 weeks after planting.	22
4.3 effects of AMF and Rhizobia on root weight	23
4.4 effects of AMF and Rhizobia on nodule number.....	24
4.5 effects of AMF and Rhizobia on nodule weight.....	25
4.6 effects of co-inoculating AMF and Rhizobia on fresh biomass	26
4.7 effects of co-inoculating AMF and Rhizobia on dry biomass	27
CHAPTER 5	28
5.0 Introduction	28
5.1 Effects of AMF and Rhizobia on plant Height	28
5.2 Effects of AMF and Rhizobia on root length.....	29
5.3 Effects of AMF and Rhizobia on root weight.....	30
5.4 Effects of AMF and Rhizobia on Nodulation	31
5.5 Effects of AMF and Rhizobia on Biomass	31
CHAPTER SIX.....	33
6.0 Conclusion.....	33
6.1 Recommendations	33
REFERENCES	34
Appendix.....	42

LIST OF TABLES

Table 4.1: Effects of AMF and Rhizobia on plant height at 2 weeks interval from 2 weeks up to 8 weeks after planting

1.....**Error! Bookmark not defined.**

Table 4.2: Effects of AMF and Rhizobia on root length at 8 weeks after planting

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Fig 4.3 Effects of AMF and Rhizobia on root weight

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Fig 4.4 Effects of AMF and Rhizobia on module number

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Fig 4.5 Effects of AMF and Rhizobia on nodule weight

2.....**Error! Bookmark not defined.**

Fig 4.6: Effects of co-inoculating AMF and Rhizobia on fresh biomass

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Fig 4.7: Effects of co-inoculating AMF and Rhizobia on dry biomass

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LIST OF ABBREVIATIONS

AMF – Arbuscular Mycorrhizal Fungal.

Um- micrometer.

T- treatment.

CV – coefficient of variation

SED-Standard error of difference

LSD -List significant difference

P-Value

CHAPTER ONE

1.1 Background of the study

Cowpeas (*Vigna unguiculata* L. Walp.) is a legume crop that originated in Africa, where they were domesticated from their wild progenitor *V. unguiculata* subsp. This crop was domesticated in regions such as Ethiopia, West Africa, South Africa and Central Africa it was later introduced to other countries like India, Europe and America (Boukar *et al.* 2015). Cowpeas is a warm season legume crop that is mostly grown by smallholder farmers for human consumption, animal feed and soil conservation purposes. Cowpeas grow in a wide range of temperatures from 18 degrees Celsius to 35 degrees Celsius through all their stages but with an optimum of 35 degrees Celsius (Diaz-Urbano *et al.*,2023)The crop adapts to sandy loam soils with a pH of 5.5 to 7.0 cowpeas is also a drought tolerant legume that can be grown in "low fertility conditions due to their ability to fix nitrogen with the soil bacterium Bradyrhizobium" (Barros *et al.*, 2022).

Cowpeas is an annual crop that grows vigorously having a strong taproot system. Cowpeas have erect semi-erect spreading, and prostrate or climbing like growth habits with purpose branching. CBC2 is a cowpea variety that is known for its early maturity, high yield potential, disease resistance and drought tolerance characteristics. This variety is one of the most commonly grown varieties in Zimbabwe. It provides a reliable source of nutrients for the people of Zimbabwe especially as an essential protein supplement. It also plays an important role for smallholder farmers in soil fertility and conservation it increases soil fertility due to biomass residue provided by cowpeas. This also enhances organic matter.

"Cowpea production is constrained by many biotic and abiotic factors including low soil fertility and a wide range of factors such as disease, insects and parasitic weeds"by Ayoola, J.B, Therasa

Adoga and unavailability of improved seeds (Horn *et al.*, 2022). Among these factors low soil fertility is a potential threat to the decline of cowpea production in Zimbabwe. Cowpeas have the ability to fix atmospheric nitrogen into the soil to be ready to use as nitrogen, thereby providing nitrogen to both the soil and the crop thereby increasing soil fertility (Birla *et al.*, 2017). Cowpeas produce nitrogen for the plant as a macronutrient leaving out other important nutrients that are essential to the plant for example phosphorus. In order to improve the yield of cowpeas the use of biofertilizers is of importance. These biofertilizers include Rhizobia and Arbuscular Mycorrhizal Fungi.

Biofertilizers are natural products that contain beneficial living organisms that improve soil fertility and plant growth by increasing the availability of nutrients to crops (Mandal *et al.*, 2025). The microorganisms colonize the rhizosphere of the plant and increase the plant and increase the supply of availability of primary nutrients or growth stimulus to the target crop (Ritika and Utpal, 2014). Biofertilizers have demonstrated significant potential as a sustainable and environmentally friendly source of plant nutrition for example rhizobia. These biofertilizers are widely used to enhance nutrient availability, improve growth and increase yield in a sustainable and cost effective way.

Arbuscular mycorrhizal fungi and Rhizobia are biofertilizers that have different modes of action, (Vasma and Chaudhary 2019) yet both help tend to do the job of enhancing nutrients in the soil and their availability. Arbuscular mycorrhizal fungi (AMF) are soil borne fungi that form a symbiotic relationship with the roots of over 80% of terrestrial plants (Vasma and Chaudhary 2019).

AMF creates structures called arbuscules, which facilitate the exchange of nutrients between fungus and host plant. Through this association AMF has the ability to uptake essential nutrients particularly phosphorus and other mineral elements thereby improving carbon transfer and overall plant vigour (Singh *et al.*, 2024). The colonization of the roots of the plant by AMF may raise the concentration of different macro and micronutrients leading to increasing plant growth in our legume plant cowpeas (Vasma and Chaudhary 2019)).

In order for a plant to have good plant vigour, AMF is not the only biofertilizer that could improve plant growth there is also the presence of rhizobia that improves plant growth. Rhizobia are biological nitrogen fixing bacteria that fix atmospheric nitrogen to a form that can be used by plants (Fahde *et al.*, 2023). In return the plant hosts provide rhizobia with a source of carbon thereby showing a symbiotic relationship. Nitrogen is an essential macronutrient needed for plant growth and development. This nutrient plays a crucial role in plant physiological processes like plant photosynthesis (Ruscitti *et al.*, 2017).

The ability of cowpeas (*Vigna unguiculata* L. Walp.) to fix nitrogen in the soil to a readily available nutrient improves plant growth and reduces the use of inorganic fertilizer, which, if used in excess, will cause an ecological change in the environment (Pathak 2023). The use of biofertilizers AMF and Rhizobia will improve the growth of cowpeas (*Vigna unguiculata* L. Walp.) as these microorganisms have the ability to fix phosphorus and nitrogen in the soil to readily available nutrients. This project aims to show the effect of AMF and Rhizobia on plant growth when inoculated to cowpeas (Mpongwana *et al.*, 2023).

1.2 Problem Statement

Cowpeas is a vital pulse crop for food security and population health around the globe with major nutritional and nutraceutical qualities (Abede K 2023). Cowpeas are an important source of nutrients, providing both macro and micronutrients in the human diet. In Zimbabwe cowpeas is a crop that is mostly grown by small scale farmers as a source of food and income (Chingwara V 2021). This legume plant provides a good ground cover, reducing soil erosion and also "fixing up to 80% nitrogen, controlling weeds and reducing the need for and cost of fertilizer" (Abebe and Alemayehu, 2002). However, the production of cowpeas is affected by poor soil fertility especially low levels of nitrogen and other macronutrients like phosphorus. The use of inorganic fertilizer has negative effects on the environment as it is also expensive for smallholder farmers. There is an urgent need to develop cost effective, sustainable and environmentally friendly agricultural methods to improve cowpea yield. Research has shown that soil microorganisms like AMF and Rhizobia form a symbiotic relationship with legume plants the specific effects of their co-inoculation on cowpeas remain insufficiently understood (Wahab *et al.*, 2023). Existing research offers an incomplete insight on "the dual inoculation of AMF and Rhizobia" (Khater *et al* 2026) in the essence of macronutrients nitrogen and phosphorus, plant physiology and soil health (Razafintsalama *et al.*, 2022). Rhizobia is supplying nitrogen only however, other macronutrients may also be a limiting factor to the optimum yield of cowpeas. This study aims to give an insight into the effects of AMF and Rhizobia inoculation on cowpea growth and development and how the co-interaction of these soil microorganisms can improve the uptake of macro and micronutrients from the soil.

