EFFECTS OF BIO-FERTILIZER AND PHOSPHORUS APPLICATION ON GROWTH, YIELD, YIELD COMPONENTS, AND PHOSPHORUS NUTRITION OF COWPEAS GROWN IN TWO DIFFERENT SOIL TYPES.

By

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DECLARATION

I, Ntombifuthi Shane Mashele (Student No: 20025347) hereby declare that this dissertation for the degree of Master of Science (MSc.) in Agriculture (Soil Science) submitted to the Department of Plant and Soil Sciences, Faculty of Science, Engineering, and Agriculture at the University of Venda has not been previously submitted for any degree at this or other universities. I also declare that this dissertation is my original work, except where references are made and duly cited as such.

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Signature

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As the supervisor and co-supervisor of the candidate, we agree to the submission of this dissertation without any reservations.

Prof. J.J.O. Odhiambo
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DEDICATION

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop that is native to Africa. This crop is cultivated for food, animal feed, and organic manure. Cowpea also contributes nitrogen (N) to the soil through the process of biological nitrogen fixation. Among other quality properties, cowpea leaves and grain contain essential nutrient elements and protein. The production of cowpeas, however, faces obstacles that reduce their productivity. This is despite the significant benefits cowpeas provide to people, animals, and soils. Cowpea productivity is limited by the lack of essential nutrient elements in croplands, particularly phosphorus (P). One way by which we could address low P in croplands is the addition of P-fertilizers. However, because cowpea crop is primarily grown by unemployed small-holder farmers and that P fertilizer is scarce, expensive, and inefficient, its ability to exhibit high growth and yield is limited by low P levels in soils. Alternatively, the availability of P in soils could be improved by adding phosphate solubilizing bacteria and inoculating with rhizobial inoculants. This study investigated the effects of rhizobial inoculants, P fertilizer, and phosphate-solubilizing bacteria addition on growth and yield-related parameters of cowpeas cultivated in soils with varying characteristics.

Field experiments were conducted at the Agriculture Research Council - Tropical and Subtropical Crops (ARC-TSC) and the Sabie River area during the 2019/20 and 2020/21 summer seasons. ARC-TSC and Sabie River sites have different soil types. That is, the former has Hutton soils (sandy soil) while the latter has Glenrosa (loam-sandy soil) soil type. The field study was conducted using a 2 x 5 factorial experiment layout in a Randomised Complete Block Design (RCBD) replicated three times. The experimental design was done in two locations, namely Sabie River (Glenrosa soil type) and ARC (Hutton soil type). The said designs had five treatments, and these were Control (C) - no fertilizer, P, *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + P (BR + P +BIO). The experiment was conducted under rain-fed conditions. During the flowering growth stage, biomass, including shoot, root, number of root nodules, and weight were determined. At harvest stage, shoot biomass, number of pods/plant, grain yield, shoot and grain P uptake, and available P in the rhizosphere were determined. Data were subjected to a normality test and analysed using a Genstat software, 24th edition program. Where significant differences within the treatments were observed, a comparison of mean was made using the least significant difference (LSD) method at a 5% significance interval.

Both the soil type and treatment had significant ($p \leq 0.01$) effect on all measured cowpea parameters during the 2019/2020 and 2020/21 planting seasons. Hutton soil at the ARC site produced significantly higher shoot and root dry matter, number and weight of nodules, number of pods, and grain yield than Glenrosa soil at Sabie River site. Results show that the addition of Phosphate solubilizing bacteria + Bradyrhizobium + Superphosphate significantly improved shoot dry matter, root dry matter, number of nodules, nodule dry weight, number of pods, and grain yield of cowpea relative to the control. However, BR+P+BIO significantly differed from BR+P and BIO+P in terms of the shoot dry and root dry matters, number of nodules, nodule dry weight, number of pods, and grain yield of cowpea in both soil types. Compared to the other treatments, BR+P+BIO application enhanced P nutrition of shoots and grains, and available P in rhizosphere soil.

Study findings suggest that using bio-fertilizer and P application could be a viable strategy to improve crop yield and P nutrition in cowpea cultivation. Further research and field trials, however, might be necessary to optimize application rates and assess long-term effects on soil fertility, crop performance, and the importance of considering soil types in agricultural practices. These findings contribute valuable insights for sustainable agricultural practices to enhance cowpea production and ensure food security. This has implications for sustainable agricultural practices that enhance crop yields and nutrient use efficiency in cowpea production.

Keywords: Cowpea, bio-fertilizer, phosphorus, soil type, yield-related parameters, phosphorus nutrition.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS/ACRONYMS

ARC-TSC	Agricultural Research Council – Tropical and Sub-tropical Crop
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
BR	Bradyrhizobium
CEC	Cation Exchange Capacity
DAFF	Department of Agriculture, Fisheries and Forestry
DM	Dry matter
EC	Electrical conductivity
EDTA	Ethylene Diamine Tetra Acetic
FSSA	Fertilizer Society of South Africa,
HCl	Hydrochloric acid
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
ISFM	Integrated soil fertility management
LSD	Least significant difference
NH_4OAc	Ammonium acetate
P	Phosphorus
PSB	Phosphate Solubilizing Bacteria
RCBD	Randomised Complete Block design
SP	Superphosphate
SSA	Sub-Saharan Africa

CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND TO THE STUDY

The utilization of bio-fertilizers in contemporary agricultural practices signifies a fundamental change towards sustainable and environmentally friendly farming approaches. This change has attracted attention and interest from researchers and farmers due to advantages associated with adding bio-fertilizers. Bio-fertilizers are obtained from organic sources like plant extracts, compost, or microbial inoculants (Kumar, 2022). Thus, bio-fertilizers are a natural and environmentally friendly alternative. When added to the soil, bio-fertilizers enhance its fertility by improving the activities of beneficial micro-organisms and soil structure (Itelima *et al*., 2018). Bio-fertilizers also increase the availability of essential nutrient elements in soil and their uptake by plants while reducing reliance on chemical inputs (Itelima *et al*., 2018).

The advantage of using bio-fertilizers is that they improve plant nutrition through various mechanisms. For example, inoculating seeds with bio-fertilizers such as rhizobial inoculants improves the ability of legumes to contribute N to soils through the process of symbiotic nitrogen fixation. Furthermore, adding bio-fertilizers stimulates soil activity (Bhardwaj *et al.,* 2014). In addition to that micro-organisms improve the ability of plant roots to absorb essential nutrients, resulting in improved growth, development, and yield (Bhardwa *et al.,* 2014). Certain bio-fertilizers help to improve solubility, availability, and uptake of P. P is an essential nutrient element that is crucial in physiological processes of plants (Rychter, 2005). These physiological processes are, *inter alia*, energy transfer, photosynthesis, and nutrient transport (Rychter, 2005). The availability of P in soils, however, is limited (Margenot *et al.,* 2016). This limit is apparent in P-deficient soils mainly found in Sub-Saharan Africa (Margenot *et al.,* 2016). P-deficiency in croplands is largely caused by geological factors. These factors are low P minerals, fixation to soil particles, acidity of soils, run-off and erosion, and the addition of lower than recommended rates of P-fertilizers (Alewell *et al.,* 2020). To address P-deficiency, sustainable soil management and balanced fertilisation strategies are essential (Rowe *et al*., 2016).

Literature that focuses on synergistic effects of bio-fertilizer and P application on crop yield is abound (Aloo *et al.,* 2022). In particular, the co-addition of bio- and P-fertilizers was found to promote root development, improve nutrient absorption, and overall plant performance (Mahanta *et al*., 2014). Singh *et al*. (2018), for example, showed that adding bio- and P-fertilizers significantly increased root length, root surface area, and root dry matter in maize plants. In general, increased root development is associated with improved uptake of nutrients, which in turn enhances plant growth and/or yield (Fageria & Moreira, 2011).

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This study focused on cowpea crop (also known as the black-eyed pea). Cowpea is a leguminous crop widely cultivated for its nutritional value and adaptability to diverse agro-ecological conditions (Kebede & Bekeko, 2020). Cowpea is valued for its protein-rich leaves, pods, and grains that are widely eaten by people (Asiwe, 2009a; Sheahan, 2012; Muranaka *et al.,* 2016; Maseko *et al*., 2020). Cowpea crops are also used as animal fodder. Despite cowpea leaves and grain displaying anti-nutritive factors, these structures are reportedly required for their palatability (Bala & Hassan 2023), quality, compatibility when grown with other diverse crops, and their greater ability to combat soil erosion (Shah & Wu 2019). Cowpea contributes N to the soil through the process of symbiotic nitrogen fixation, a nutrient that limit productivity in low-input small-holder croplands (Gyaneshwar *et al*., 2011). As is the case with many legumes, however, growth, development, and yield of cowpea are hindered by P-deficiency in soils (Kyei-Boahen *et al*., 2017). Furthermore, the decreases in cowpea growth, development, and yield are caused by poorly controlled weeds, diseases, and crop pests, among others. Collectively, these factors (especially P-deficiency) necessitate attention if we are to produce quality crops in productive croplands.

Consequently, there is a need to adopt sustainable soil management practices. One such practice is the application of bio-fertilizers like *Bradyrhizobium* inoculant, phosphate solubilizing bacteria (PSB), and P-fertilizers. It is evident that *Bradyrhizobium* inoculation alongside P-fertilizers significantly improves cowpea growth, root proliferation, nodulation, and grain yield (Stamford *et al*., 2013; Kyei-Boahen *et al.,* 2017; Madzivandila, 2020). Nonetheless, these practices are under-utilized by resource-poor small-holder farmers due to the high cost of P-fertilizers and scarcity of bio-fertilizers in rural areas. Nevertheless, Africa has seen an increase in the number of companies that import and/or manufacture bio-fertilizers. In this context, the availability of bio-fertilizers in retail shops has so far improved. It is, therefore, not surprising that the commercial crop farmers have adopted the co-adding bio- and inorganic fertilizers approach (Mapope & Dakora, 2016).

Most importantly, PSB improves the production of cowpea. For example, dual inoculation of PSB + Rhizobium culture outperformed PSB only application in that the former helped to increase growth and yield-related parameters (Kalegore *et al.*, 2018). Similarly, PSB increases the yield of cowpea by increasing the availability of P in soil. P availability in soil is essential in that it supports plant growth and development. Bio-fertilizers, particularly microbial inoculants, contain living micro-organisms. When used to inoculate seeds, bio-fertilizers enhance germination and other processes to improve growth and quality of plants and soil (Singh *et al*., 2019; Aloo, 2020). For example, there is improved growth, yield, and yield-related parameters (Chaudhary *et al.,* 2016). In that case, quality parameters were recorded with 40 kg P//ha + PSB application compared to PSB only (Chaudhary *et al.,* 2016).

Despite these potential benefits, widespread adoption of PSB bio-fertilizers in cowpea production is limited. It is limited partly due to lack of awareness and education, limited access to quality bio-fertilizers, cost consideration, perceived rules or uncertainty, and lack of extension support as well as lack of will to invest therein (Sansinenea, 2021). To encourage the use of PSB bio-fertilizers in cowpea production, there is a need to do awareness campaigns, enhance access to quality bio-fertilizers, lower costs, address perceived dangers, and provide extension support through training and education programs (Barragán-Ocaña & del Carmen del-Valle-Rivera, 2016; Raimi, *et al.,* 2017).

Even though P is abundant in soil, most of it is adsorbed, hence its individual or combined application with other soil amendments. There is limited information about effects of co-adding PSB, rhizobial inoculation, and P-fertilizers on growth and yield of cowpea grown in South Africa. This study hopes to help address this gap in literature on co-adding PSB, rhizobial inoculation, and P-fertilizers. That is, this study investigated effects of bio-fertilizers and P application on yield-related parameters and cowpea P nutrition. The study examined these effects across two different soil types. In doing so, the study acknowledged the influence of soil characteristics on nutrient availability and crop response. Understanding these interactions is vital for optimizing agricultural practices to improve crop productivity, especially in regions where soil fertility is a limiting factor. Insights gained from this study might inform agricultural strategies aimed at maximizing cowpea productivity while minimizing negative environmental impact. Achieving the foregoing would help support food security and sustainable development in agricultural systems.

1.2 PROBLEM STATEMENT

Cowpea production in South Africa is constrained by P deficiency, which limits biological nitrogen fixation, growth, and grain yield. Although P-fertilizers and rhizobial inoculants are known to improve cowpea productivity, their effectiveness is often restricted by low P availability and high cost of fertilizers for small-holder farmers. There is limited research about the combined application of PSB, rhizobial inoculants, and P-fertilizers in South African soils. This knowledge gap hinders the development of sustainable and cost-effective strategies to enhance cowpea productivity under low-P conditions.

Similarly, there is not much information about effects of co-adding PSB with rhizobial inoculants and P-fertilizers on cowpea growth and yield in South Africa. This is despite the fact that the production of cowpea is limited by P deficiency. Poor availability of P in croplands and poor efficacy of P-fertilizer contribute to low cowpea productivity. When cowpea is cultivated on low P soils, the result is low yield, deformation of top soil, and low quality of leaves and grain. The general approach to address low P problem in soils is the application of P-fertilizers. This could be done through exogenous means using single or double super-phosphate granular fertilizers. P-fertilizers, unfortunately, are too expensive and scarce for small-holder farmers. To solve this conundrum, alternative solutions to low P in soils are urgently needed. Alternative solutions would provide relatively improved cowpea growth, yield, and its quality. One such approach is to co-add P-fertilizers with rhizobial inoculation and bio-fertilizers.

1.3 MOTIVATION FOR THE STUDY

When P-fertilizers, rhizobial inoculants, and P-solubilising bacteria are co-applied, they significantly increase soil fertility, enhance nutrient efficiency and, therefore, improve crop yield and quality. Additionally, there is an increased interest in locally produced and sold bio-inputs from researchers and crop farmers in Africa. This is due to an increase in the number of publications about the above issue. Astonishingly to this researcher though, not much has been studied about effects of co-adding P-fertilizers, rhizobial inoculants, and PSB individual or in combination on cowpea crops. This gap in literature suggests a need for in-depth empirical research to investigate effects of applying these bio-inputs on cowpea growth and yield. Results from such research would benefit both the commercial and small-holder crop farmers in Africa.

In essence, co-adding P-fertilizer, rhizobial inoculant, and PSB could reveal unique biological traits. In this way, new knowledge would be created.