1.3 Justification

Crop production is increasingly being reduced by poor soil fertility the high cost of chemical fertilizer and environmental problems caused by excessive fertilizer use. These challenges are common in smallholder farming systems whose land has low soil nutrient levels which often limit early plant growth and establishment. There is therefore a need for affordable and sustainable methods that can improve plant growth while maintaining soil health (FAO, 2017; Vanlauwe *et al.*, 2019). AMF and Rhizobia are beneficial soil microorganisms that form a symbiotic relationship with plants. Rhizobia fix atmospheric nitrogen and make it readily available to plants through root nodules while AMF improve the uptake of nutrients especially phosphorus by extending the effective root surface area (Singh *et al* 2017). The role played by these microorganisms is important during the early stages of plant growth when nutrient demand is high and root systems are still developing (Ritika and Utpal, 2014). Root biomass are crucial indicators of root development and plant growth. Well developed roots improve the plant capacity to access water and nutrients which supports healthy shoot growth. Previous studies have shown that AMF inoculation can significantly increase root biomass by improving nutrient availability and root efficiency (Çekiç, Ünyayar, and Ortas, 2012). Plant height measured after emergency to full development is a useful indicator of early vegetative growth and successful establishment. Early height of the plant reflects improved nutrient uptake and plant vigor. Rhizobia inoculation has shown to enhance early shoot growth in legumes due to improved nitrogen availability from biological nitrogen fixation. Leaf broadness is closely related to leaf area and photosynthetic capacity. Broader leaves allow greater light interception, leading to increased photosynthesis and biomass production. Increased photosynthesis is a result of the availability of nitrogen in the soil to the plant. Although AMF have been promoted to promote leaf expansion by improving plant nutrient status and water use efficiency (Miransari, 2011). This study is justified as it aims to evaluate the effects of AMF and Rhizobia inoculation both

individually and in combination, on early plant growth parameters such as root mass, plant height, nodule weight and number of nodules and plant biomass. The findings of this project will contribute to knowledge on sustainable soil fertility management and support the use of microorganisms that are beneficial to the environment and in crop production (Khater *et al.*,2026).

1.4 Objectives

1.4.1 Main objectives

To examine the effects of arbuscular mycorrhizal fungi and rhizobia inoculation on plant growth.

1.4.2 Specific objectives

- To evaluate the effectiveness of co-inoculating AMF and Rhizobia on plant biomass.
- To assess the effectiveness of AMF and Rhizobia on plant growth.
- To evaluate the effectiveness of AMF and Rhizobia co-inoculation on nodulation.

1.5 Hypothesis

H0: Co-inoculation with AMF and Rhizobia singularly or in combination has no effect on plant biomass.

H1: Co-inoculation with AMF and Rhizobia singularly or dual significantly increases plant biomass,

H0: Co-inoculation with AMF and Rhizobia singularly or dual has no effect on plant growth.

H1: Co-inoculation with AMF and Rhizobia singularly or in combination has an effect on plant growth.

H0: Co-inoculation with AMF and Rhizobia singularly or dual has no effect on nodulation.

H1: Co-inoculation with AMF and Rhizobia singularly or dual has a great effect on nodulation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cowpea Origins

Cowpeas (*Vigna unguiculata* L. Walp.) is a type of bean plant that belongs to the Fabaceae family (Prasad Dixit *et al.*, 2025). The cowpea came from Africa where people first started to grow it thousands of years ago. The cowpea is a type of legume did not just appear as a crop overnight. It grew slowly from wild hybrids that worked well in the Savannah region of Africa. The early farmers who lived in these areas made it what it is today. Farmers in this area started planting these wild plants once they learned that they could survive the severe temperatures, bad soils and drought that are common in many parts of Africa. People finally picked the seeds that do the best which are the ones with bigger seeds, better flavours and more reliable yields. This gradual process of choosing plants from around the world changed wild cowpea into the crop we know today.

Cowpeas came from West Africa and spread across the continent through migration and trade. People brought cowpea seeds with them when they moved which enabled the crop adapt to many types of farming and living. Later cowpea spread from Africa to other parts of the world. It was taken to Asia especially India where it became an important pulse crop. The crop also made its way to the Americas most likely via the trans Atlantic slave trade. There it became part of the indigenous farming system and diets. Cowpea is now grown in many tropical and subtropical areas all over the world. But it is still closely linked to Africa, both as its place of origin and as a crop that has changed throughout time as farmers have had to adapt it to harsh conditions (Boukar *et al.* 2015).

2.2 Cowpea Climatic requirements

Cowpea is a warm season crop that performs best in hot conditions. The crop requires an optimal temperature between 25 and 35 degrees Celsius. It is very sensitive to frost and low temperatures. The crop requires a moderate rainfall of 400 to 800 mm therefore making it highly drought tolerant crop suitable for semi arid areas. Cowpea needs plenty of sunlight for optimal flowering and pod formation. The crop prefers well-drained sandy loam to loamy soils it can tolerate poor soils better than many crops. The crop does not do well in waterlogged or heavy clay soils, as they reduce growth and nodulation. It requires a pH of 5.5 to 7.0 cowpea crops can tolerate slightly acidic soils to neutral soils.

Very acidic soils may limit nutrient availability and nitrogen fixation. In order for cowpea to fully establish it requires adequate moisture during germination and flowering availability of too much water causes root rot disease and insufficient water affects flowering and pod set. Cowpea fixes its nitrogen through Rhizobium bacteria. It also requires the application of phosphorus and small amounts of potassium and micronutrients. Cowpeas are sensitive to weed competition especially in early stages they also need to be managed carefully for pest and diseases for examples pest like aphids and pod borers (Barros *et al.*, 2022).

2.3 Cowpea production in Zimbabwe

Cowpea is a key grain legume in Zimbabwe, especially within the smallholder farming system. It assists a lot with food security, nutrition and soil fertility and it grows best in the country semi arid areas where it is mostly grown with rain. In drier areas like Bindura, it is often planted as an intercrop with maize or sorghum or as a single crop. Matove and Gasura (2018) state that the crop demands low input conditions which means it doesn't need much fertilizer or water.

Cowpeas is a very important for food security especially in sub Saharan Africa. It is often used as feed, grain and vegetables which is good since it is a reliable source of food in a region that is prone to drought. It is a dependable crop for rural families since it may grow well even when it does not rain much. Cowpeas are a great source of both macronutrients and micronutrients and they are also very high in protein. People in Zimbabwe can keep their standard of living because cowpeas are grown and sold in local and regional markets. This crop also creates jobs in growing, processing and marketing.

Lastly, cowpea production improves soil fertility and health through biological nitrogen fixation, reducing the need for expensive fertilizer often used. It acts as a cover crop and component in a crop rotation system (Kim *et al.*, 2025). Cowpea (*Vigna unguiculata*) is still a very useful grain legume especially in sub Sahara Africa, where it can grow well in adverse agro ecological conditions like drought and poor soils. The literature consistently highlights its dual role in enhancing food and nutritional security while contributing to sustainable agricultural system through biological nitrogen fixation and soil improvement. Its rich protein content, along with essential vitamins and minerals makes it a important dietary component for rural and urban populations alike

Cowpea can naturally fix nitrogen from the air by interacting with a rhizobium though several studies show that his process is frequently hindered by undesirable native microbial population and soil conditions. By applying biofertilizers such efficient rhizobia inoculants, arbuscular mycorrhizal fungi and other microorganisms that help nodulation, nutrient availability and the overall performance of crops. In comparison to inorganic fertilizers, biological inputs provide a more sustainable, affordable and eco friendly option particularly for small holder farmers. In order to maximize soil health and support long term agricultural sustainability it is essential to

promote the use of biofertilizers in conjunction with appropriate soil and crop management techniques.

The literature generally demonstrates how crucial it is to enhance market access, research and extension assistance in order to fully benefit from the advantages of cowpea farming. Promoting its broader production and utilization can enhance the resilience of small holder farming system while significantly contributing to food security, nutrition and sustainable agricultural development.

2.4 Biofertilizers

Biofertilizer is a type of fertilizer that contain living microbes in them. When this fertilizer is applied on seeds, plants, or soil they help the host plant grow by giving it more primary nutrients. Biofertilizer are a better choice for the environment and also for long term use compared to chemical fertilizer as they improve soil health.

Arbuscular mycorrhizal fungi are microorganisms that live in the soil and work together within roots of most crops (Nicholas Mawira Gitonga 2012). AMF enhances plant access to soil nutrients especially phosphorus by extending roots systems within fungal hyphae (Smith, 2009). Phosphorus is an important part of the crop biogenetic of living cells.

It is necessary for the growth of new tissue and the movement of genetic information within the plant. Many studies have shown that inoculation with AMF enhances root development by increasing both root mass and weight (Mapunda *et al.*,2025). A well developed system improves the plant ability to absorb water and nutrients leading to improved shoot and plant health (ÇekiÇ, Ünyayar, and Ortas 2012). AMF additionally helps plants deal with the environmental stressors like drought by helping them take in water more effectively and making soil structures better. AMF has been shown to influence both above and belowground parameter including plant

height and plant biomass. Increased phosphorus availability through AMF symbiosis supports cell division and elongation leading to an improved early plant height.