P is essential for cowpea growth and yield, but P-fertilizers are costly for small-holder farmers with limited resources. The use of PSB, which has the potential to increase P availability to plants, would significantly benefit farmers. Such a benefit would be achieved through efficient use of applied P-fertilizers when used in conjunction with *Bradyrhizobium* inoculant. Doing so would increase nodulation and N fixation. These two products have the potential to significantly increase cowpea growth and yield. Cowpea growth and yield could contribute to improved farmer income, food security, and nutritional security. Study results would demonstrate whether the use of a combination of bio-fertilizer Bradyrhizobium and P-fertilizer improves cowpea growth and yield. Cowpea growth and yield would help address food security and nutritional needs of resource-poor farmers in particular and the general population at large.

1.4 AIM AND OBJECTIVES OF THE STUDY

1.4.1 Aim of the study

Therefore, the purpose of this study was to determine how co-application of P and these bio-fertilizers (PSB and *Bradyrhizobium* inoculants) affects growth, yield, and P nutrition of cowpea grown in two different soil types under rain-fed conditions in Mpumalanga Province of South Africa.

1. 4.2 Specific study objectives

i. To determine effects of bio-fertilizers and P application on nodulation, root, and shoot biomass at flowering.
ii. To determine the effects of bio-fertilizers and P application on grain yield, shoot, and root biomass at harvest maturity.
iii. To determine the effects of bio-fertilizers and P application on the P content of the shoot at flowering and the grain at harvest maturity.
iv. To determine the effects of bio-fertilizers and P application on P content in rhizosphere soil.

1.5 HYPOTHESES

i. The co-application of bio- and P-fertilizers will improve nodulation, root, and shoot dry matter at flowering.
ii. The co-application of bio- and P-fertilizers will increase grain yield, shoot, and root dry matter at harvest maturity.
iii. The co-application of bio- and P-fertilizers will increase the P uptake of the shoot at flowering and grain at harvest maturity.
iv. The co-application use of bio- and P-fertilizers will increase the rhizosphere soil P content.

CHAPTER 2: LITERATURE REVIEW

2.1 ORIGIN, DESCRIPTION, AND IMPORTANCE OF COWPEA

2.1.1 Origins of cowpea

Cowpea belongs to the legume crop family (*Fabaceae)*, sub-family *Papilionoideae*, and its tribe is *Phaseoleae*, its order is *Fabales*, genus is *Vigna*, and is of *unguiculata* type (Oyewale & Bamaiyi, 2013). It is an *herbaceous* annual legume food crop native to the tropical regions of Africa (Müller *et al.,* 2017). Cowpea is classified as a dicotyledonous plant (Nounagnon, 2024). This legume naturally grows in bushes of South and West Africa (Gonçalves *et al*., 2016; Karapaonos, 2017; Lazaridi, 2017). Countries in Eastern and Southern Africa are regarded as the primary centres of diversity, while West and Central Africa are secondary centres of diversity (Khoury, 2016). Cowpea crop is highly valuable to both humans and animals, as well as to soil. This crop is predominantly consumed in rural and peri-urban areas of the Global South. Its significance extends beyond food security, as it provides proteins to rural families (Opitz, 2016). In addition to the above, cowpea crop serves as a source of animal feed (Gonçalves, 2016) and helps generate cash for farmers through its production (Nwagboso, 2024). Additionally, cowpea crop can fix nitrogen, meeting 80% of its growth requirements through atmospheric nitrogen fixation (Mndzebele *et al.,* 2020). In this case, cowpea reduces the need for nitrogen fertilizers and their associated costs. Its potential as a companion crop is also significant, as residual nitrogen from its leaf litter, roots, and root nodules (Okereke *et al*., 2017) can benefit cereal-legume cropping systems.

2.1.2 Cowpea description

Genus *Vigna* comprises more than 80 species, which are subdivided into six groups: *Vigna*, *Comosae*, *Macrodontae*, *Reticulatae*, *Liebrechtsia,* and *Catiang* (Maxted *et al.,* 2004). Globally, cowpea is commonly referred to as *V. unguiculata* (Vacu *et al.,* 2025). Cowpeas are herbaceous annual plants typically planted in summer due to their morphology (Nounagnon *et al.,* 2024; Badiane *et al.,* 2014). The crop has semi-prostrate, semi-erect, erect, or climbing growth forms, depending on its genotypic characteristics. According to Omundi (2020), cowpeas can thrive in a variety of soil types, ranging from sandy to heavy and nutrient-poor soils. Njonjo (2018) stated that cowpeas can grow in a variety of temperatures, with an average of 28°C as ideal.

Depending on the photoperiod, cowpea can take anywhere from 60 to 150 days to mature. Singh (2005) indicated that cowpea leaves can be divided into four classes, vis, sub-globose, sub-hastate, globose, and hastate/lanceolate. At distal extremities of lengthy peduncles, cowpea flowers are arranged in alternating pairs on racemes, with two blooms in each inflorescence. Cowpea blossoms can be yellow, brown, or dark purple in colour. Mensah (2021) states that cowpea seeds may be kidney, ovoid, crowder, globose, or rhomboid in shape. Cowpeas can also be white, cream, green, buff, red, brown, or black in colour. Cowpea root system is robust and thick (Adu *et al.*, 2019).

2.1.3 Importance of cowpeas

Above-ground parts of cowpea are used for human consumption. For example, young and tender fresh green leaves are harvested for use as vegetables. Fresh pods are harvested for consumption, while dry grain is used to prepare various dishes. In Africa, cowpea accounts for more than half of plants consumed by humans daily. Cowpea leaves and grain contain essential nutrient elements, carbohydrates, and proteins (Sheehan, 2012; Asif *et al.,* 2013). Given its high nutritional value, cowpea has the potential to address nutritional and food security in South Africa (Gerrano *et al.,* 2015; 2017).

Generally, fresh and dry shoots of cowpea are used as animal feed. Dried above and below-ground parts of cowpea are spread on cropland soils as manure. Cowpea root nodules largely contribute N to soils through symbiotic nitrogen fixation (Belane *et al*., 2011). Given the above, it is clear that the crop possesses important attributes of value, especially in small-holder cropping systems, where little or no fertilizer is added to cropland soils. Due to its multi-purpose uses, short growing period, and relative tolerance to drought, cowpea is an ideal crop for improving agricultural sustainability and food security in marginal areas.

2.2 COWPEA PRODUCTION IN SUB-SAHARAN AFRICA

Cowpea is cultivated annually across the world on an estimated 14.5 million hectares of land, with a total annual production of 6.5 million metric tons (Kebede & Bekeko, 2020). For the past three decades, global cowpea production has grown at an average rate of 5%, with 3.5% annual growth in area and 1.5% growth in yield (Boukar *et al*., 2016). The area extension accounted for 70% of total growth during that period (Boukar *et al*., 2016). Nigeria contributes the largest tonnage of cowpea production in Africa (Mamiro *et al.,* 2011). According to FAO (2016), the area under cowpea cultivation in 2014 was estimated to be 12.3 million ha of land. Of that land, approximately 10.6 million hectares of bulk production are done in West African countries such as Niger, Burkina Faso, Mali, and Senegal (FAOSTAT, 2016). Niger, Burkina Faso, Benin, Mali, Cameroon, Chad, and Senegal are net exporters of cowpeas, while Nigeria, Ghana, Togo, Côte d'Ivoire, Gabon, and Mauritania are net importers of cowpea in Africa (Nwagboso *et al*., 2024).

Limpopo, Mpumalanga, North-West, and KwaZulu-Natal Provinces contribute the lion's share of cowpea production in South Africa (Department of Agriculture, Forestry, and Fisheries (DAFF), 2011). In South Africa, the most universally improved genotypes of cowpea are PAN 311 and IT-18 (Gerrano *et al.,* 2019). Small-holder crop farmers mainly cultivate unimproved landraces that are bartered among communities, provinces, and countries (Van Niekerk & Wynberg, 2017). This partly explains the widespread low grain yields and poor nutritional value of the prototype in question. As a result, cowpea requires significant improvements in terms of yield enhancement and reduction of production constraints (Asiwe, 2009b).

Compared to the anticipated yields of 1,500–3,000 kg/ha, cowpea yields have been poor, ranging from 100 to 599 kg/ha (Gbaye & Holloway, 2011; Horn & Shimelis, 2020; Gerrano *et al.,* 2020; Shegro *et al.,* 2020). Usage of unimproved local cultivars, low soil fertility, drought, and other biotic and abiotic stressors are frequently linked to low yield. Only three varieties, specifically Nakare (IT81D-985), Shindimba (IT89KD-245-1), and Bira (IT87D-453-2), which were originally imported from the International Institute of Tropical Agriculture (IITA), are currently available in South Africa (Horn & Shimelis, 2020).

2.3 LIMITATIONS OF COWPEA PRODUCTION IN SUB-SAHARAN AFRICA

Production of cowpea in South Africa is limited by several factors. These factors include, among others, use of unimproved varieties, lack of knowledge of good agronomic practices, shortage of high-quality seeds for planting, inadequate cowpea markets, and insufficient storage facilities (Asiwe, 2009b). Other limiting factors are drought and biotic stress (for example, weeds, diseases, and insects/pests) (Asiwe, 2009b). Further constraints include lack of government funding and low productivity of cowpeas (Asiwe, 2009b). Limited knowledge of effective agronomic practices and non-accessibility of good seed varieties for producing cowpeas in South Africa, particularly in the provinces of KwaZulu-Natal, Limpopo, and Mpumalanga, were also explained by Adebowale (2011). Additionally, low soil fertility, combined with unsuitable agricultural soils, poses a significant challenge in Sub-Saharan Africa.

Ogbazghi *et al*. (2016) reported that approximately 55% of croplands in Africa are unsuitable for sustainable agriculture due to several factors. These factors include being derived from infertile and highly weathered parent material, acidic soils, and high nutrient fixation (Javed *et al*., 2022). Collectively, these factors contribute to poor availability of P in croplands. Ogbazghi *et al*. (2016) found that different types of soils in agro-ecological zones of Southern Africa exhibit distinct patterns in water and nutrient storage capacity, organic matter content, and nutrient depletion.

2.4 INTEGRATED SOIL FERTILITY MANAGEMENT: AN APPROACH TO IMPROVING LOW FERTILITY SOILS

Maintaining soil fertility is a pivotal factor in ensuring long-term sustainability of food production. Indiscriminate use of chemical fertilizers, alongside inadequate soil management practices and environmentally harmful methods, has led to depletion or destruction of soil organisms (Geisseler & Scow, 2014; Ebrahimi *et al.,* 2016). To address these issues, Integrated Soil Fertility Management system (ISFM) was developed as a set of practices that combine judicious use of fertilizers, organic inputs, and improved crop varieties with a thorough understanding of how to adapt these practices to local conditions. Primary goal of ISFM is to optimize agronomic efficiency of applied nutrients while simultaneously enhancing crop productivity and minimizing potential environmental impacts (Vanlauwe & Zingare, 2011).

ISFM scheme involves strategic and sustainable use of a combination of organic and inorganic fertilizers to enhance crop productivity. This approach incorporates both physical and biological measures of soil and water conservation, as well as tailored technologies designed for specific agronomic and socio-economic conditions. The objective of ISFM procedure is to address nutrient imbalances and organic matter degradation while minimizing adverse environmental effects of fertilizer use (Sommer *et al.,* 2013).

2.5 ROLE OF P APPLICATION ON COWPEA GROWTH, YIELD, AND P-NUTRITION

Small-holder crop farmers in South Africa often apply lower rates of P fertilizer than recommended despite the prevalence of P deficiency in many small-scale croplands in the country (Mohale *et al.,* 2014). P is a crucial nutrient that plays a significant role in symbiotic nitrogen fixation process, extracellular enzyme secretion, and arbuscular mycorrhiza association in legumes (Hedin *et al*., 2009). Application of P-fertilizers can enhance plant growth, photosynthesis, drought tolerance, biological nitrogen fixation, yield, and plant quality (Blackshaw & Brandt, 2009; Mapfumo, 2011; Jansa *et al.,* 2011).

P forms an essential component of various biomolecules, including membrane proteins, lipids, and nucleic acids. It is critical in various cellular processes such as respiration and photosynthesis (Hawkesford *et al.,* 2023). This essential nutrient element is involved in the formation of sugar-phosphate intermediates, which are essential for the transfer of energy and maintenance of cellular structures (Moseley & Grossman, 2009). Deficiency in P in croplands results in stunted growth of plants and dark green coloration of leaves, as well as the formation of necrotic spots (areas of dead tissue) (Abbas *et al.,* 2021). Addition of P-fertilizer considerably boosts growth and yield parameters such as plant height, leaf area, and number of branches and leaves (Kwon *et al*., 2019). Application of P reportedly improves grain filling and seed formation (Haruna, 2011), and increases the number and weight of nodules of legume food crops (Kyei-Boahen *et al*., 2017). Nodule weight of IT99 K-573-1-1 variety was detected at P-fertilizer application of 80 kg/ha, while IT99 K-573-2-1 exhibited a rate of 40 kg/ha (Kumar, 2023). A significant increase in nodulation of cowpeas has been documented (Agboola & Obigbesan, 2001; Mokwunye & Bationo, 2002; Olaleye *et al*., 2011). These studies suggest that adding P-fertilizers to legumes improves their nodulation and nitrogen fixation (Haruna & Aliyu, 2011). To ensure optimal agricultural productivity, it is essential to maintain sufficient levels of P in soil through the application of available forms of the element (Muindi, 2019; Selim, 2020). Moreover, maintaining productive soils requires use of appropriate fertilizers that provide available forms of P (White & Brown, 2010; Balemi & Negisho, 2012).

2.6 ROLE OF BIO-FERTILIZERS TO IMPROVE CROP PRODUCTION

Biofertilizers are defined as substances that contain living cells or organisms that colonize rhizosphere or root interior of plants, promoting plant growth, and enhancing soil fertility by increasing availability of essential nutrients (Mohammadi & Sohrabi, 2012). Of these, microbial inoculants or bio-formulations are crucial in ISFM and are also vital components of organic farming. There is a claim that adding bio-fertilizers can reduce the need to add fertilizers, reduce cost associated with fertilizers while preventing environmental pollution and supporting sustainability (Kumar *et al.*, 2013; Kumar *et al.*, 2015). Without any doubt, application of bio-fertilizers can improve crop yields by increasing nutrient uptake. In fact, benefits of such extend beyond the above, as bio-fertilizers are critical in maintaining long-term soil fertility and sustainability.

Bio-fertilizers contribute to nutrient cycling transformation, increase organic matter content, maintain soil pH through the release of organic acids, and promote sustainable soil management. These benefits are distinct from those of other products such as green manure, manure, intercrop, and organic-supplemented chemical fertilizers (Bhattacharyya & Jha, 2012; Halpern *et al*., 2015). These products are not the same as bio-fertilizers. Bio-fertilizers contain living cells or organisms that colonize rhizosphere or root interior of plants to promote plant growth and enhance soil fertility. They are vital components of integrated nutrient management and organic farming. Use of bio-fertilizer can improve crop yields and reduce the amount and cost of fertilizers.

Utilization of bio-fertilizers has the potential to significantly enhance soil fertility by fixing atmospheric nitrogen, solubilizing insoluble phosphates, and synthesizing plant growth-promoting substances within soil (Mazid & Khan, 2015). Seed inoculation with N and PSB can significantly improve plant growth and biomass, resulting in an estimated yield increase of between 16% and 60% (Fatimah *et al.,* 2021). Use of bio-fertilizers with PSB improves soil fertility and food security (Stamford *et al.,* 2013). Nonetheless, this practice remains underutilized among small-holder farmers, particularly in resource-poor communities. To address concerns regarding food and nutritional security, this study investigated the use of bio-fertilizers with PSB to enhance cowpea production, grain yield, and biomass.

2.7 EFFECTS OF *BRADYRHIZOBIUM* INOCULATION ON COWPEA GROWTH AND YIELD

Few attempts have been made to determine how different cowpea varieties react to Bradyrhizobia inoculation, despite the fact that cowpea is a crop that increases soil fertility and contributes to food security (Yoseph *et al.,* 2017). Given that cowpea is cultivated by small-holder crop farmers, there is no evidence of its inoculation with rhizobial inoculants as an agronomic technique. To achieve food and nutritional security in South Africa, innovative cultural practices such as Bradyrhizobium inoculation, which serves as source of nitrogen (N), can improve grain yield and mineral nutrition (Yoseph *et al.,* 2017).

Farmers in the Global South struggle to address infertility of croplands, especially those used to grow food legumes. Co-application of fertilizers with bio-fertilizers to legumes could mitigate this issue by improving symbiotic nitrogen (Janati *et al*., 2021). While the process occurs through lightning as well as biological nitrogen fixation, the latter contributes more of N (Barth *et al*., 2023). Biological N_2 fixation has three categories, and these are free-living N2 fixation, associative N_2 fixation, and symbiotic N_2 fixation. Free-living N_2 fixation involves free-living bacteria (Azotobacter, Bacillus, Clostridium, Klebsiella) that inhabit bulk soil and occur without a direct symbiosis with plants. Associative N_2 fixation involves bacteria, which colonize either rhizosphere or endosphere of grasses and cereal plants. Symbiotic N_2 fixation involves symbiotic bacteria that live in symbiotic relationship with legume plant species.

Cowpea contributes N to soil, largely through the latter's types of N2 fixation processes. In fact, N contributed by cowpea through symbiotic interaction with soil or commercial bacteria is enhanced by inoculating seeds with rhizobial inoculants (Chianu *et al.,* 2011). N_2 fixation type of biosphere receives more than 170 million tons of fixed nitrogen from biological nitrogen fixation. Jalal *et al*. (2022) asserted that bacteria that live in symbiotic relationships with legumes and some non-legume plants provide 80% of stable biologically fixed nitrogen. Even so, one can regulate the amount of BNF that contributes to N cycle by adjusting a range of biological, dietary, environmental, and physical variables (FAO, 2010; 2012; 2013). Vidigal *et al.* (2019) reasoned that legumes are essential to agricultural systems of the tropics, especially in Sub-Saharan Africa. On the other hand, their adoption has not yet had the revolutionary effect that it has in temperate zones.

Legumes provide a significant amount of N, but their full potential is still unrealized, especially in tropical regions. Worse still, not much is known about how legumes in tropical Africa fix N. By supplying N to soil, biological N fixation maintains or improves soil fertility in agricultural systems. In turn, that increases soil productivity, both directly and indirectly. In poorer nations, growing cereals and legumes together is a customary farming method. Generation of more N and dry matter might result in benefits from mixed cropping. Cowpea crops obtain large portion of their N from the atmosphere and are typically more effective at fixing N (Ayalew & Yoseph, 2022). Its capacity to fix atmospheric N would contribute to enhancing soil fertility and raising crop yields in future. In addition to fixing atmospheric N and favourably influencing soil's N balance, cowpea is high in protein. Cowpea's support of small-scale agricultural systems is essential to maintaining long-term crop fertility. If done correctly, inoculation can help establish effective N-fixation on newly planted seeds. Particularly in areas where a legume from cross-inoculation group has never been grown before or where unfavourable soil conditions such as drought or acidity, have significantly decreased the population of N-fixing bacteria in soil.

2.8 EFFECT OF PSB ON COWPEA GROWTH AND YIELD

Cowpea contributes significantly to food and nutritional security, particularly for low-income households in Africa. The crop is also widely consumed by rural people in South Africa as it is commonly cultivated in rural communities. However, deficiency of essential nutrient elements in croplands, especially P, impairs cowpea growth. Consequently, cowpea yield and nutritional quality are adversely affected. Since bio-fertilizers fix atmospheric nitrogen and increase crop P availability, they significantly reduce the need for synthetic fertilizers (Selvakumar *et al.,* 2012). Bio-fertilizers have significant and advantageous impact on crop productivity and plant's ability to absorb nutrients from soil. Factors such as soil fertility, soil carbon content, soil biodiversity, and climate affect the action of these inoculants. Release of inorganic P is facilitated by PSB, which also enhances availability of P to developing plants. After hydrolyzing insoluble substances, PSB bacteria enhance organic and inorganic P content. PSB releases low molecular weight organic acids that chelate cations' hydroxyl and carboxyl groups to form a link with phosphate, converting inorganic P into accessible P. Gupta *et al.* (2014) pointed out that bacteria from genera *Rhizobium, Pseudomonas*, and *Bacillus* play significant function in solubilization of P. PSBs could regulate P in P-deficient soils. That is why Panhwar *et al.* (2012) proposed using PSBs in crop production. Although the role of PSB in increasing plant P availability is well studied in various crops, there is currently a dearth of information regarding its potential application in treating cowpea seeds.

2.9 EFFECTS OF SOIL TYPE ON COWPEA PRODUCTION

Crop agriculture in small-holder sector of Sub-Saharan Africa is notorious for low crop productivity. One of the reasons for this is soil fertility-related variables, particularly the variable soil types such as Hutton (dystrophic) and Glenrosa. The former is prevalent in high rainfall and sub-humid areas. The latter is found in arid and semi-arid areas. Related to the variable type of soil are soil texture and its organic matter, which affect production of grain legumes (Wei *et al*., 2020). Soil texture and organic matter content are key determinants of the rate of decomposition, a process that affects availability of organic substrates and net mineralization (Mouhamad *et al*., 2015). Among the types of soil texture, clay hinders the quick decomposition and breakdown of decomposable organic molecules (Mouhamad *et al*., 2015). Influence of soil texture on organic matter decomposition is explained by protective effect of clay against organic matter degradation. The latter is achieved by the formation of complexes between metal ions associated with large clay surfaces and high CEC (Ugwu & Igbokwe, 2019). Compared to sandy soils, clays have higher levels of organic matter (Mtangadura *et al*., 2017). When soils have a low organic matter content, they tend to store fewer mineral nutrients such as N, P, and some trace elements (Regassa *et al.,* 2023).

2.10 GAP IN KNOWLEDGE AND CONTRIBUTION OF THE STUDY

Although increased growth and yield of cowpea have been demonstrated with the addition of P-fertilizers, small-holder farmers rarely use them due to their scarcity and high costs. Cultivation of food legume crops such as cowpeas using the co-adding P-fertilizer, rhizobial inoculation, and P-solubilising bacteria approach increases availability of P in soil, its uptake, and accumulation, as well as cowpea growth and yield. This approach can potentially enhance cowpea growth and yield, thereby improving the income of farmers and providing food and nutritional security. To address food security and nutritional needs of resource-poor farmers and the general public, results of this study are crucial. They would help improve our knowledge about effects of using a combination of bio-fertilizer, Bradyrhizobium, and P-fertilizer on cowpea growth and yield. Research conducted in various agro-ecologies across Sub-Saharan Africa has shown that co-application of Bradyrhizobium inoculation and P-fertilizers enhances cowpea grain yield. On the other hand, adoption and use of bio-fertilizers containing PSB have increased in the Global South. Unfortunately, very little is known about how these PSBs, combined with P-fertilizers and *Bradyrhizobium* inoculants, affect cowpea growth and yield in South Africa. Though cowpea significantly contributes to food security in many areas, there are still obstacles concerning maximization of its productivity. A crucial element that impacts growth and productivity of cowpeas is accessibility of vital nutrients, including P. Cowpea cultivation is hindered in many agricultural systems by low soil fertility, particularly low levels of P. Utilizing bio-fertilizers is a viable and sustainable way to improve soil fertility and encourage plant development. Nevertheless, little is known about the effectiveness of bio-fertilizers, especially when combined with P application. Such lack of knowledge includes how bio-fertilizers affect yield-related metrics and cowpea P nutrition in various soil types.

CHAPTER 3: RESEARCH METHODOLOGY AND RESEARCH DESIGN

3.1 DESCRIPTION OF EXPERIMENTAL SITES

3.1.1 ARC SITE

The seasonal mean temperature at ARC site ranges between 10.14°C and 31.18°C for minimum and maximum temperatures, with an annual rainfall of 445.2 mm. Soils at ARC-TSC are classified as Hutton soil form, with an average clay content of 22%, a pH of 5.98 (KCl), and P content of 33 mg/kg.

3.1.2 SABIE RIVER SITE

Seasonal average minimum temperature at Sabie River site was 8.89 °C, and maximum temperature was 30.75 °C, with an annual rainfall of 480.9 mm. Soils at Sabie River site are classified as Glenrosa soil form, with an average clay content of 32%, a pH of 5.51(KCl), and P concentration of 7 mg/kg.

Experiments were conducted in two sites as shown and described below.

Figure 1: Site 1 – Agricultural Research Council – Tropical and Subtropical Crops (ARC TSC)

Source: Google Maps.

Figure 2: Site 2-Ndlovu farm–Sabie River.

Source: Google map

9

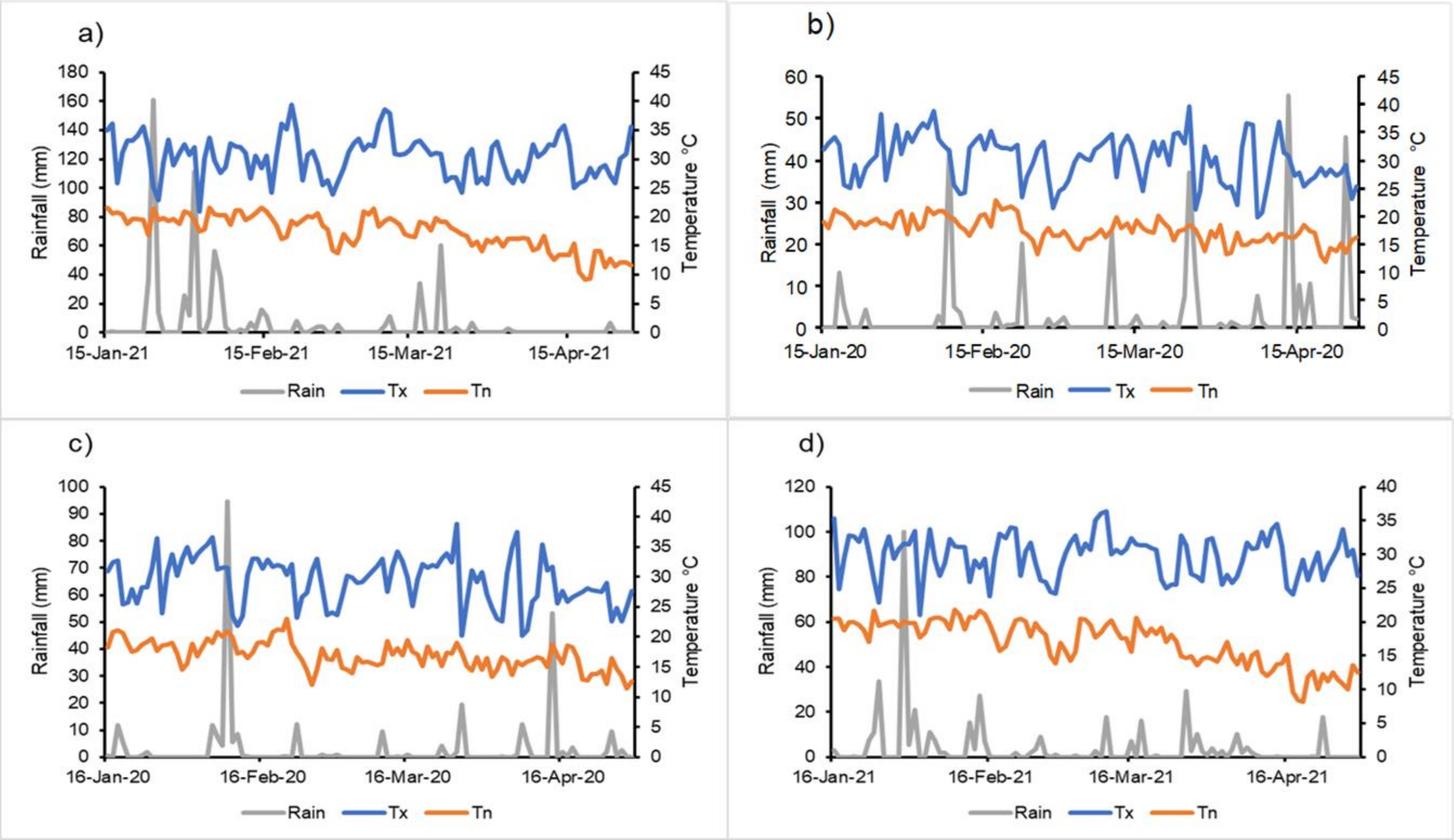

Figure 3: Weather data for the 2019/20 season 1 and 2020/21 season 2 at the ARC (A and B) and Sabie River (C and D) in South Africa.