AMF has been proven to affect both above and below ground factors, such as the height of plants and the growth of their leaves. AMF symbiosis increases the availability of phosphorus which helps cells divide and grow longer which leads to a better early plant. The relationship AMF have with plant roots allows for the plant to benefit from vital nutrients and mineral salts this leads to the plant developing broader leaves due to increased photosynthetic capacity and biomass accumulation (Shrivastava, Mahajan, and Varma, 2021).

Rhizobia are gram-negative bacteria that exist in a way that helps either these bacteria and leguminous plants. They dwell in root nodules and change nitrogen from the air into ammonia that plants may use. This method of fixing nitrogen is very crucial for helping plants grow especially in soils that do not have enough nitrogen (Vance, 2001). It has been shown that rhizobia inoculation can help legumes grow in several ways such as by making their roots grow making more biomass and making them taller. Better nitrogen availability encourages vegetative growth and helps plants get established early. Researchers have found that plants inoculated with efficient Rhizobia strains have taller shoots and bigger leaves than plants that are not inoculated (Heinrich *et al.*, 2021). Nitrogen is particularly crucial for the growth of leaves. Enough nitrogen helps leaves grow and make chlorophyll, which makes photosynthesis happen more. Plants that have been inoculated with rhizobia tend to have broader leaves and more biomass, which helps them grow better overall.

AMF and Rhizobia have different roles in promoting plant growth studies have examined their combined effects (Parihar *et al.*, 2011). Co-inoculation of Rhizobia and AMF has been reported to produce synergistic effects particularly in leguminous crops. The phosphorus and other nutrients scavenged by AMF are essential for the energy-intensive process of nitrogen fixation

carried out by Rhizobium essentially AMF helps fuel the Rhizobium (Hu *et al.*,2026). AMF helps plants take in more phosphorus which is important for transferring energy during nitrogen fixation. Rhizobia, on the other hand provide nitrogen that plants need to flourish (Xavier and Germid, 2003). Studies show that simultaneous inoculation can make plants taller have more roots and have better leaf growth than single inoculation. (Mortimer, Pérez-Fernández, and Valentine 2008) show that plants receiving both AMF and Rhizobia showed superior early growth due to improved nutrient balance and enhanced physiological processes (Kumar *et al.*,2022).

The effectiveness of co-inoculation varies based on soil properties, plant species and the microbial strains utilized. Some studies have showed different results which shows how important it is to do site specific studies to find the best ways to inoculate (Vanlauwe *et al.*, 2015). Although the positive effects of AMF and Rhizobia are well documented there is limited research on their combined influence on early plant growth parameters such as root mass, plant height at two weeks after emergency, and nodulation weight under local conditions. This knowledge gap highlights the need to further study information that can support sustainable crop production.

CHAPTER THREE

METHODOLOGY AND EXPERIMENTAL DESIGN

3.1 Site Description

The research project was conducted at the Soil Productivity Laboratory in Marondera during the 2025 to 2026 cropping season. This site lies in region 2 of Zimbabwe's agricultural ecological zones, this region is characterized by coarse sandy soil, sandy clay loams and clays with an annual rainfall of 750–1000 mm/year and a mean annual temperature of 16 to 19 degrees. All these characteristics make the sites a suitable place to grow CB2 cowpeas.

3.2 Experimental Design

The trial was laid out in a completely randomized design (CRD), with eight treatments replicated three times. Twenty four pots will be used for this practical experiment. The treatments consist of arbuscular mycorrhizal fungi inoculation, rhizobium inoculation, no control treatment and rhizobia plus AMF as follows:

- T1: zero control (no AMF, no rhizobium or inorganic fertilizer)
- T2: Rhizobium inoculation
- T3: AMF inoculation
- T4: AMF+Rhizobium Inoculation
- T5: Rhizobia+ Compound D
- T6: AMF+ Compound D
- T7: Compound D

- T8: AMF+ Rhizobia+ Compound

Table 3.1 Experimental Design Summary

Design factor	Description
Experimental design	Completely randomized design
Crop	<i>Vulgaris unguiculata</i>
Variety	CB2
Number of treatments	8
Number of replicates	3
Total of plots	24
Experimental unit	Greenhouse
Blocking factor	None

3.3 Isolation and Quantification of mycorrhizal

Soil was collected from the rhizosphere zone of about zero to twenty cm depth of two different trees, one a *Eucalyptus* tree and another soil sample from a *Brachystegia spiciformis* tree with each soil sample weighing 500 g. The soils were air dried separately from each other and plant debris was removed. 250 g of soil was weighed and mixed with 1 litre of water and stirred thoroughly 15 times. The heavy particles were allowed to settle for 30-60 seconds. The solution was decanted through a series of sieves of 500 μm , 250 μm , 100 μm , and 45 μm ; a 45 μm sieve was used instead of a 50 μm sieve. Residue was retained on the smallest sieve by rinsing with clean water to remove soil particles. Sieve residue was collected and transferred in a test tube. The test tube was centrifuged with water at 2000 rpm for 5 minutes. The floating debris with supernatant was discarded and in a 50-60% sucrose solution, the supernatant was decanted through a 45 μm sieve and rinsed with distilled water thoroughly. The spores collected were put into a petri dish using a pipette. The spores collected were put under a microscope for identification based on size, shape and presence of subhyphae. The spores collected from each different trees were kept in different test tube, from the observation made on the microscope the

soil that was taken from the *Eucalyptus* tree had more spores compared to the soil taken from the *Brachystegia spiciformis*

3.4 Agricultural Practices

3.4.1 PH Testing

Soil used for growing cowpeas was collected from the fields at the station and tested for pH. The field at which the soil was collected from was firstly divided into uniform sections and from each section samples of soils were collected for pH testing. A soil auger was used to dig soil from a depth of 0-20cm which is the rooting zone for cowpeas. The soil was collected using the random sampling method, the soil collected from each section was put in different bags in order for us to see which part of the field had the most definite pH for cowpeas. Soil from anthills was avoided in order to reduce biased results. Collected soil samples from the field were air dry and stone debris and roots were removed. The soil was sieved to obtain fine particles, 15 grams of soil was measured and put into a beaker and 35 ml of distilled water was used to make a mixture. The mixture was stirred thoroughly to form a suspension, the mixture was left to settle for 15-30 minutes. The pH meter was calibrated using a standard buffer solution, the electrode was calibrated using distilled water and wiped gently with tissue paper. After calibration the electrode was insert into the soil suspension and readings for each sample were taken. The field had a pH range from 5.2 to 7. 0 making it suitable for growing cowpeas.

3.4.2 Germination Test

CB2 cowpeas seeds were tested for germination first before planting the seed. 100 seeds were used to test for germination. Cotton wool was placed in a petri dish as medium, water was used

to make the cotton wool dump. The seeds were evenly placed on the moist medium and were placed in the incubator with necessary environmental conditions required for germination. The seeds were checked daily for 7 days and water was added when necessary to maintain moisture throughout the testing period. Seed germination was recorded on the 8th day, seeds with emerged radical were counted and recorded. 100 seeds were used to test for germination and only 87 of them germinated giving a germination percentage of 87%. The percentage of seed germination was determined by the formula,

Germination % = sprouted seeds in petri dishes $\times$ 100% divided by the total number of seeds.

3.4.3 Pot Preparation

Firstly uniform pots were selected for planting, the pots were soaked in a 10% bleach solution for 30 minutes and rinsed three to four times with distilled water. The growing media which was the soil was sterilized in an autoclave for 30 minutes at 121 degrees Celsius. Each pot was consistently filled with the same amount of soil and clearly labelled with its identifier. After pot filling the pots were watered with distilled water and two seed were sowed in each pot. Each pot has its own treatment and the treatment were replicated three times giving a sum of twenty four pots. A standard nutrient solution with less phosphorus and nitrogen was applied for this was crucial for arbuscular mycorrhizal fungi and Rhizobia colonization.

3.4.4 Management

The plants were watered everyday the first 2 weeks to help the seeds emerge. After the vegetative stage the plants were now watered thrice a week. After 2 weeks compound D was applied at certain treatments which include treatment 5, treatment 6, treatment 7 and treatment 8. Hand

weeding was used as a physical method of removing weeds from the pots. The plants were checked for pest infestation from time to time. After 8 weeks the plants were uprooted for root measurements and nodule counting and weighing.