Legend: T ˚C: Maximum temperature; T ˚C: Minimum temperature; Rain: Rainfall (mm).

3.2 SOIL SAMPLING AND ANALYSIS

3.2.1 Description and characteristics of soils

Soil of the experimental plot at ARC location is Hutton soil type. Soil at Sabie River location is Glenrosa soil type (Soil Classification Working Group, 1991). Glenrosa soil comprises an Orthic A topsoil horizon. Its subsoil horizon is classified as Lithocutanic B. In contrast, Hutton soil comprises Orthic A topsoil horizon that overlies a red apedal B subsoil horizon (overlying unspecified material). At each study location, plot area was ploughed. Thereafter, topsoil was randomly collected across the plot surface area. That is, topsoil was collected from five randomly selected spots, at a depth of between 0 - 20 cm. Samples from each location were pooled together to obtain a composite sample. Each composite sample was air-dried and sieved (2 mm). Samples were used to determine soil texture using the hydrometer method (Bouyoucos, 1962). Soil pH was measured in 1:2.5 soil-to-solution ratio using a 1 M potassium chloride (KCl) solution (Thomas, 1996). P was measured using Bray 1 method (Bray & Kurtz, 1945), and cation exchange capacity (CEC) was measured using ammonium acetate extraction method (Chapman, 1965). Concentrations of extractable cations (Ca, Mg, K, andNa) were extracted using 1 M NH_4OAc (ammonium acetate, pH 7) (Okalebo *et al.,* 2002). Micronutrients (Fe, Cu, Zn, and Mn) were extracted using 0.25 M ethylene diamine tetraacetic acid (EDTA) (Garcia *et al.,* 1993).

3.3 EXPERIMENTAL SET-UP

Cowpeas contribute to solubilisation and availability of P through the activity of acid and alkaline phosphatases (Makoi *et al*., 2010). Field study was conducted using a 2 x 5 factorial experiment layout in Randomised Complete Block Design (RCBD) replicated three times. Experimental design had two factors: namely Sabie River location (Glenrosa soil type) and ARC location (Hutton soil type), and five treatments, namely Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

These factors are site (soil types) and biofertiliser treatments. Sites (soil types) were Hutton and Glenrosa. Fertilisers were namely control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P BIO). Cowpea cultivar seeds [Glenda] were obtained from Agricol in Potchefstroom. Seeds were treated with liquid bio-fertilizers prepared using stock solution of 10 ml of Rhizovital in 80 ml of water. Approximately 9 ml of stock solution was added to 1 kg of cowpea seeds in a plastic bag and thoroughly mixed to ensure that bacteria were evenly distributed throughout seeds. Seeds were then placed in shade for few minutes to absorb liquid into their seed coat. Seeds were planted as soon as they were dry enough to handle.

Seed inoculation with *Bradyrhizobium* was achieved by weighing 0.5 kg of cowpea seeds into plastic bag and adding mixed content of 20 ml (comprising 0.5 litres of water with a sachet, 1-1.5 g) of stimulym as sticker and dissolving it. Seeds and stimulant solution were thoroughly mixed. Then, 5 g of peat-based inoculant (according to manufacturer's recommendation) was applied to bagged seeds and thoroughly mixed until all seeds were completely covered with inoculant. Seeds were treated in the field before planting. To minimize contamination, non-inoculated plots were planted first.

During planting, superphosphate fertilizer was band applied in all plots at the rate of 50 kg p/ha, banded 5 cm away (side) and 5 cm below seed row. Only the control plot did not receive this type of fertilizer. Cowpea seeds were sown manually in holes with depth of 1.5 cm. Space between plots was 1 m. Replicates had spacing of 1 m in between as well. Each plot measured 4 m in length, with 4 rows having an inter-row spacing of 70 cm and an intra-row spacing of 10 cm, respectively. Area of each plot was 11.2 m^2. In total, there were 20 plots with five treatments. These treatments were replicated four times. Weeding was manually done (using a hoe), two weeks after plants were planted. Thereafter, it was done four - five weeks after planting. To control pests and diseases, cypermethrin was used to spray aphids on cowpeas. Spraying was done in both locations during the two planting seasons.

To manage aphid infestations on cowpeas, cypermethrin (active ingredient: 200 g/L EC formulation) was applied as an insecticidal spray. The chemical was prepared at a concentration of 0.5 ml per litre of water (equivalent to 100 g a.i/ha) following the manufacturer's recommendations. Spraying was carried out using knapsack sprayer fitted with a fine nozzle to ensure even coverage of both upper and lower leaf surfaces. Applications were made once per week during the cropping season, or as soon as aphid populations reached economic threshold levels (early signs of infestation on tender shoots and leaves). Spray regime was implemented consistently at both experimental sites and across the two planting seasons. Protective clothing (gloves, mask, and overalls) was used during mixing and application to ensure operator safety. Spraying was always conducted in early morning or late afternoon to minimize chemical drift and reduce harm to beneficial insects.

3.4 DATA COLLECTION

3.4.1 Plant material sampling and analysis

3.4.1.1 Determination of dry matter and nodulation

At flowering stage, six cowpea plants were randomly sampled from each experimental plot from middle rows. Individual plant stands were uprooted, and above-ground material, used as shoots, was placed into a sample paper bag. Material below-ground root nodules on each root were counted, and numbers were recorded and then put into a sample paper bag. Plant materials in sample paper bags were placed in hot (65°C) oven for three consecutive days until constant weight was achieved. Weight of each material was determined by its dry weight. Nodules on each root were counted and recorded. Root nodules were stored in sterile phosphate-buffered saline (PBS) solution to maintain their integrity. Nodules and shoot material were then separately placed into paper bags and oven-dried at 65°C for three consecutive days until constant weights were attained. Their individual dry weights were then determined.

3.4.1.2 Grain yield and dry matter at physiological maturity

At physiological maturity, ten plants were randomly selected and sampled from middle rows of each plot. The pods on each plant were counted and threshed. Seeds were weighed to determine grain yield. Dry weight of threshed pods from each plant was determined. Above-ground dry matter weight was also determined. Harvest index was calculated as the ratio of economic yield to above-ground dry matter and expressed as percentage.

Grain Yield = weight of sample (kg) /area of sample (m^2) x 10 000 m^2 (1).

Where: Yg = Grain yield in kilograms per hectare (kg/ha), W_{sample} = Total weight of threshed grain from sample in kilograms (kg), A_{sample} = Area of harvested sample in square meters (m^2), 10,000 = Conversion factor from square meters to hectares (10,000 m^2=1 ha).

HI = Yg/B (2).

Where: HI= Harvest Index (%), Yg = Economic yield based on grain yield (kg), B= above-ground dry matter (kg).

3.4.1.3 Sample collection and P concentration determination in leaves and grain

During flowering stage, top-canopy leaves were plucked from selected plants in middle rows of each experimental plot. Fresh green leaf material of each plant was placed in sample paper bag, and then oven-dried at 65˚C for three consecutive days to constant weight. Correspondingly, grain samples were collected from middle row plants in each experimental plot. Grain of each plant was oven-dried to constant weight for three consecutive days. Leaf and grain samples were ground into fine powder. Concentration of P in shoot and grain samples was determined by ashing 1 g of pounded sample into porcelain crucible at 500˚C overnight. The process was followed by dissolving ash in 5 ml of 6 M HCl and placing it in an oven at 50˚C overnight for 30 minutes before adding 35 mL of de-ionised water. Combined mixture was filtered with Whatman No. 1 filter paper. Concentration of P in plant was determined using inductively coupled plasma (ICP) analysis (Giron, 1973).

3.4.2 Soil sample and analysis

3.4.2.1 Collection of rhizosphere soil and determination of P in rhizosphere soils

To account for variability, four plants were selected from each experimental plot to serve as representatives. Top layer of soil near cowpea plant base was carefully removed without disturbing roots. Soil auger was used to dig a hole approximately 20 cm deep. This depth was where most of the root activity occurred. Roots were gently shaken to remove bulk soil, which was then placed into a clean plastic bag. Rhizosphere soil was collected from multiple plants at each sampling point. Samples were all placed into one clean container to create a homogeneous sample. Homogenous sample was air-dried for 24 hours. It was then sieved using a 2 mm sieve. Available P was determined using Bray1 method (Bray & Kurtz, 1945).

3.5 DATA ANALYSIS

Soil analysis and plant parameter data collected were subjected to variance analysis using GENSTAT 24 program (VSN International, 2025). That is, GenStat for Windows 24th Edition, VSN International, Hemel Hempstead, UK.<URL to GenStat website or “Genstat.co.uk>. Where significant differences within treatments were observed, comparison of means was conducted using the least significant difference (LSD) test at a 5% significance level.

CHAPTER 4: RESULTS

4.1 EXPERIMENT SITE SOILS: SELECTED PHYSICAL AND CHEMICAL PROPERTIES

Topsoil at the two sites differed in both texture and nutrient status (Table 5). At ARC site, Hutton soil was classified as sandy, whereas at Sabie River site, Glenrosa soil was sandy loam. Although both soils were slightly acidic (pH 5.51 and 5.98, respectively), and had generally low CEC values indicative of limited nutrient retention, their contrasting textures and nutrient profiles strongly influenced cowpea performance. Higher clay and silt fractions in Glenrosa (9.6% and6.4%, respectively) contributed to greater CEC (4.81 cmolc/kg), which enhanced its ability to hold base cations such as Ca, Mg, K, and Na. However, this soil was severely limited in P (7 mg/kg), which constrained growth despite its relatively richer nutrient base. In contrast, Hutton soil, with its very sandy texture (94% sand) and low CEC (2.47 cmolc/kg), was poor in most base nutrients but contained substantially higher available P (33 mg/kg). This higher native P availability appears to explain superior cowpea performance at ARC site, despite its soil's lower nutrient-holding capacity. Results, therefore, suggest that P availability, rather than general cation supply, was the most critical factor determining treatment response and growth outcomes across the two sites.

Table 1: Selected physicochemical properties of Hutton and Glenrosa soil types at ARC and Sabie River experimental sites.

Parameters	Unit	Glenrosa (Sabie River)	Soil fertility status	Hutton (ARC)	Soil fertility status
pH (KCl)		5.51	Slightly acidic	5.98	Slightly acidic
Available Nitrogen	mg/kg	31	low	21	low
Calcium	mg/kg	617	Moderate	293	Low
Magnesium	mg/kg	177	Moderate	103	Moderate
Potassium	mg/kg	82	Moderate	52	Low
Sodium	mg/kg	14	Low	6	Low
Phosphorus	mg/kg	7	Low	33	High
Zinc	mg/kg	1.0	Low	3.0	Low
Copper	mg/kg	2.0	Low	3.8	Low
Manganese	mg/kg	27	Low	21	Low
Iron	mg/kg	23	Low	15	Low
CEC	$cmol_c$/kg	2.47	Low	4.81	Low
Textural classification					
Clay (%)		9.6	3.2		
Sand (%)		84	94		
Silt (%)		6.4	2.8		
Soil Textural Class		Loam Sand	Sand		

4.2 THE 2019/20 PLANTING SEASON COWPEA DRY MATTER, NODULATION, AND GRAIN YIELD

Interactive effect of soil type and bio-fertilizer treatment had highly significant ($p \leq 0.01$) effect on all measured growth and yield-related parameters (Table 2). Cowpea grown in sandy Hutton soil produced significantly greater shoot dry weight and root dry weight, number of root nodules, nodule dry weight, number of pods/plant, and dry weight of pods, and grain yield than that obtained from loamy sand Glenrosa soil type of Sabie River site. Shoot dry weight of cowpea grown at ARC location was higher (almost 2-fold) during flowering (2000 kg/ha) and harvest (1891 kg/ha) stages compared to that of plants grown at Sabie River site (1049 and 950 kg/ha at flowering and harvest, respectively). Root dry weight of plants planted at ARC location was almost five times greater than that of their counterparts at Sabie River site. Number and dry weight of root nodules of cowpea planted at ARC site were markedly higher (48 and 13 g/plant, respectively) compared to those of the plants grown at Sabie River site (31 and 2 g/plant, respectively). Number of pods/plant determined in cowpeas planted in sandy soil was 48% higher than that of cowpeas planted in loamy-sand soil. Grain yield produced by plants grown in sandy soil was 67% greater than that of those grown in loamy sand soil.

Adding BR + P + BIO increased shoot dry weight as measured during flowering and harvest stages, compared to all study treatments. As expected, cowpea planted without the application of either agro input (control) had the least shoot dry weight, as determined during flowering and harvest stages. Application of BIO + P and BR + P revealed similar shoot dry weight, as determined during flowering and harvest stages. But, shoot dry weight was significantly enhanced compared to that raised with the addition of P. A similar trend was observed for root dry weight as measured during flowering stage. Adding BR + P + BIO significantly improved the number of nodules/plant and nodule dry weight. Application of BR + P particularly increased dry weight of root nodules. Meanwhile, non-treatment controls formed least root nodules (13) and nodule dry weight (35 g/plant). By contrast, adding BR + P and BR + P + BIO significantly enhanced the number of pods/plant relative to non-treatment controls, which exhibited the least (27) pods/plant. Adding BR + P and BR + P + BIO notably increased cowpea grain yield when compared to that obtained from treated plants. Control treatment provided lowest yield (1524 kg/ha).

Table 2: Cowpea shoot and root dry matter, nodule number, dry weight, number of pods, pod weight, and grain yield of 2019/20 planting season.