3.4.5 Data Collection

Plant height data was collected every two weeks from planting until eight weeks of uprooting the plant the data was collected using a string and ruler. The plants were not straight during measurement so a string was first used to follow the stem from the base of the plant to the tip of the tallest leaf. The length of the string was then measured using a ruler calibrated in centimeters. The height of two plants were recorded separately and the average plant height was calculated using the formula $\text{Average} = \text{plant height 1} + \text{plant height 2} \text{ divided by } 2$, two is the number of plants grown in the pot. Root length data was collected at the end of the experiment that was at 8 weeks. The roots were uprooted carefully from the pot and soil was washed of carefully without damaging the roots. A string was first used to measure the roots then the length of the string was measured using a ruler then calibrated to centimeters from the end of the stem to the tip of the longest root. The average root length for each pot was measured using the formula $\text{Average} = \text{root length of plant 1} + \text{root length of plant 2} \text{ divided by } 2$.

Nodule number and weight were collected as part of data to be analysed. The nodules were detached carefully from the roots and were counted manually. Each pot had two plants, nodules from both plants were counted separately and recorded and then average number of nodule were calculated using the formula $\text{Average} = \text{nodules on plant 1} + \text{nodules on plant 2} \text{ divided by } 2$. The nodules from the same pot were then mixed together after physical counting for weighing the mass of nodules in grams.

Fresh and dry biomass data was collected at the end of the experiment to determine the growth performance of the plants. Fresh biomass was determined immediately after harvesting the plants. Plants from the same pots were weighed on a digital balance at once to determine their biomass, after fresh biomass was taken the plants were oven dried at 65 degrees Celsius to 70 degrees Celsius. This was done to remove moisture from the plant tissue after oven drying the plants, the dried samples were then weighed using a digital balance and the dry weights were recorded in grams.

3.4.6 Data Analysis

Data collected was entered on Microsoft excel in a spreadsheet manner. The experiment was analysed using the completely randomized design before analysis of data, the data from each treatment and replicate were entered into the GenStat 18th edition in a spreadsheet format. The collected data was analysed using Analysis of variance by the GenStat statistical software. ANOVA was used to determine a significant differences among the treatments for the measured parameters such as plant height, root length, nodules number, nodule weight and fresh and dry biomass.

ANOVA separated the total variation observed in the experiment into variation due to treatments and variation due to experimental error, the treatment means were compared at a 5% level of significance . The significant differences were detected among treatments, and mean separation was performed using the Least Significant Difference test at the 5% probability level.

CHAPTER 4

RESULTS

4.1 effects of AMF and Rhizobia on plant height at 2 weeks interval from 2 weeks up to 8 weeks after planting

Plant height was measured at two weeks from planting up to eight weeks after planting. Table 4.1 presents the mean plant height of all eight treatments across the four measurements periods.

Table 4.1: Effects of AMF and Rhizobia on plant height at 2 weeks interval from 2 weeks upto 8 weeks after planting

Treatment	Week 2	Week 4	Week 6	Week 8
T1	5.33 ^{bc}	15.42 ^c	20.82 ^c	23.58 ^e
T2	5.97 ^{abc}	18.38 ^{abc}	24.83 ^{ab}	32.98 ^b
T3	5.83 ^{abc}	16.92 ^{bc}	20.9 ^c	27.48 ^d
T4	5.6 ^{abc}	19.25 ^{abc}	24.9 ^{ab}	29.27 ^{cd}
T5	6.32 ^{ab}	19.73 ^{ab}	26.65 ^a	35.07 ^a
T6	6.47 ^{ab}	20.17 ^{ab}	23.92 ^b	29.57 ^c
T7	6.98 ^a	20.95 ^a	23.53 ^b	27.87 ^d
T8	5.25 ^c	17.7 ^{abc}	25.72 ^{ab}	35.23 ^a
Grand mean	5.97	18.56	23.91	30.13
P-value	0.025	0.012	0.001	0.001
SED	0.456	1.278	1.14	1.003
LSD	0.979	2.742	2.446	2.151
CV%	9.4	8.4	5.8	4.1

At 2 weeks, plant height ranged from 5.25cm to 6.98cm with T8 having the least plant height mean and T7 having the highest plant height mean. Analysis of variance revealed significant differences among treatments ($p=0.025$). The zero control treatment (T1) recorded a mean height of 5.33 cm, which was significantly lower than T7 (compound D only) and T6 (AMF plus compound D) but was statistically similar to T2, T3, T4, T5 and T8.

At week 4, plant height ranged from 15.42cm to 20.95cm with T1 having the lowest plant height mean and T7 having the highest plant height mean. The significant difference among treatments was $p=0.012$. The zero control treatment produced the shortest plants while T7 recorded the tallest plants at this growth stage. Treatments that received compound D (T5, T6, T7) generally showed greater plant heights compared to treatments without inorganic fertilizer.

At week 6, there was a highly significant differences among treatments $p=0.001$. Plant height ranged from 20.82cm to 26.65cm with T1 having the lowest plant height mean and T5(Rhizobia plus Compound D) having the highest plant height mean. T5 produced the tallest plants followed, by T8 (AMF plus Rhizobia plus Compound D) at 25.72cm. The control treatment consistently showed the lowest plant height. The coefficient of variation decreased to 5.8% indicating reduced variability among replicates as the experiment progressed.

At week 8, a very highly significant difference was recorded among treatments $p<0.001$. Plant height ranged from 23.58cm (T1) to 35.23cm (T8). T8 (AMF plus Rhizobia plus Compound D) produced the tallest plants closely followed by T5(Rhizobia plus Compound D) at 35.07cm. T2 (Rhizobia alone) produced plants of 32.98 cm, while T3 (AMF alone) produced plants with an average mean height of 27.48 cm but than treatments receiving both biofertilizer and inorganic fertilizer. The zero control treatment consistently produced the shortest plants throughout the experimental period. The low CV% of 4.1at 8 weeks indicates high precision of the experimental date.

4.2 Effects of AMF and Rhizobia on root length at 8 weeks after planting.

Root length was measured at end of the experiment after 8 weeks. Table 4.2 presents the mean root length for all treatments.

Table 4.2: Effects of AMF and Rhizobia on root length at 8 weeks after planting

Treatment	Root length (cm)
T1	17.67 ^a
T2	20.18 ^a
T3	20.8 ^a
T4	19.15 ^a
T5	19.35 ^a
T6	20.27 ^a
T7	18.98 ^a
T8	22.13 ^a
Grand mean	19.82
P-value	0.233
SED	1.532
LSD	3.286
CV%	9.5

Root length was measured at 8 weeks after termination of the experiment. Root length ranged from 17.67 cm to 22.13cm . T1 had the shortest root length of 17.67cm while T8 had the longest root length of 22.13cm, although T1(zero control) recorded the shortest plant roots and T8 (AMF plus Rhizobia plus Compound D) recorded the longest plant roots analysis of variance showed no significant differences among treatments $p=0.233$. The CV% of 9.5 indicates moderate variability in root length measurements. The lack of significant difference suggests

that neither biofertilizer inoculation or inorganic fertilizer application had a statistically detectable effect on root elongation under the conditions of this experiment.

4.3 Effects of AMF and Rhizobia on root weight

Analysis of variance revealed highly differences n root weight among treatments ($p < 0.001$).

Figure 4.1 illustrates the variation in root weight across treatments.

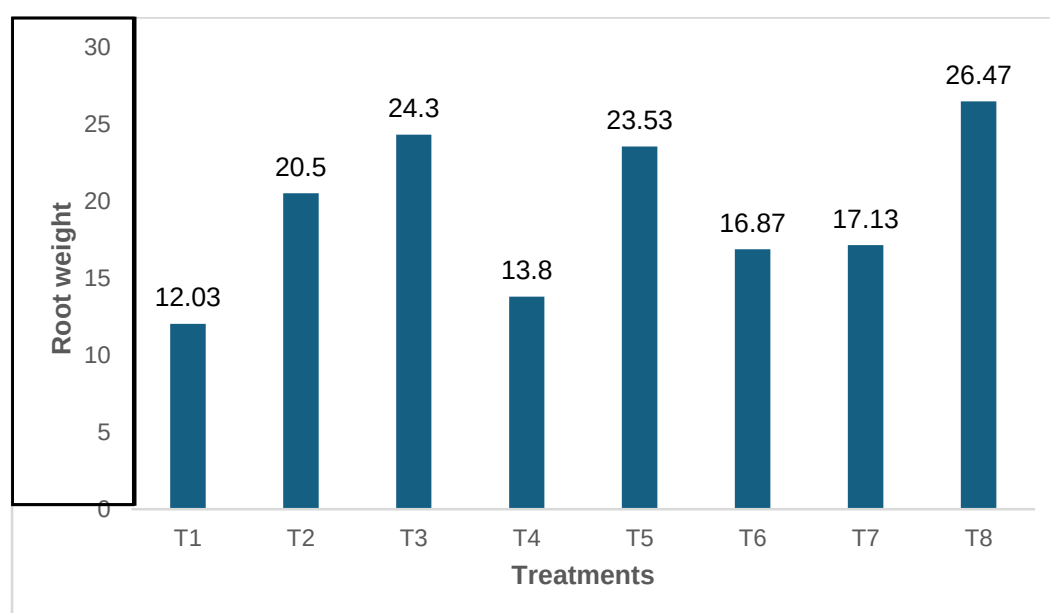


Figure 4.1: Effects of AMF and Rhizobia on root weight

The zero control treatment (T1) recorded the lowest root weight, while treatments receiving AMF (T3, T4, T6, T8) generally showed increased root weight. The highest root weights were observed at T8 (AMF plus Rhizobia plus Compound D), followed by T3 (AMF alone) and T6 (AMF plus Compound D) . The significant differences among treatments ($p < 0.001$, Appendix 6) indicates that AMF inoculation, either alone or in combination with Rhizobia and inorganic, positively influenced root biomass accumulation compared to the zero control.

4.4 effects of AMF and Rhizobia on nodule number

Nodule number was significantly influenced by the treatment $p=0.004$. Table 4.3 presents the mean number of nodules per plant for each treatments.

Table 4.3: Effects of AMF and Rhizobia on nodule number

Treatment	Nodule number
T1	11.2 ^d
T2	28.8 ^b
T3	24.3 ^{bc}
T4	28.2 ^b
T5	39.2 ^a
T6	22.5 ^{bc}
T7	17 ^{cd}
T8	41 ^a
Grand mean	26.5
P-value	0.004
SED	6.23
LSD	13.36
CV%	28.8

Nodule number ranged from 11.2 (T1) to 41.0 (T8). The zero control treatment produced the fewest nodules, while T8 (AMF plus Rhizobia plus Compound D) produced the highest number of nodules. T5 (Rhizobia plus Compound D) also recorded a high nodule count of 39.2.

Treatments with rhizobia inoculation (T2, T4, T5, T8) generally showed a higher nodule number compared to treatments without rhizobia. The CV% of 28.8% indicated that a relatively high variability in nodule count among replicates, which is common for nodulation data due to the biological nature of the symbiotic process.

4.5 Effects of AMF and Rhizobia on nodule weight

Nodule weight showed significant differences among treatments $p=0.007$. Figure 4.2 presents the mean nodule weight for each treatment.

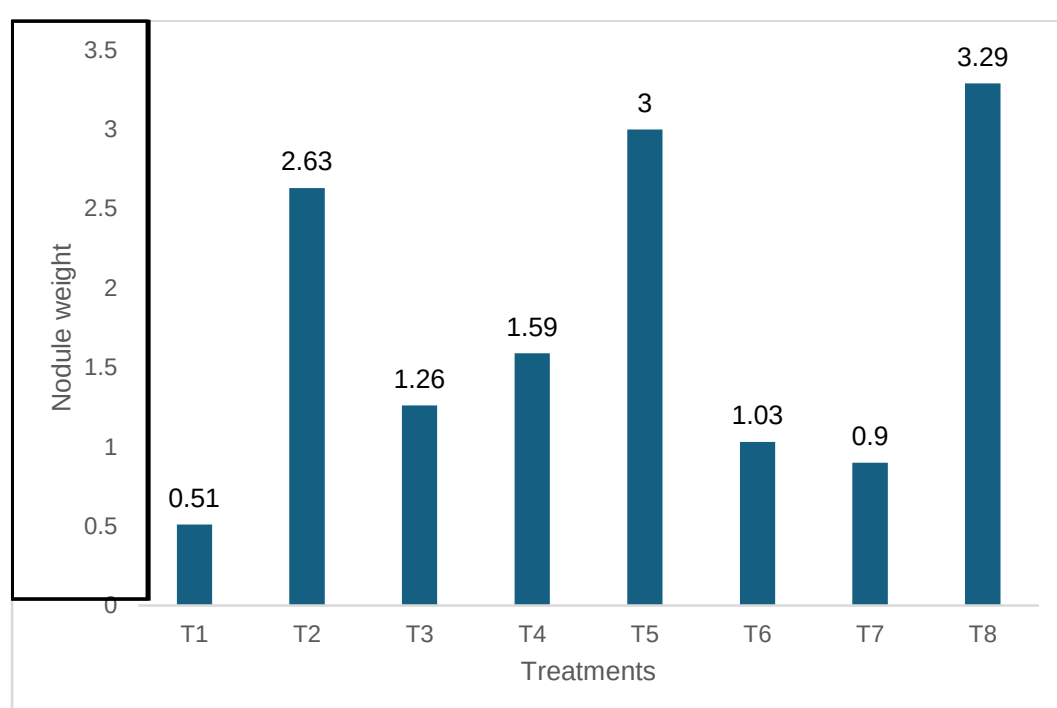


Fig 4.2: Effects of AMF and Rhizobia on nodule weight.

Nodule weight followed a similar pattern to nodule number. The zero control treatment recorded the lowest nodule weight, while treatments containing rhizobia inoculation (T2, T4, T5, T8) produced higher nodule weights. T8 (AMF plus Rhizobia plus Compound D) and T5 (Rhizobia plus Compound D) recorded the highest nodule weights. The significant difference ($p=0.007$, Appendix 8) indicate that rhizobia inoculation effectively enhanced nodule development and

biomass, while AMF co-inoculation further improved nodule wrightin some treatment combinations.

4.6 effects of co-inoculating AMF and Rhizobia on fresh biomass

Fresh biomass was determined immediately after harvest at 8 weeks after planting. Highly significant difference were observed among treatments ($p < 0.001$). Figure 4.3 illustrates the fresh biomass for all treatments.

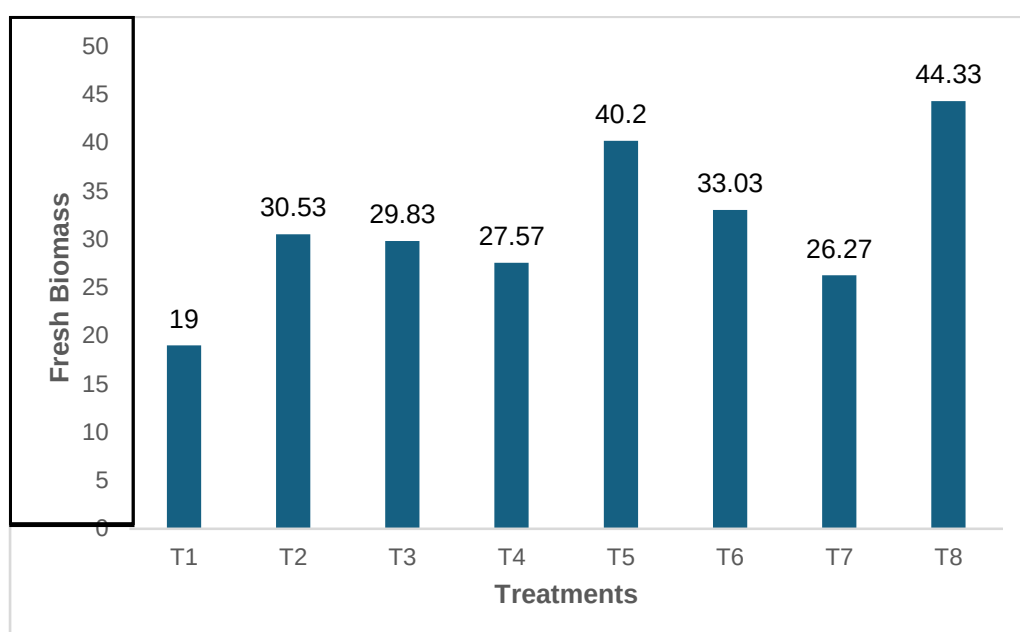


Fig 4.3: Effects of co-inoculating AMF and Rhizobia on fresh biomass.

Fresh biomass ranged from the lowest to the highest treatment, T5 (Rhizobia plus Compound D) and T8 (AMF plus Compound D plus Rhizobia). Treatments receiving both biofertilizer and inorganic fertilizer (T5, T8) produced substantially greater fresh biomass compared to the no control and single inoculated treatments. The very low CV%, Appendix 9 indicates high precision of the experimental data.

4.7 effects of co-inoculating AMF and Rhizobia on dry biomass

Dry biomass was measured after the plant samples were oven dried at 65-70 degrees Celsius, this showed a highly significant difference among treatments ($p < 0.001$). Figure 4.4 presents the dry biomass for all treatments.