	Shoot dry matter (kg/ha)		**Root dry matter (kg/ha)**	**Nodule (number/plant)**	**Nodule dry weight (g/plant)**	**Pods (number/plant)**	**Pods weight g/plant**	**Grain yield (kg/ha)**
	Flowering stage	**Harvest maturity**	**Flowering stage**					Harvest maturity
Soil type (ST)								
Hutton (ARC)	2009a	1891a	518a	48a	89a	46a	36a	2345a
Glenrosa (Sabie River)	1049b	950b	104b	31b	70b	31b	26b	1404b
Treatment (TR)								
control	1127d	1018d	199d	13c	35e	27c	22d	1524d
P	1328c	1226c	257c	25b	54d	38b	27d	1664cd
BIO+P	1535b	1423b	335b	25b	74c	40b	31c	1899bc
BR+P	1649b	1562b	354b	30a	100b	44a	36b	2079ab
BR+P+BIO	2005a	1874a	409a	32a	134a	46a	39a	2206a
SED	131	150	28	403	9.4	403		272
p-values								
Soil type (ST)	<.001	<.001	<.001	<.001	<.001	<.0001	<.0001	<.001
Treatments (TR)	<.001	<.001	<.001	<.001	<.001	<.0001	<.0001	<.001
ST*TR	0.015	0.014	<.001	0.244	<.001	0.0253	0.032	0.225
CV (%)	8.6	10.5	8.9	11	11.9	10.5	9.1	14.5

Values with dis-similar letters in a column indicate that means are significantly different at $p<0.05$ and those values with similar letters are non-significant. Abbreviations: Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO). SED-standard error of variation; CV-coefficient of variation.

Interactive effect of soil type and treatment (BR + P +BIO) was highly significant ($p \leq 0.01$) on shoot dry weight as obtained during flowering and harvest stages, and cowpea root dry matter, nodule dry weight, pod weight, and pod number grown in both study locations (see Table 2; Fig. 4). Cowpea grown in Glenrosa soil type at Sabie River site produced higher shoot dry weight at both flowering stage (Fig. 4a: a) and harvest stage (Fig. 4a:b) across all treatments (control P, BIO + P, BR + P, and BR + P + BIO). In contrast, cowpea grown in Hutton soil at ARC site showed lower shoot dry weight.

Significant soil and treatment interaction was observed for cowpea shoot dry weight at both flowering and harvest stages. Among the treatments, combined application of BR + P + BIO consistently produced highest shoot dry weight, which was significantly greater than that of the control in both soils. Similar trend was observed for root dry matter (Fig. 4b: a), nodule dry weight (Fig. 4b: b), number of pods (Fig. 4c: a), and pod weight (Fig. 4c: b) at harvest time. Moreover, both BR + P + BIO (10 g/plant) and BR + P treatments increased nodule dry weight (at flowering) compared to that of the control (4 g/plant) planted in both Glenrosa and Hutton soil types (see Fig. 4b: b). BIO-P and P showed non-significant difference from each other in both Glenrosa and Hutton soil types. In addition, treatment BR + P + BIO significantly improved pod dry weight compared to that of non-treatment control plants grown in both soil types. BR + P, BIO + P, and P also significantly increased pod dry weight, with their effectiveness ranked from highest to lowest (Fig. 4).

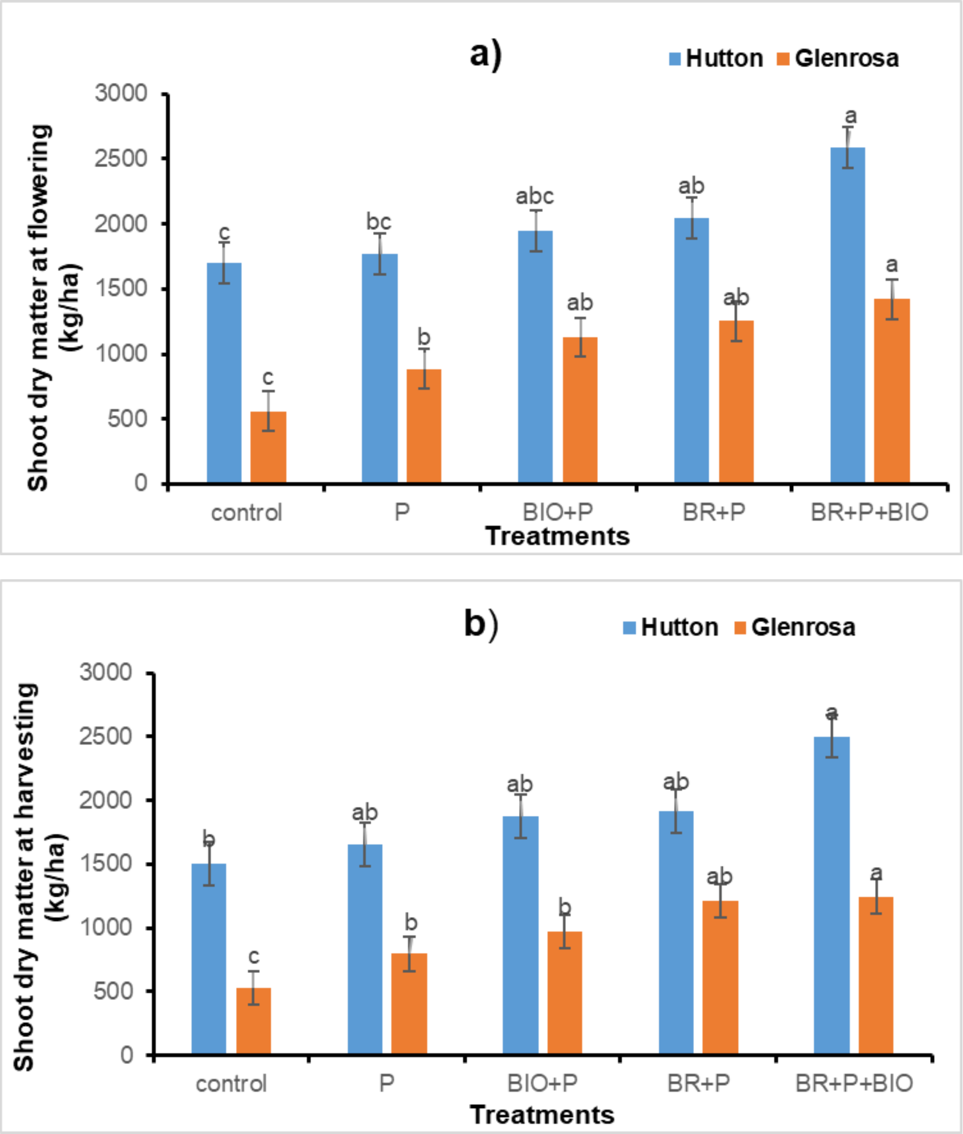

Figure 4a: Two-way analysis showing interactive effects of Hutton soil (sand) at ARC and Glenrosa soil (loamy sand) at Sabie River site, and treatments on a) shoot dry matter at flowering stage, and b) shoot dry matter at harvest of cowpea in the 2020 season. Each bar represents the mean (n = 5). Treatments show significant differences at $p \leq 0.05$. Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

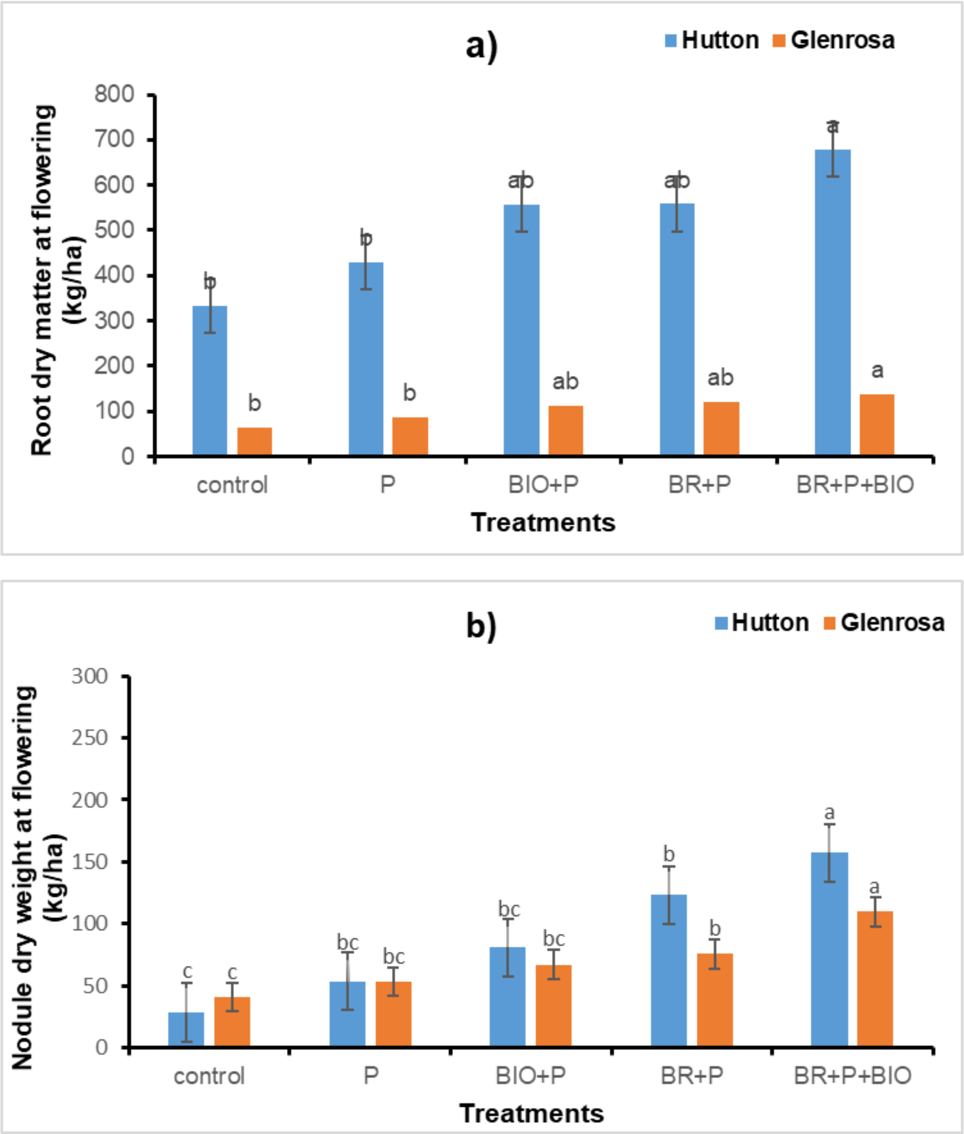

Figure 4b: Two-way analysis showing the interactive effects of the Hutton soil type of the ARC and Glenrosa soil type of Sabie River, and treatments on a) root dry matter at flowering stage, and b) nodule dry weight of cowpea in the 2020 season. Each bar represents the mean (n = 5). Treatments show significant differences at p≤0.05. Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

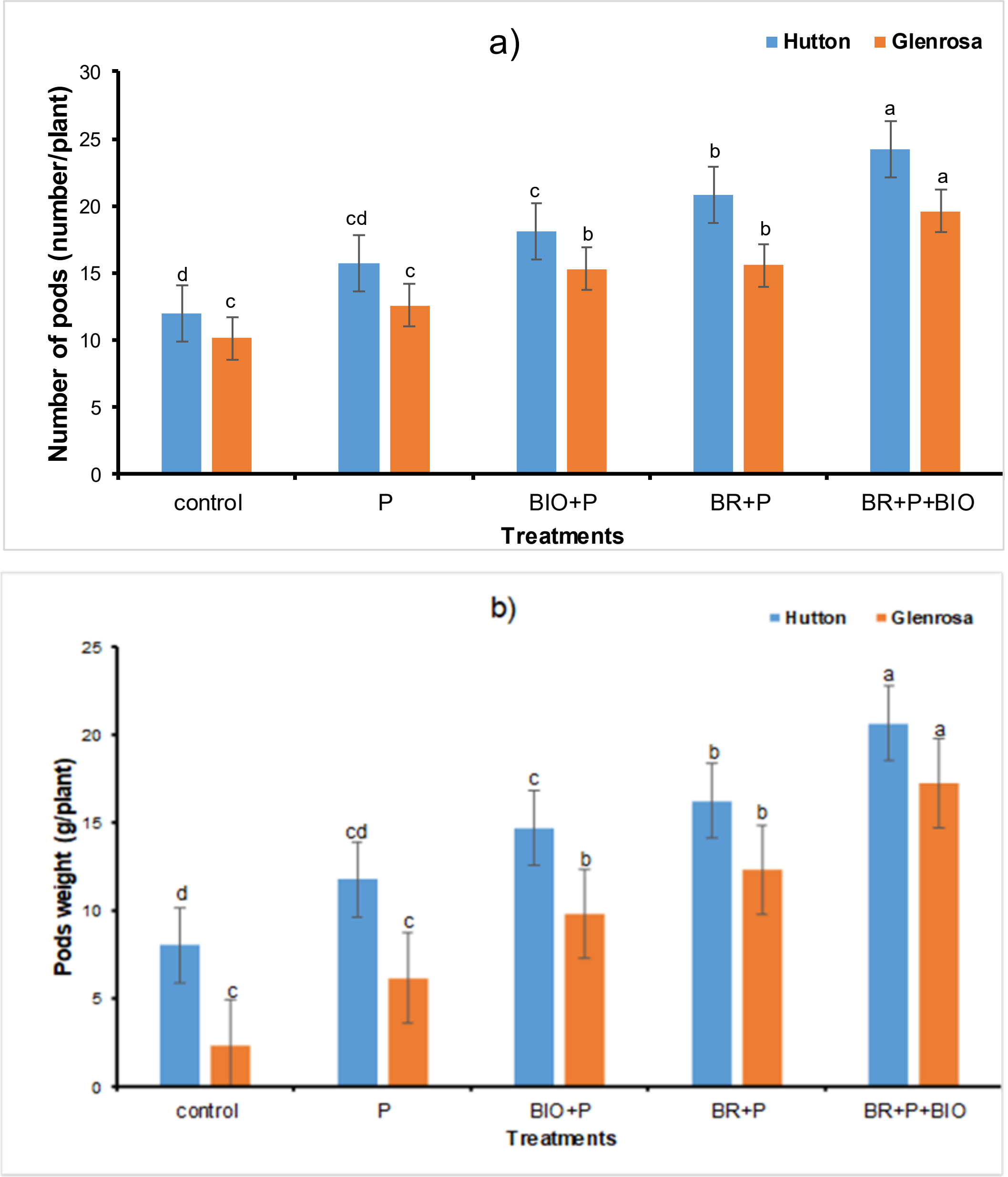

Figure 4c: Two-way analysis showing the interactive effects of the Hutton soil type at the ARC site and the Glenrosa soil type at Sabie River, and treatments on: a) shoot dry matter at flowering stage, pods number, and b) pod weight of cowpea in the 2020 season. Each bar represents the mean (n = 5). Treatments show significant differences at $p \leq 0.05$. Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

4.3 COWPEA DRY MATTER, NODULATION, AND GRAIN YIELD OF THE 2020/21 SEASON

Interactive effect of soil type and treatments was significantly different ($p \leq 0.01$) on all growth and yield parameters (Table 3). Plants grown in Hutton soil form had significantly higher shoot and root dry weights, number of root nodules, and nodule dry weight, number of pods, pod dry weight, and grain yield compared to those of plants planted in loamy-sand at Sabie River site. Cowpea grown in Hutton soil at ARC produced significantly greater shoot dry matter at flowering (1151 kg/ha) and harvest (1083 kg/ha) stages relative to that of plants grown at Sabie River site at flowering (1031kg/ha) and harvest (866 kg/ha) stages. However, interactive effect of soil type and treatments showed no significant differences on root dry weight. Notably, root dry mass in sandy soil (261 kg/ha) was significantly higher compared to that of cowpea grown in Glenrosa soil type (238 kg/ha). Number of pods produced by plants planted in sandy soil was 20% higher than that of plants grown in loamy-sand soil, while grain yield of cowpea grown in sandy soil was 49% higher than that from loamy-sand soil.