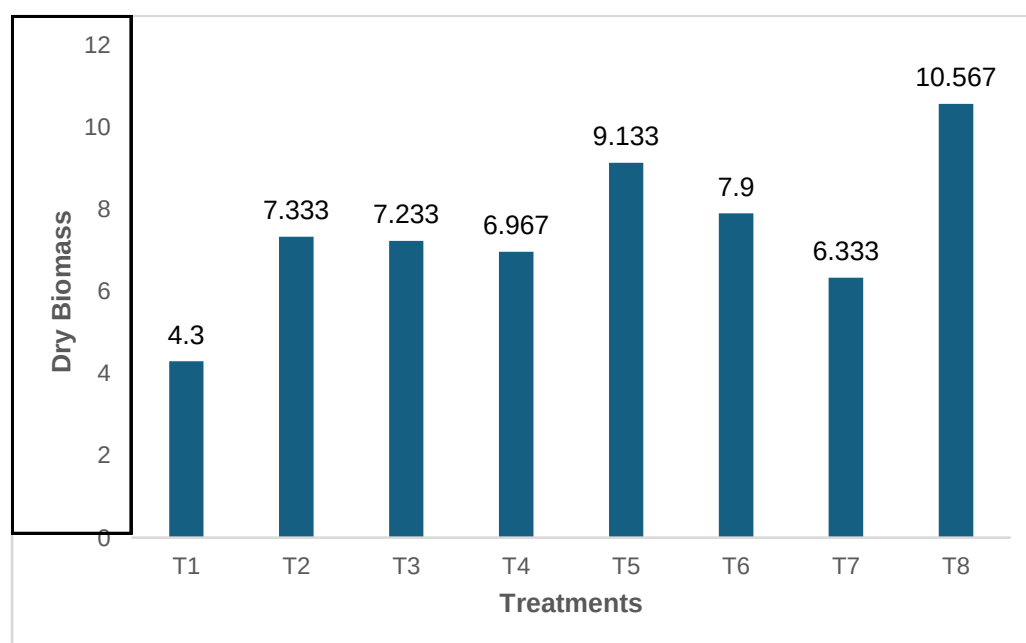


Fig 4.4: Effects of co-inoculating AMF and Rhizobia on dry biomass.

The pattern of dry biomass accumulation was consistent with fresh biomass. The zero control treatment produced the lowest the lowest dry biomass, while T8 (AMF plus Rhizobia plus Compound D) and T5 (Rhizobia plus Compound D) produced the highest dry biomass. Treatments receiving AMF alone (T3) or Rhizobia (T2) produced intermediate dry biomass values. The highly significant p value (0.001, Appendix 10) confirms that biofertilizer inoculation, particularly when combined with inorganic fertilizer, significantly enhanced biomass accumulation in cowpeas.

CHAPTER 5

DISCUSSION

5.0 Introduction

This chapter discusses, interprets and analyses the findings obtained from the study on the effects of Arbuscular Mycorrhizal Fungi and Rhizobia on plant growth of cowpeas. These results are interpreted in relation to the study objectives and compared with findings from previous research. This chapter examines the influence of AMF and Rhizobia, both individually, in combination and in combination with Compound D on growth parameters, fresh and dry biomass and overall plant performance. The discussion provides a deeper understanding of the observed trends, relationship, patterns and explains how the findings contribute to knowledge in the area of study.

5.1 Effects of AMF and Rhizobia on plant Height

The results presented in Table 4.1 demonstrates that AMF and Rhizobia inoculation had significant effects on plant height at all measurement intervals (2,4,6 and 8 weeks after planting). The p-values ranged from 0.025 at week less than 0.001 at week 8, indicating increasingly significant treatment effects as the plant matured. At 8 weeks, the tallest plants were observed in T8 (AMF plus Rhizobia plus Compound D) with a mean height of 35.07cm closely followed by T5 (Rhizobia plus Compound D) at 35.07cm. These treatments were significantly taller than the zero control (T1) which recorded only 23.58cm . The superior performance of combined treatments supports the hypothesis (H1) that co-inoculation significantly increases plant growth compared to uninoculated plants.

These results are consistent with (Mortimer, Pérez-Fernández, and Valentine 2008) who reported that plants receiving both AMF and Rhizobia showed superior early growth due to improved nutrient balance and enhanced physiological processes. The synergistic effect observed can be explained by the complementary roles of these biofertilizer, rhizobia provides readily available nitrogen through biological nitrogen fixation which helps in the protein and enzyme synthesis, cell division and tissue growth, while AMF enhances phosphorus uptake which is essential for energy transfer during nitrogen fixation (Xavier and Germid, 2003). Treatment 8 was a combination of AMF and Rhizobia plus Compound D, compound D is important because it supply phosphorus for root establishment before nitrogen fixing nodules mature (Kutamahufu *et al.*, 2022).

The relatively high coefficient of variation at week 2 was 9.4% compared to week 8 which was at 4.1%, this CV variance suggest that early growth was more variable, possibly due to differences in the time required for microbial colonisation and establishment of symbiotic relationships. While the experiment progressed, treatment effects became more uniform indicating successful root colonisation across replicates.

5.2 Effects of AMF and Rhizobia on root length

Table 4.2 presents the effects of treatments on root length at 8 weeks after planting. Although T8 (AMF plus Rhizobia plus Compound D) recorded the longest roots (22,13cm)while T1(zero control) recorded the shortest root length (17.67cm), the p-value of 0.233 indicates that these differences were not statistically significant at the 5%level. The fact that significant variation on plant height and biomass occurred independently of changes in root length is an interesting

observation. This may suggest that under the experimental conditions, the biofertilizer prioritised above ground growth over root elongation.

Alternatively, the eight week growing periods may not have been sufficient to manifest significant differences in root length, as root architecture changes may occur over long time scales.

According to (ÇekiÇ, Ünyayar, and Ortas 2012) AMF inoculation can significantly increase root biomass by improving nutrient availability and root efficiency, but the present study shows no significant difference in root length this may be possible that AMF and Rhizobia influenced root system architecture increasing root branching and fine root production rather than primary root elongation.

5.3 Effects of AMF and Rhizobia on root weight

Figure 4.1 illustrates the effect of treatments on root weight. The results showed a highly significant treatment effect ($p < 0.001$) while the LSD of 11.07 indicates substantial differences among treatments. Treatments receiving both AMF and Rhizobia particularly T5 and T8 exhibited greater root weight compared to the zero control and single inoculation treatments. This finding aligns with the work of (Shrivastava, Mahajan, and Varma, 2021) who demonstrated that AMF symbiosis enhances root development by increasing both root mass and weight. A well developed root system improves the plant ability to absorb water and nutrients, leading to improved shoot and plant health.

The difference between root length and root weight is notable. This suggest that while biofertilizer treatments did not necessarily produce longer roots they produced denser and more

robust root systems with a greater biomass. This is ecologically because root weight is often a better indicator of nutrient and water acquisition capacity than root length alone.

5.4 Effects of AMF and Rhizobia on Nodulation

Table 4.3 presents the effects of treatments on nodule number with a significant p-value of 0.004. T8 (AMF plus Rhizobia plus Compound D) produced the highest number of nodules (41 nodules), followed by T5 (Rhizobia plus Compound D) with 39.2 nodules and the T1 (zero control) produced only 11.2 being the treatment with the least number of nodules.

Similarly, figure 4.2 shows that nodule weight followed a comparable pattern with T8 and T5 achieving the highest nodule weights. The results for nodule weight confirmed a significant treatment effect with a p-value=0.007. These results shows that Rhizobia Inoculation, especially when combined with AMF and appropriate phosphorus fertilization (compound D), significantly enhances nodulation. This is consistent with (Vance, 2001) who emphasised that phosphorus availability is crucial for the energy intensive process of nitrogen fixation. AMF facilitates phosphorus uptake, thereby supporting more robust nodule formation and function.

The high CV% for nodule number 28.8% indicates that there was a considerable variability within treatments, which may be attributed to differences in the effectiveness of root colonisation or competition between native and introduced microbial strains.

5.5 Effects of AMF and Rhizobia on Biomass

Figure 4.3 and 4.4 show the effects of treatments on fresh and dry biomass respectively, both parameters showed highly significant treatment effects ($p < 0.001$, Appendices 9 and 10). The highest fresh biomass was recorded in T8 (AMF plus Rhizobia plus Compound D) and T5 (Rhizobia plus Compound D) substantially out performing the zero control and single

inoculation treatments. These findings strongly support the hypothesis that co-inoculation with AMF and Rhizobia significantly increases plant biomass compared to uninoculated plants.