Cowpea grown with the addition of BR + P + BIO, BR + P, and BIO + P produced a significantly higher ($p<0.001$) shoot dry weight at flowering, while adding BR + P + BIO, BR + P increased shoot dry weight during harvest stages when compared to all other treatments. Plants raised as non-treatment controls produced the least shoot dry weight at flowering and harvest stages. Application of BIO + P and BR + P showed similar shoot dry weights when measured during flowering and harvest growth stages. But, these were significantly higher compared to those of plants provided with P-fertilizer. Similar trend was observed with shoot dry weight, which was also noted for root dry matter at flowering. Cowpea supplied with the BR + P + BIO provided greater number of root nodules and nodule dry weight as determined during flowering stage, compared to that of cowpea supplied with other treatments. Treatment BR + P + BIO had a significantly higher number of pods/plant and pod weight (19 g/plant) than all other treatments. Control treatment had the least (11) number of pods/plants. Treatment BR + P + BIO had a significantly higher grain yield (1893 kg/ha) than all other treatments. Control treatment had lowest yield (518 kg/ha).

Table 3: Cowpea shoot and root dry matter, nodule number, dry weight, number of pods, pod weight, and grain yield of the 2020/21 planting season.

	Shoot dry matter (kg/ha)		**Root dry matter (kg/ha)**	**Nodules (number/plant)**	**Nodule dry weight (g/plant)**	**Number of pods (number/ plant)**	**Pods weight (g/plant)**	**Grain yield /(kg/ha)**
	Flowering stage	**Harvest maturity**		**Flowering stage**			**Harvest maturity**	
Soil Type (ST)								
Hutton (ARC)	1151a	1083a	261a	29a	12.6a	18a	14a	1426a
Glenrosa (Sabie River)	1031b	866b	238b	7b	2.4b	15b	10b	956b
Treatment (TR)								
control	872c	779c	175c	6e	4d	11e	5e	518e
P	1067b	925b	241b	8d	6.4c	14d	9d	896d
BIO+P	1135ab	970b	265ab	13c	8b	17c	12c	1224c
BR+P	1149ab	1094a	270ab	27b	8.7b	18b	14b	1425b
BR+P+BIO	1233a	1105a	297a	34a	10.4a	22a	19a	1893a
SED	101	86	39	237	1.3	130		105
p-values								
ST	<.001	<.001	0.067	<.001	<.001	<.0001	<.0001	<.001
TR	<.001	<.001	<.001	<.001	<.001	<.0009	<.0001	<.001
ST *TR	<.001	0.861	0.079	<.001	0.005	0.0717	0.111	0.146
CV (%)	9.3	8.8	15.4	13.4	17.9	7.9	8.3	8.8

Values with dissimilar letters in a column indicate that the means are significantly different at $p<0.05$. Values with similar letters are non-significant. Abbreviations: Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO). ED-standard error of variation; CV-coefficient of variation.

Interactive effect of soil type and treatment was highly significant ($p \leq 0.01$) on shoot dry matter at flowering stage, as well as nodule number and nodule dry weight in the 2020/21 season (Table 3; Fig. 5). Cowpea grown in Glenrosa soil type and that grown in Hutton soil type produced more shoot dry matter at flowering stage in BR+P+BIO treatment. This is compared to the control and P treatment in the 2020/21 season (Fig. 5a). There were no significant differences, however, between treatments BR+P and BIO+P in Glenrosa soil type when compared to BIO+P treatment in Hutton soil type treatment and control (Fig. 5b-a). In both Glenrosa and Hutton soil types, number of nodules at flowering stage was significantly higher in BR+P+BIO treatment than in control and P treatment (Fig. 5b-b).

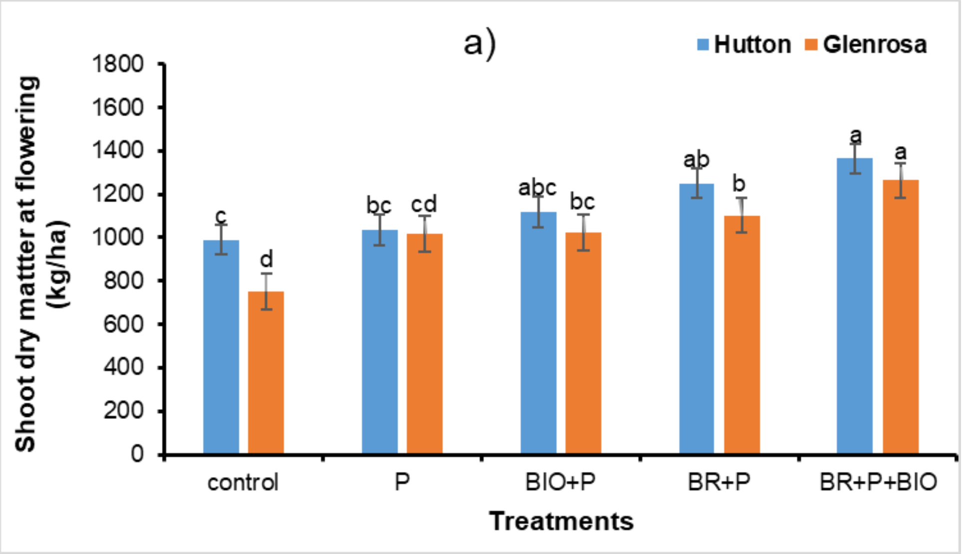

Figure 5a: Two-way analysis showing interactive effects of soil type (Hutton- ARC and Glenrosa-Sabie River) and treatments on: a) shoot dry matter at flowering stage of cowpea in the 2020/21 season. Each bar represents the mean (n = 5). Treatments show significant differences at ≤0.05. Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

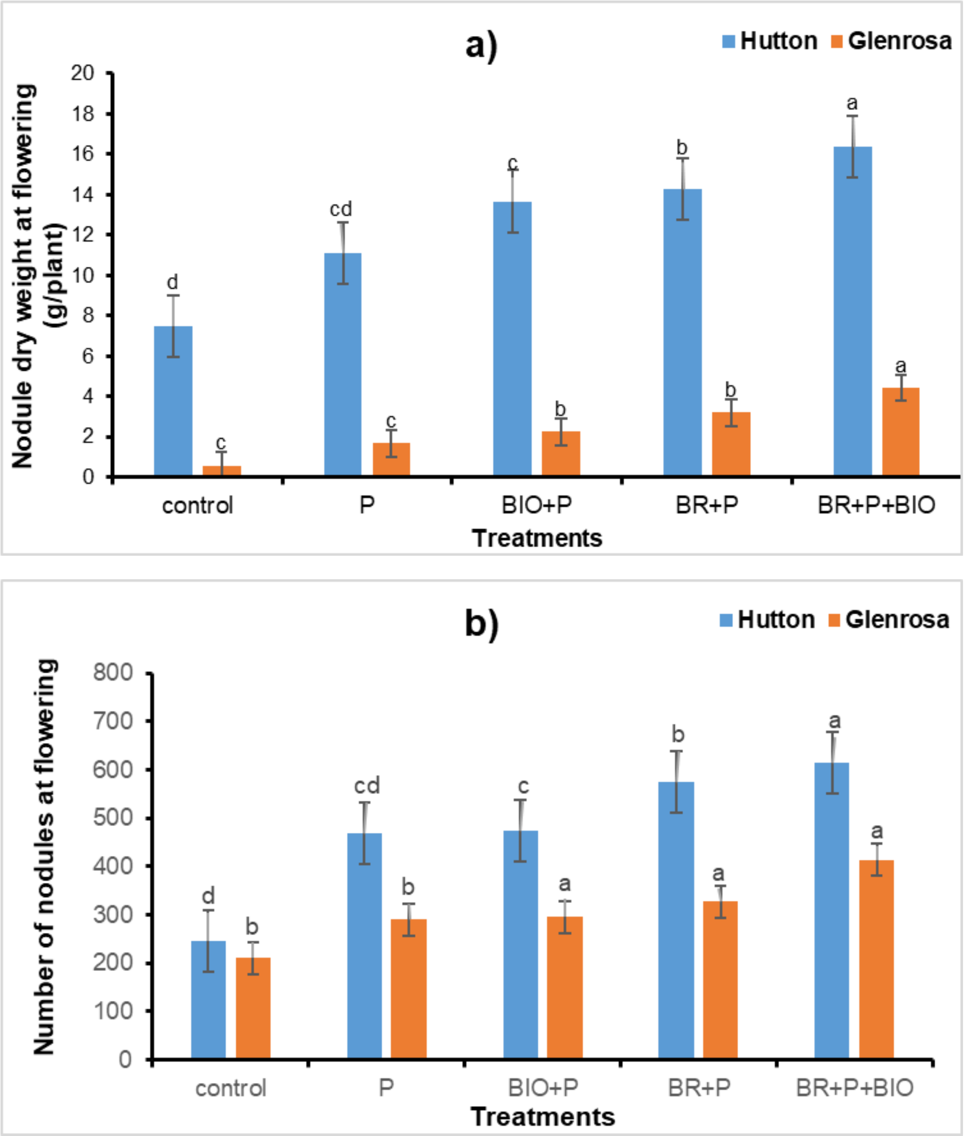

Figure 5b: Two-way analysis showing interactive effects of soil type (Hutton- ARC and Glenrosa-Sabie River) and treatments on: a) nodule dry matter at harvest, c) number of nodules at flowering stage, and b) pods weight of cowpea in the 2020/21 season. Each bar represents the mean (n = 5). Treatments show significant differences at ≤0.05 Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

4.4 EFFECTS OF SOIL TYPE AND TREATMENT ON COWPEA P NUTRITION AND AVAILABLE P IN RHIZOSPHERE SOIL IN THE 2019/20 AND 2020/21 SEASONS.

4.4.1 Available P in rhizosphere soil

Soil type and treatment had highly significant ($p \leq 0.01$) effect on rhizospheric P concentration (Table 4). Concentration of P in rhizosphere of cowpea grown in Hutton was significantly higher in the 2019/20 (232.6 mg/kg) and 2020/21 (143.7 mg/kg) (232.6) seasons compared to that of plants grown in Glenrosa soil type (65.1 mg/kg) in the 2019/20 and 2020/21 seasons. In both cropping seasons, adding BR + P + BIO to cowpea significantly increased the P in rhizosphere relative to that of plants supplied with other treatments. Rhizospheric P of control plants was the least in both seasons (102.4 mg/kg in 2019/20 and 64.9 mg/kg in 2020/21). Yet, other treatments (P, BIO + P, and BR + P) had intermediate values, with a general increase in available P in order control, P, BIO + P, and BR + P in both seasons (Table 4).

Table 4: The effect of soil type and different treatments on the rhizosphere soil available P in the 2019/20 and 2020/21 seasons.

	Soil available P mg/kg **2019/20 season**	**Soil available P mg/kg** **2020/21 season**
Soil type (ST)		
Hutton (ARC)	232.6a	143.7a
Glenrosa (Sabie River)	65.1b	60.8b
Treatment (TR)		
control	102.4d	64.9d
P	130.7c	91.5c
BIO+P	145.6bc	107.8b
BR+P	167.2b	114.7b
BR+P+BIO	198.3a	132.3a
SED	23	15
p-values		
ST	<.001	<.001
TR	<.001	<.001
ST*TR	0.158	0.04
CV (%)	15.2	14.7

Values with dissimilar letters in column. The means are significantly different at $p<0.05$. Values with similar letters are non-significant. Abbreviations: Control (C) - no fertilizer, P, *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P + BIO). SED-standard error of variation; CV-coefficient of variation.

Interactive effect of soil type and treatment was highly significant ($p\leq0.01$) on rhizospheric P concentration in the 2020/21 season (refer to Table 4; Fig. 6a). There was a general gradual increase in P in rhizosphere of plants grown in both Glenrosa and Hutton soil types (see Fig. 6a) and control, P + BIO + P, BR + P, and BR + P + BIO. Meanwhile, rhizosphere P of cowpea grown in Hutton soil was higher compared to that of their counterparts planted in Glenrosa soil type.

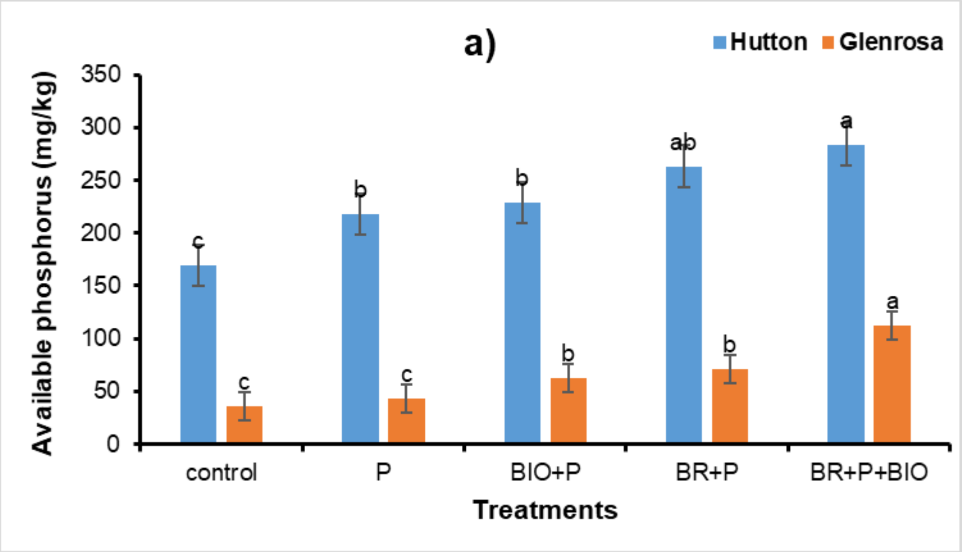

Figure 6: Two-way analysis showing interactive effects of soil type (Hutton- ARC, and Glenrosa-Sabie River) and treatment on; a) available phosphorus in rhizosphere soil of cowpea in the 2020/21 season. Each bar represents the mean (n = 5). Treatments show significant differences at p≤0.05. Control (C) - no fertilizer, P, *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO).