The superior performance of T5 (Rhizobia plus Compound D) relative to T4 (AMF plus Rhizobia without Compound D) suggest that adequate phosphorus availability is critical for maximising the benefits of dual inoculation. Compound D which contains phosphorus likely enhanced the energy supply required for nitrogen fixation as discussed by (Kutamahufu *et al.*, 2022).

The synergistic effect observed are consistent according to (Kavidia et al., 2021) who reported that co-inoculation with rhizobia and arbuscular mycorrhizal fungi lead to synergistic growth effects in cowpea. The combination of improved nitrogen availability from Rhizobia and enhanced phosphorus uptake from AMF creates a positive feedback loop that is better phosphorus status supports more effective nitrogen fixation, which in turn supports greater photosynthetic capacity and biomass accumulation.

Treatment with AMF alone (T3) produced moderate improvements over the zero control while Rhizobia alone (T2) produced better results than AMF alone, reflecting the critical role of nitrogen as a limiting nutrient in the experimental soil. However, the best results were consistently achieved when both biofertilizer were applied, either with or without additional compound D confirming the complementary nature of these symbiotic relationship,

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS.

6.0 Conclusion

This study has demonstrated that AMF and Rhizobia inoculation whether applied singularly or in combination significantly improves the growth of cowpeas CB2 variety. The most pronounced effects were observed with dual inoculation combined with compound D fertilizer (T8), which produced the greatest plant height, root weight, nodule number, nodule weight and biomass. This research contributes to the knowledge on sustainable soil fertility management and support the use of beneficial microorganisms in crop production. For small holder farmers in Zimbabwe and similar agro ecological zones biofertilizer represents a viable, cost effective and environmentally friendly strategy for improving cowpeas productivity and ensuring food security.

6.1 Recommendations

The use of AMF and Rhizobia biofertilizer is an affordable, sustainable and environmentally friendly strategy for improving cowpea productivity by adapting dual inoculation farmers can

reduce production costs by no longer depending on expensive inorganic fertilizer. The use of both AMF and Rhizobia creates a synergy that provides both phosphorus and nitrogen to the plant.

REFERENCES

A. Kavadia,. Co-Inoculation with Rhizobia and Arbuscular Mycorrhizal Fungi Alters Mycorrhizal Composition and Lead to Synergistic Growth Effects in Cowpeas That Are Fungal Combination Dependent. 151, Part A (2021): 941–48.

Abebe, Belete Kuraz, and Mulugeta Tesfaye Alemayehu. “A Review of the Nutritional Use of Cowpea (*Vigna Unguiculata* L. Walp) for Human and Animal Diets.” *Journal of Agriculture and Food Research* 10 (December 2022): 100383. <https://doi.org/10.1016/j.jafr.2022.100383>.

Badiane, François Abaye, Made Diouf, and Diaga Diouf. “Cowpea.” In *Broadening the Genetic Base of Grain Legumes*, edited by Mohar Singh, Ishwari Singh Bisht, and Manoranjan Dutta. Springer India, 2014. https://doi.org/10.1007/978-81-322-2023-7_5.

Barros, Juliane Rafaela Alves, Miguel Julio Machado Guimarães, Welson Lima Simões, Nataniel Franklin De Melo, and Francislene Angelotti. “Temperature: A Major Climatic Determinant of Cowpea Production.” *Acta Scientiarum. Agronomy* 45 (September 2022): e56812. <https://doi.org/10.4025/actasciagron.v45i1.56812>.

BHATTACHARJEE, RITIKA, and UTPAL DEY. Biofertilizer, a Way towards Organic Agricultural:A Review. (Kenya , Nairobi) 8 (2014).

Birla, Jinendra, B. M. Patel, P. M. Patel, Y. A. Tamboli, and Devendra Patil. “Yield and Quality of Cowpea [*Vigna Unguiculata* (L.)Walp] as Influenced by Organic Sources of Nitrogen.”

Co-Inoculants for Improve Plant-Physiological Responses in Sustainable Agriculture.”
Biological Control 182 (July 2023): 105223. <https://doi.org/10.1016/j.biocontrol.2023.105223>.

Fageria, Nand Kumar. The Role of Plant Roots in Crop Production. 0 ed. CRC Press, 2012.
<https://doi.org/10.1201/b12365>.

Fageria, Nand Kumar. The Role of Plant Roots in Crop Production. 0 ed. CRC Press, 2012.
<https://doi.org/10.1201/b12365>.

Giovannetti, M., and B. Mosse. “AN EVALUATION OF TECHNIQUES FOR MEASURING
VESICULAR ARBUSCULAR MYCORRHIZAL INFECTION IN ROOTS.” New Phytologist
84, no. 3 (1980): 489–500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>.

Horn, Lydia N., Selma N. Nghituwamhata, and Ueitele Isabella. “Cowpea Production
Challenges and Contribution to Livelihood in Sub-Saharan Region.” Agricultural Sciences 13,
no. 01 (2022): 25–32. <https://doi.org/10.4236/as.2022.131003>.

Horn, Lydia N., Selma N. Nghituwamhata, and Ueitele Isabella. “Cowpea Production
Challenges and Contribution to Livelihood in Sub-Saharan Region.” Agricultural Sciences 13,
no. 01 (2022): 25–32. <https://doi.org/10.4236/as.2022.131003>.

Hui, Qiuling, Sumei Li, Zhe Chen, et al. “Inoculation With Salt-Tolerant Rhizobia and AMF
Regulates K⁺ /Na⁺ Homeostasis in Robinia Pseudoacacia L. Alleviating Salinity.” Plant, Cell &
Environment 49, no. 7 (2026): 4116–33. <https://doi.org/10.1111/pce.15621>.

Introduction of Cowpeas by Global Scientific Journal . volume 9, no. 10 October 2022 (n.d.).

Kavadia, Athanasia, Michalis Omirou, Dionysia A. Fasoula, Filippa Louka, Constantinos
Ehaliotis, and Ioannis M. Ioannides. “Co-Inoculations with Rhizobia and Arbuscular
Mycorrhizal Fungi Alters Mycorrhizal Composition and Lead to Synergistic Growth Effects in

Cowpea That Are Fungal Combination-Dependent.” *Applied Soil Ecology* 167 (November 2021): 104013. <https://doi.org/10.1016/j.apsoil.2021.104013>.

Khater, Ahmed Kassem, Ezekiel Mugendi Njeru, and Stephen Mwangi Githiri. “Association of Soil Physicochemical Properties with Bacterial Microbiome Structures in Cowpea Farms from Semiarid Eastern Kenya.” Preprint, In Review, April 2, 2026. <https://doi.org/10.21203/rs.3.rs-9264911/v1>.

Khater, Ahmed Kassem, Ezekiel Mugendi Njeru, and Stephen Mwangi Githiri. “Association of Soil Physicochemical Properties with Bacterial Microbiome Structures in Cowpea Farms from Semiarid Eastern Kenya.” Preprint, In Review, April 2, 2026. <https://doi.org/10.21203/rs.3.rs-9264911/v1>.

Kim, Dong-Kwan, Kingsley Ochar, Kanivalan Iwar, Bo-Keun Ha, and Seong-Hoon Kim. “Cowpea (*Vigna Unguiculata* L.) Production, Genetic Resources and Strategic Breeding Priorities for Sustainable Food Security: A Review.” *Frontiers in Plant Science* 16 (July 2025): 1562142. <https://doi.org/10.3389/fpls.2025.1562142>.

Kumar, Satish, Diksha, Satyavir S. Sindhu, and Rakesh Kumar. “Biofertilizers: An Ecofriendly Technology for Nutrient Recycling and Environmental Sustainability.” *Current Research in Microbial Sciences* 3 (2022): 100094. <https://doi.org/10.1016/j.crmicr.2021.100094>.

Kutamahufa, Marilyn, Lincon Matore, Gabriel Soropa, Nilton Mashavakure, Ezekia Svotwa, and Arnold B. Mashingaidze. “Forage Legumes Exhibit a Differential Potential to Compete against Maize and Weeds and to Restore Soil Fertility in a Maize-Forage Legume Intercrop.” *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* 72, no. 1 (2022): 127–41. <https://doi.org/10.1080/09064710.2021.1998593>.

Mandal, Debjani, Subhamoy Ghosh, Aditi Guha, et al. "Role of Microbial Biofertilizer in Plant Growth Promotion." In *Microbial Biotechnology: Integrated Microbial Engineering for B3 – Bioenergy, Bioremediation, and Bioproducts*. Elsevier, 2025. <https://doi.org/10.1016/B978-0-443-23796-6.00005-5>.

Mapunda, Hellen B., Stephane Declerck, Kelvin M. Mtei, and Akida I. Meya. "Arbuscular Mycorrhizal Fungi Inoculation Enhanced Banana Plant Growth and Fruit Yield in Central-Northern Tanzania." *Discover Sustainability* 6, no. 1 (2025): 869. <https://doi.org/10.1007/s43621-025-01183-z>.