4.4.2 P content of shoot and grain

Soil type and treatment had a highly significant ($p \leq 0.01$) effect on both the shoot and grain P content in the 2019/20 and 20/2021 seasons (see Table 5). Cowpea grown in Hutton soil at ARC had higher shoot (5738 mg/kg) and grain P content (12091 mg/kg) than that grown in Glenrosa soil type. Compared to control and P, BR+P+BIO, BIO + P, and BR+ P treatments had more P content (5120 mg/kg) in shoot in the 2019/20 season. In the 2020/21 season, the trend remained consistent, with BR+P+BIO treatment having highest P content (4668 mg/kg) in shoot dry matter and (11430 mg/kg) in grains. This is compared to control being lowest (3567 mg/kg in shoot dry matter) and control, P, BIO+P (8792 mg/kg) being lowest in grains.

Table 5: Effect of soil type and different treatments on P content in shoot dry matter and cowpea grain in the 2019/20 and 2020/21 seasons.

	P content in shoot dry matter (mg/kg)	**P content in grains (mg/kg)**	**P content in shoot dry matter (mg/kg)**	**P content in grains (mg/kg)**
	2019/20 season		**2020/21 season**	
Soil type (ST)				
Hutton (ARC)	5738a	12091a	5144a	10975a
Glentosa (Sabie River)	3436b	10203b	3140b	8940b
Treatment (TR)				
control	3794c	9831c	3567d	8792c
P	4291b	10554bc	3860cd	9140c
BIO+P	4819a	10768bc	4234bc	9710bc
BR+P	4911a	11660b	4380ab	10716ab
BR+P+BIO	5120a	12920a	4668a	11430a
SED	311	1216	401	1088
p-values				
ST	<.001	<.001	<.001	<.001
TR	<.001	<.001	<.001	<.001
ST*TR	0.095	0.072	0.434	0.001
CV (%)	6.8	10.9	9.7	10.9

Values with dissimilar letters in column, the means are significantly different at p<0.05 and values with similar letters are non-significant. Abbreviations: Control (C) - no fertilizer, P, *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P +BIO). SED-standard error of variation; CV-coefficient of variation.

Interactive effect of soil type and treatment was highly significant (p ≤ 0.01) on P concentration of grain in the 2020/21 season (see Table 3; Fig. 6b). There was general trend of increasing P content in grain among treatments in both soils in order control, P, BIO + P, BR + P, BR + P + BIO, with amounts being slightly lower for Glenrosa soil type.

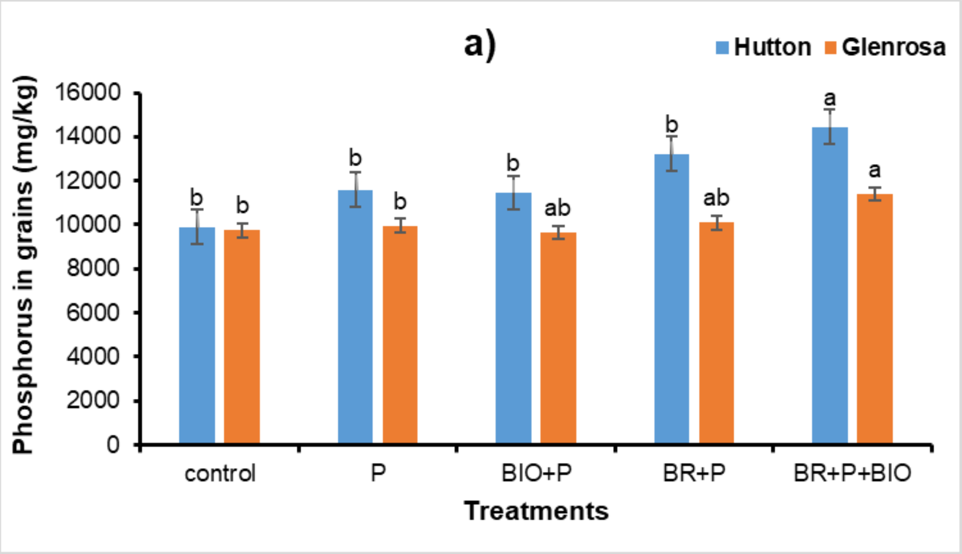

Figure 7: Two-way analysis showing interactive effects of soil type Hutton and Glenrosa and treatments on: a) P concentration in grains of cowpea in the 2020/21 season. Each bar represents the mean (n = 5). Treatments show significant differences at p≤0.05. Control (C) - no fertilizer, Phosphorus (P), *Bradyrhizobium* + Phosphorus (BR + P), Bio-fertilizer + Phosphorus (BIO + P), and *Bradyrhizobium* + Bio-fertilizer + Phosphorus (BR + P + BIO).

CHAPTER 5: DISCUSSION

The study evaluated effect of bio-fertilizer and P application on growth, yield components, and P nutrition of cowpea grown in locations with contrasting soil properties. Results highlight the role of differences in properties of soil types in efficacy of bio-inputs on growth, yield, and quality of cowpea as well as nutrients in those soils. For example, adding bio-fertilizers and P substantially improved shoot dry and root dry weights, nodule dry weight, nodule number, pod number, pod dry weight, and grain yield. Distinct variations were observed between Hutton and Glenrosa soil types. In general, it was observed that cowpeas grown in ARC soil outperformed those grown in Sabie River site across all growth and yield parameters. Distinct variations were observed between Hutton and Glenrosa soil types. Glenrosa soil at Sabie River site, which is loamy sand with higher clay (9.6%) and CEC (4.81 cmolc/kg), contained relatively higher levels of nitrogen, calcium, magnesium, potassium, and sodium but was limited in P (7 mg/kg). In contrast, Hutton soil at ARC site is coarse sandy soil with very low clay content (3.2%), low CEC (2.47 cmolc/kg), and generally poor in most nutrients, though it had comparatively higher available P (33 mg/kg) and micronutrients such as zinc and copper. In general, cowpeas grown in ARC soil outperformed those at Sabie River site across growth and yield parameters. This variation was attributed not only to soil nutrient differences but also to climatic conditions, as rainfall and temperature were higher at ARC site than at Sabie River site.

Additionally, there were differences in physicochemical properties of the two soil types. That is, ARC soil type is Hutton, with very high agricultural potential that is suitable for cowpea production. That soil type is also fertile and has high native P content presence in it. That creates a more favorable environment for bio-fertilizer and P application. On the other hand, Sabie River site soil type is Glenrosa. It is too shallow and hence unsuitable for agricultural production. That soil type has low native P content, which potentially restricts availability of nutrients and microbial activity essential for cowpea growth and yield. Thus, P was higher in Hutton soil compared to that of Glenrosa soil type in both the 2019/20 and 2020/21 seasons.

Observed increases in growth-related parameters of cowpea grown in Hutton soil could be attributed to high concentration of available P (33 mg/kg), which is considered sufficient for crop production. In addition, soil pH (5.98) was within a range favorable for cowpea grown in well-drained soils. Studies conducted in Cameroon, South Africa, and China (Ossom & Rhykerd, 2007; Mndzebele *et al.*, 2020; Gikonyo *et al.,* 2022) demonstrate that soil pH is a key variable with significant impact on cowpea growth, productivity, and yield. Addition of P-fertilizers, especially as triple superphosphate, increased level of soil pH, availability of mineral-bound P, and number of root nodules in soils (Phares *et al.,* 2020). Cowpea response to application of P was an indicator of optimum levels required to drive plant growth (Dugje *et al.,* 2009).

Application of BR + P + BIO significantly increased cowpea shoot, root dry matter, and grain yield in both soils during the 2019/20 and 2020/21 seasons. Increased dry matter was due to combined effects of BR + P + BIO on plant growth. For example, P plays key role in cell division and development of growing plant tip, thereby increasing plant growth (Liu, 2021). Rhizobial inoculants contribute to increased yield of legume crops, that is, at times between 10% and 28%. This increase was realised in peanut, soybean, black gram, pigeon pea, chickpea, and green gram (Arumugam *et al.*, 2021). Bio-fertilizers contain living micro-organisms that colonize rhizosphere, promoting plant growth and contributing to availability and uptake of mineral nutrients (Malusa & Vassilev, 2014; Fasusi *et al.,* 2021). Similarly, increased growth was possibly caused by the ability of rhizobial inoculants to produce plant growth-promoting substances such as auxins, gibberellins, and cytokinins, resulting in higher plant potency and growth (Xiao-Ping & Xi-Gui, 2006; Raspor *et al.,* 2021). Results obtained in this study are comparable to those of other reports, which indicate increased grain yield and grain P content associated with PSB, bio-fertilizer, and addition of P-fertilizer (Martins *et al.,* 2003; Mahanta, 2008; Musa *et al.,* 2011; Bilal *et al.,* 2021). Findings demonstrate benefits of co-applying PSB, bio-fertilizer, and P fertilization on cowpea growth and yield.

BR + P + BIO increased P content in shoots and grain of cowpea grown in Hutton soil type compared to that grown in Glenrosa soil type during the 2019/20 and 2020/21 cropping seasons. In this study, a higher concentration of P was recorded in shoots and grains of BR + P and BIO + P treated cowpeas planted in Hutton and Glenrosa soil types (see Fig. 3a). Accumulation of increased P in cowpea grain supplied with BR + P and BIO + P could have been due to improved availability of P in soil. Increased P content in cowpea shoots and grain was mostly expressed in BR + P + BIO treatment. This was probably due to the role that combined fertilizers played in cowpea growth and production. P application increased shoot P by enhancing root proliferation and energy transfer required for growth and yield. Applying P, Bradyrhizobium, and bio-fertilizer to soils could improve their fertility and availability of P. Doing so would enhance its uptake by roots and its accumulation in grains. If supply, interception, and uptake of P in soils are improved, partly through improvement of root biomass, its concentration in the above-ground parts also improves. That could explain its increased concentration in cowpea shoots and grain.

Uptake of P from soil is followed by its transportation to plant organs. Phytohormones present in bio-fertilizers contribute to enhanced efficacy of P-fertilizer (Raspor *et al.,* 2021). Therefore, there is an increase in plant growth and yield due to enhanced symbiotic nitrogen fixation (Xiao-Ping & Xi-Gui, 2006; Malhotra *et al.,* 2018; Raspor *et al.,* 2021). In croplands with low P levels, crops such as legumes undergo various morphological and physiological changes (Mitran *et al.,* 2021; Mitra *et al*., 2021; Malhotra *et al.,* 2018). This explains poor growth and yield of cowpea in low-P Sabie River site compared to high-P Hutton soils.

Bio-fertilizers contain living microorganisms that colonize rhizosphere and directly or indirectly promote plant growth by supplying essential nutrients (Malusa & Vassilev, 2014; Fasusi *et al.,* 2021). Results of this study are consistent with previous findings that application of PSB, bio-fertilizers, and P-fertilizers enhances cowpea and other legume growth, shoot development, and yield (Patel *et al.,* 2018; Srinivasamurthy & Dayamani, 2014; Namlı *et al*., 2017; Bationo *et al*., 2012; Unkovich *et al.,* 2010).

To address this challenge, farmers should adopt sound agronomic practices that include addition of lower-than-recommended rates of P-fertilizers in conjunction with bio-fertilizers (Ogola *et al*., 2023). As revealed above, combined application of the P-fertiliser with rhizobial inoculation increases accumulation of P in soils. Doing so leads to better uptake and accumulation by above-ground tissue, and hence increased growth and yield.

Results of this study demonstrate the importance of cultivating cowpeas with combination of agricultural inputs for sustainable production. Findings also show that co-added inputs can be considered an integrated soil fertility management approach that could maximise crop productivity. Most importantly, cowpea production requires combination of inorganic and bio-fertilizers for improved productivity, especially in low-nutrient croplands. Overall, production and consumption of cowpea crop have the potential to improve food and nutritional security in poor rural communities. This study also shows that bio- and P-fertilizers have positive effects on cowpea growth and yield-related parameters. Another key finding is the importance of considering soil types in agricultural practices, as variations in soil characteristics can impact effectiveness of applied fertilizers.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study aimed to assess the impact of bio-fertilizer, Bradyrhizobium inoculants, and P application on growth, yield, and P nutrition of cowpeas in two distinct soil types. Findings clearly indicate that combined use of these inputs significantly enhanced soil P availability, plant growth parameters, and yield-related parameters, particularly in sandy Hutton soil. This supports the study's aim by demonstrating the potential of integrated nutrient management practices to address P deficiencies, a critical limitation for small-holder farmers. These findings offer practical methods for enhancing crop yields, particularly in areas with P deficiencies. Small-holder farmers can utilize synergistic combination of bio-fertilizers, Bradyrhizobium, and P fertilizers to enhance soil fertility and crop yields, while reducing their dependence on chemical inputs. This method is particularly relevant for sandy soils with elevated drainage such as Hutton, which shows increased reactivity to treatments. For loamy soils such as Glenrosa, the technique remains advantageous but may necessitate supplementary amendments or increased application rates.

Despite positive results, some restrictions must be acknowledged. The study was conducted over two planting seasons under field experimental conditions, which may not fully reflect the long-term effects or real-world variability. Soil factors such as organic matter content and microbial populations, which vary greatly between regions, may influence treatment success. Furthermore, the study did not include potential environmental consequences such as nutrient leaching or runoff.