Mapunda, Hellen B., Stephane Declerck, Kelvin M. Mtei, and Akida I. Meya. "Arbuscular Mycorrhizal Fungi Inoculation Enhanced Banana Plant Growth and Fruit Yield in Central-Northern Tanzania." *Discover Sustainability* 6, no. 1 (2025): 869. <https://doi.org/10.1007/s43621-025-01183-z>.

Mapunda, Hellen B., Stephane Declerck, Kelvin M. Mtei, and Akida I. Meya. "Arbuscular Mycorrhizal Fungi Inoculation Enhanced Banana Plant Growth and Fruit Yield in Central-Northern Tanzania." *Discover Sustainability* 6, no. 1 (2025): 869. <https://doi.org/10.1007/s43621-025-01183-z>.

Matova, P. M., and E. Gasura. "Yield and Stability of New Cowpea Varieties in Zimbabwe." *African Crop Science Journal* 26, no. 2 (2018): 277. <https://doi.org/10.4314/acsj.v26i2.9>.

Miransari, Mohammad. "Arbuscular Mycorrhizal Fungi and Nitrogen Uptake." *Archives of Microbiology* 193, no. 2 (2011): 77–81. <https://doi.org/10.1007/s00203-010-0657-6>.

Mpongwana, Sanele, Alen Manyevere, Johnfisher Mupangwa, Conference Thando Mpendulo, and Chuene Victor Mashamaite. "Foliar Nutrient Content Responses to Bio-Inoculation of Arbuscular Mycorrhizal Fungi and Rhizobium on Three Herbaceous Forage Legumes."

Frontiers in Sustainable Food Systems 7 (October 2023): 1256717.
<https://doi.org/10.3389/fsufs.2023.1256717>.

Parihar, Manoj, Amitava Rakshit, Alok Adholeya, and Yinglong Chen, eds. *Arbuscular Mycorrhizal Fungi in Sustainable Agriculture: Inoculum Production and Application*. Springer Nature Singapore, 2024. <https://doi.org/10.1007/978-981-97-0296-1>.

Pathak, Rakesh. *Genetics, Physiology and Cultivation of Moth Bean, Cowpea and Horse Gram*. Springer Nature Singapore, 2023. <https://doi.org/10.1007/978-981-19-9956-7>.

Prasad Dixit, Girish, Harsh Kumar Dikshit, Gyan Prakash Mishra, and Muraleedhar S. Aski, eds. *Fundamentals of Legume Breeding: A Text for Students and Practitioners*. Springer Nature Singapore, 2025. <https://doi.org/10.1007/978-981-96-7838-9>.

Ritika, Bhattacharjee, and Dey Utpal. “Biofertilizer, a Way towards Organic Agriculture: A Review.” *African Journal of Microbiology Research* 8, no. 24 (2014): 2332–43. <https://doi.org/10.5897/AJMR2013.6374>.

Ruscitti, Marcela, María Arango, and José Beltrano. “Improvement of Copper Stress Tolerance in Pepper Plants (*Capsicum Annuum* L.) by Inoculation with Arbuscular Mycorrhizal Fungi.” *Theoretical and Experimental Plant Physiology* 29, no. 1 (2017): 37–49. <https://doi.org/10.1007/s40626-016-0081-7>.

Shrivastava, Neeraj, Shubhangi Mahajan, and Ajit Varma, eds. *Symbiotic Soil Microorganisms: Biology and Applications*. Vol. 60. *Soil Biology*. Springer International Publishing, 2021. <https://doi.org/10.1007/978-3-030-51916-2>.

Silahooy, Charles. “The Effect of Various Types of Animal Manure on The Growth and Production of Mustard (*Brassica Juncea* L.) on Ultisol Soil.” *International Journal of Scientific Multidisciplinary Research* 4, no. 3 (2026): 223–34. <https://doi.org/10.55927/ijsmr.v4i3.11>.

Singh, Dhananjaya Pratap, Harikesh Bahadur Singh, and Ratna Prabha, eds. *Plant-Microbe Interactions in Agro-Ecological Perspectives*. Springer Singapore, 2017. <https://doi.org/10.1007/978-981-10-5813-4>.

Smith, Alison M., George Coupland, Liam Dolan, et al. *Plant Biology*. 0 ed. Garland Science, 2009. <https://doi.org/10.1201/9780203852576>.

Vance, Carroll P. “Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources.” *Plant Physiology* 127, no. 2 (2001): 390–97. <https://doi.org/10.1104/pp.010331>.

Vanlauwe, B., M. Hungria, F. Kanampiu, and K. E. Giller. “The Role of Legumes in the Sustainable Intensification of African Smallholder Agriculture: Lessons Learnt and Challenges for the Future.” *Agriculture, Ecosystems&Environment* 284 (November 2019): 106583. <https://doi.org/10.1016/j.agee.2019.106583>.

Varma, Ajit, and Devendra K. Choudhary, eds. *Mycorrhizosphere and Pedogenesis*. Springer Singapore, 2019. <https://doi.org/10.1007/978-981-13-6480-8>.

Varma, Ajit, and Devendra K. Choudhary, eds. *Mycorrhizosphere and Pedogenesis*. Springer Singapore, 2019. <https://doi.org/10.1007/978-981-13-6480-8>.

Wahab, Abdul, Murad Muhammad, Asma Munir, et al. “Role of Arbuscular Mycorrhizal Fungi in Regulating Growth, Enhancing Productivity, and Potentially Influencing Ecosystems under

Abiotic and Biotic Stresses.” *Plants* 12, no. 17 (2023): 3102.
<https://doi.org/10.3390/plants12173102>.

Xavier, Lisette J. C., and James J. Germida. “Bacteria Associated with *Glomus Clarum* Spores Influence Mycorrhizal Activity.” *Soil Biology and Biochemistry* 35, no. 3 (2003): 471–78.
[https://doi.org/10.1016/S0038-0717\(03\)00003-8](https://doi.org/10.1016/S0038-0717(03)00003-8).

Appendix

Appendix 1: Analysis of variance

Variate: Plant_height_at_2_weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Block stratum		2	2.4944	1.2472	3.99	
Block.*Units* stratum						
Treatment		7	7.4191	1.0599	3.39	0.025
Residual		14	4.3756	0.3125		
Total		23	14.2891			

Appendix 2: Analysis of variance

Variate: Plant_height_at_4_weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Block stratum		2	18.585	9.293	3.79	
Block.*Units* stratum						
Treatment		7	70.494	10.071	4.11	0.012
Residual		14	34.313	2.451		

Total	23	123.392
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Appendix 3: Analysis of variance

Variate: Plant_height_at_6_weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	15.951	7.975	4.09	
Block.*Units* stratum					
Treatment	7	94.125	13.446	6.89	0.001
Residual	14	27.302	1.950		
Total	23	137.378			

Appendix 4: Analysis of variance

Variate: Plant_height_at_8_weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	29.768	14.884	9.87	
Block.*Units* stratum					
Treatment	7	343.816	49.117	32.56	<.001
Residual	14	21.120	1.509		

Total	23	394.704
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Appendix 5: Analysis of variance

Variate: Root_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	2.128	1.064	0.30	
Block.*Units* stratum					
Treatment	7	37.950	5.421	1.54	0.233
Residual	14	49.296	3.521		
Total	23	89.373			

Appendix 6: Analysis of variance

Variate: Root_weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.14	0.07	0.01	
Block.*Units* stratum					
Treatment	7	568.16	81.17	7.33	<.001
Residual	14	154.93	11.07		

Total	23	723.23
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Appendix 7: Analysis of variance

Variate: Nodule_number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	66.27	33.14	0.57	
Block.*Units* stratum					
Treatment	7	2174.91	310.70	5.34	0.004
Residual	14	815.06	58.22		
Total	23	3056.24			

Appendix 8: Analysis of variance

Variate: Nodule_weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.3159	0.6580	0.94	
Block.*Units* stratum					
Treatment	7	23.2146	3.3164	4.72	0.007
Residual	14	9.8354	0.7025		

Total	23	34.3659
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Appendix 9: Analysis of variance

Variate: fresh_biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	10.926	5.463	3.37	
Block.*Units* stratum					
Treatment	7	1336.100	190.871	117.85	<.001
Residual	14	22.674	1.620		
Total	23	1369.700			

Appendix 10: Analysis of variance

Variate: dry_biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.8258	0.4129	1.86	
Block.*Units* stratum					
Treatment	7	72.6296	10.3757	46.64	<.001
Residual	14	3.1142	0.2224		

Total	23	76.5696
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