Incorporating bio-fertilizer and P application could be considered a viable strategy to improve yield and P nutrition in cowpea cultivation. Key findings herein revealed significant increases in growth and yield-related parameters. These include shoot, root dry matter, nodule number per plant, nodule weight, number of pods per plant, pod weight, and grain yield. These increases were attributed to application of bio-fertilizer and P, particularly in soils that are P deficient. In addition, P availability and its uptake were enhanced, indicating an improved P nutrition in cowpea plants and soil. Further research and field trials, however, might be necessary to optimize application rates and assess long-term effects on soil fertility and crop performance.

6.2 RECOMMENDATIONS

The following recommendations were formulated based on study findings.

- Long-Term Impacts: Assess impacts of repeated use of bio-fertilizer and P on soil fertility, crop yield, and microbial diversity in soil for several years.
- Environmental Interactions: Understand interaction of bio-fertilizers with other nutrients like nitrogen and potassium in order to formulate balanced fertilization plan.
- Regional Variability: Conduct trials in different agro-ecological regions to test effectiveness of suggested management practices under various environmental conditions.

CHAPTER 7: REFERENCE

Abbas, S., Javed, M.T., Ali, Q., Azeem, M. and Ali, S., 2021. Nutrient deficiency stress and relation with plant growth and development. In *Engineering tolerance in crop plants against abiotic stress*. pp. 239-262. CRC Press.

Adebowale, A.S., 2011. *Evaluation of the possible application of cowpea genotypes in the farming systems of the Eastern Cape Province of South Africa* (Doctoral thesis, University of Fort Hare).

Adejumo, T.O., Florini, D.A. and Ikotun, T., 2001. Screening of cowpea cultivars for resistance to leaf smut. *Crop Protection*, *20*(4), pp. 303-309.

Agboola and Obigbesan, 2001; Agboola, A.A. and Obigbesan, G.O., 2001. Effect of different sources and levels of P on the performance and P uptake of IfeBrown variety of cowpea. Ghana Journal of Agricultural Science., 10 (1), pp.71–75.

Alewell, C., Ringeval, B., Ballabio, C., Robinson, D.A., Panagos, P. and Borrelli, P. 2020. Global phosphorus shortage will be aggravated by soil erosion. Nature Communications, 11(1), pp. 4546.

Aloo, B., Makumb, B. and Mbega, E. 2020. Plant Growth Promoting Rhizobacterial Biofertilizers for Sustainable Crop Production: The Past, Present, and Future. https://dspace.nm-aist.ac.tz/handle/20.500.12479/1913 24 May 2024.

Aloo, B.N., Tripathi, V., Makumba, B.A. and Mbega, E.R., 2022. Plant growth-promoting rhizobacterial biofertilizers for crop production: The past, present, and future. Frontiers in Plant Science, 13: 1002448.

Arumugam, V., Ismail, M.H. and Routray, W., 2021. Biofertilizers from food and agricultural by-products and wastes. *Biomolecular engineering solutions for renewable specialty chemicals: Microorganisms, products, and processes*, pp.419-448.

Asif, M., Searcy, C., Zutshi, A. and Fisscher, O.A.M., 2013. An integrated management systems approach to corporate social responsibility. Journal of Cleaner Production, 56, pp. 7–17.

Asiwe, J. a. N. 2009. Needs assessment of cowpea production practices, constraints and utilization in South Africa. African Journal of Biotechnology, 8(20). https://www.ajol.info/index.php/ajb/article/view/65978 24 May 2024.

Asiwe, J.A.N., 2009. Insect mediated outcrossing and geneflow in cowpea (*Vigna unguiculata* (L.) Walp): Implication for seed production and provision of containment structures for genetically transformed cowpea. *African Journal of Biotechnology*, *8*(2), pp. 226-230.

Asiwe, J.A.N., 2009. Needs assessment of cowpea production practices, constraints and utilization in South Africa. *African Journal of Biotechnology*, *8*(20), pp.5383–5388.
at:https://journals.plos.org/sustainability/article?id=10.1371/journal.pstr.0000123 [Accessed 29 November 2024].

Ayalew, T. and Yoseph, T., 2022. Cowpea (Vigna unguiculata L. Walp.): A choice crop for sustainability during the climate change periods. J. Appl. Biol. Biotechnol, 10(3), pp.154-162.

Badiane, F.A., Diouf, M. & Diouf, D., 2014. Cowpea. In M. Singh, I. S. Bisht, & M. Dutta, eds. Broadening the Genetic Base of Grain Legumes. New Delhi: *Springer India*: pp. 95–114. https://doi.org/10.1007/978-81-322-2023-7_5 29 May 2024.

Badiane, F.A., Diouf, M. and Diouf, D., 2014. Cowpea. In *Broadening the genetic base of grain legumes*. pp. 95-114. New Delhi: Springer India.

Bala, A.G. and Hassan, M.R., 2023. Feeding Forage Cowpea: Goats Performed Well with High Nutrient Digestibility and Nitrogen Retention. In *Goat Science-From Keeping to Precision Production*. IntechOpen.

Barragán-Ocaña, A. and del-Valle-Rivera, del C., 2016. Rural development and environmental protection through the use of biofertilizers in agriculture: An alternative for underdeveloped countries? *Technology in Society*, 46, pp. 90–99.

Barth, P., Stüeken, E.E., Helling, C., Rossmanith, L., Peng, Y., Walters, W. and Claire, M., 2023. Isotopic constraints on lightning as a source of fixed nitrogen in Earth’s early biosphere. Nature Geoscience, 16(6), pp.478-484.

Bationo, A., Waswa, B., Kihara, J., and Kimetu, J., 2012. *Advances in Integrated Soil Fertility Management in sub-Saharan Africa: Challenges and Opportunities.* Springer.

Belane, A.K., Asiwe, J. and Dakora, F.D., 2011. Assessment of N_2 fixation in 32 cowpeas (*Vigna unguiculata* L. Walp) genotypes grown in the field at Taung in South Africa, using 15N natural abundance. *African Journal of Biotechnology*, 10(55), pp. 11450–11458.

Bhardwaj, D., Ansari, M.W., Sahoo, R.K. and Tuteja, N., 2014. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, 13(1), p. 66.

Bhattacharyya, P.N. and Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, *28*, pp.1327-1350.

Bilal, S., Hazafa, A., Ashraf, I., Alamri, S., Siddiqui, M.H., Ramzan, A., Qamar, N., Sher, F. and Naeem, M., 2021. Comparative effect of inoculation of phosphorus-solubilizing bacteria and phosphorus as sustainable fertilizer on yield and quality of mung bean (*Vigna radiata* L.). *Plants*, *10*(10), p.2079

Blackshaw, R.E. and Brandt, R.N., 2009. Phosphorus fertilizer effects on the competition between wheat and several weed species. *Weed Biology and Management*, *9*(1), pp.46-53.

Boukar, O., Fatokun, C.A., Huynh, B.-L., Roberts, P.A. and Close, T.J. 2016. Genomic Tools in Cowpea Breeding Programs: Status and Perspectives. Frontiers in Plant Science, 7. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2016.00757/full 29 May 2024.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils 1. *Agronomy Journal*, *54*(5), pp.464-465.

Bray, R.H. and Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, *59*(1), pp.39-46.

Bressani R., 1985. Nutritive value of cowpea. In: Singh SR, Rachel KO, editors. Cowpea research, production and utilization. Chester, (UK): Wiley and sons, pp. 353-360

Bressani, R., 1993. Grain quality of common beans. *Food Reviews International*, *9*(2), pp.237-297

Chapman, H.D., 1965. Cation exchange capacity. In: C.A. Black, ed. *Methods of soil analysis. Part I: Physical and mineralogical properties, including statistics of measurement and sampling.* Madison: American Society of Agronomy, pp. 891–901.

Chaudhary, N., Patel, B.B. and Karnavat, R., 2016. Effect of FYM, Phosphorus, and PSB on Growth, Yield and Quality of Cowpea (*Vigna unguiculata* (L.) Walp) on Loamy Sand. *Advances in Life Sciences*, 5(8), pp.3311-3315.

Chaudhary, P., Singh, S., Chaudhary, A., Sharma, A. and Kumar, G. 2022. Overview of biofertilizers in crop production and stress management for sustainable agriculture. Frontiers in Plant Science, 13. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2022.930340/full 24 May 2024.

Chianu, J. N., Nkonya, E. M., Mairura, F. S., Akinnifesi, F. K., and Bationo, A. (2011). Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: A review. *Agronomy for Sustainable Development*, 31(1), 139-154.

Coulibaly, S., Pasquet, R.S., Papa, R. and Gepts, P.A.F.L.P., 2002. AFLP analysis of the phenetic organization and genetic diversity of Vigna unguiculata L. Walp. reveals extensive gene flow between wild and domesticated types. *Theoretical and Applied Genetics*, *104*, pp.358-366.

DAFF, 2011; DAFF. 2011. Department of Agriculture, Forestry and Fisheries. 2011. Production guidelines for cowpeas. Pretoria: Directorate of Agricultural Information Services. Available at http://www.daff.gov.za.).

DAR. 2015. Rhizobium biofertilizer. The result of research in Agricultural Research. Department of Agricultural Research. Yezin, Myanmar, pp. 14-118.

Denison, R.F. and Kiers, E.T., 2005. Evolutionary stability of rhizobium mutualism depends on legume host sanctions. *Current Plant Science and Biotechnology in Agriculture*, *41*, p. 221.

Denison, R.F. and Kiers, E.T., 2005. Evolutionary stability of rhizobium mutualism depends on legume host sanctions. *Current Plant Science and Biotechnology in Agriculture*, *41*, p. 221.

Department of Agriculture, Forestry and Fisheries (DAFF). 2011. Production guidelines for Cowpeas. Directorate Agricultural Information Services Pretoria, South Africa.

Dugje, I.Y., Omoigui, L.O., Ekeleme, F., Kamara, A.Y. and Ajeigbe, H., 2009. Farmers’ guide to cowpea production in West Africa. *IITA,* Ibadan, Nigeria, *20*, pp.12-14.

Earl, C.D. and Ausubel, F.M., 1983. The genetic engineering of nitrogen fixation. *Nutrition Reviews*, *41*(1), pp.1-6.

Ebrahimi, M., Khosravi, H. and Rigi, M., 2016. Short-term grazing exclusion from heavy livestock rangelands affects vegetation cover and soil properties in natural ecosystems of southeastern Iran. *Ecological Engineering*, *95*, pp.10-18.

Fageria, N.K. and Moreira, A., 2011. Chapter Four - The Role of Mineral Nutrition on Root Growth of Crop Plants. In D. L. Sparks, ed. Advances in Agronomy. Academic Press: 251–331. https://www.sciencedirect.com/science/article/pii/B9780123855312000049 24 May 2024.

FAO (2013, 2012, 2010). FAOSTAT. Food and Agricultural Organization of the United Nations (FAO), Rome. Retrieved from http://faostat.fao.org

FAOSTAT Database.http://faostat.fao.org/site/291/default.aspx).

FAOSTAT, 2016; FAOSTAT (2016) Food and Agriculture Organization of the United Nations (FAO).
Fasusi, O.A., Cruz, C. and Babalola, O.O., 2021. Agricultural sustainability: microbial biofertilizers in rhizosphere management. *Agriculture*, *11*(2), p.163.

Fatimah, N., Dar, S.A., Ashraf, S., Rashid, S., Mukhtar, Y. and Mir, S.H., 2021. Biofertilizers for Sustainable Agriculture-An Overview. *Journal homepage: http://www. ijcmas. com*, *10*(06), p.2021.

Fery, R.L. and Singh, B.B., 1997. Cowpea genetics: a review of the recent literature. *Advances in Cowpea Research*, p.13.

Fixen, P. E. (2005). What is the value of improving phosphorus efficiency? *Better Crops with Plant Food*, 89(2), pp.3-6.

García, G., Galvez, J.F. and De Blas, J.C., 1993. Effect of substitution of sugarbeet pulp for barley in diets for finishing rabbits on growth performance and on energy and nitrogen efficiency. *Journal of Animal Science*, *71*(7), pp.1823-1830.

Gbaye, O.A. and Holloway, G.J., 2011. Varietal effects of cowpea, *Vigna unguiculata*, on tolerance to malathion in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Journal of Stored Products Research*, *47*(4), pp. 365-371.

Geisseler, D. and Scow, K.M., 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. *Soil Biology and Biochemistry*, *75*, pp. 54-63.

Gerrano, A., Jansen van Rensburg, W., Mathew, I., Shayanowako, A., Bairu, M., Venter, S., Swart, W., Mofokeng, M., Mellem, J. and Labuschagne, M., 2020. Genotype and genotype × environment interaction effects on the grain yield performance of cowpea genotypes in dryland farming system in South Africa. *Euphytica*, 216.

Gerrano, A.S., Adebola, P.O., Jansen van Rensburg, W.S. and Laurie, S.M., 2015. Genetic variability in cowpea (*Vigna unguiculata* (L.) Walp.) genotypes. *South African Journal of Plant and Soil*, *32*(3), pp.165-174.

Gerrano, A.S., Jansen van Rensburg, W.S., Venter, S.L., Shargie, N.G., Amelework, B.A., Shimelis, H.A. and Labuschagne, M.T., 2019. Selection of cowpea genotypes based on grain mineral and total protein content. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *69*(2), pp.155-166.

Gerrano, A.S., van Rensburg, W.S.J. and Adebola, P.O., 2017. Nutritional composition of immature pods in selected cowpea [*'Vigna unguiculata'* (L.) Walp.] genotypes in South Africa. *Australian Journal of Crop Science*, *11*(2), pp.134-141.

Gikonyo, F.N., Dong, X., Mosongo, P.S., Guo, K. and Liu, X., 2022. Long-term impacts of different cropping patterns on soil physico-chemical properties and enzyme activities in the low land plain of North China. *Agronomy*, *12*(2), p.471.

Giron, H.C., 1973. Comparison between dry ashing and wet digestion in the preparation of plant material for atomic absorption analysis. *Atomic Absorption Newsletter*. 12, pp. 28-29.

Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E.A., Ferreira, L. and Rodrigues, M., 2016. Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the Science of Food and Agriculture*, 96(9), pp. 2941-2951.

Gupta, B. and Huang, B., 2014. Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. *International Journal of Genomics*, 2014(1), p. 701596.

Gyaneshwar, P., Hirsch, A.M., Moulin, L., Chen, W.M., Elliott, G.N., Bontemps, C., Estrada-de Los Santos, P., Gross, E., dos Reis Jr, F.B., Sprent, J.I. and Young, J.P.W., 2011. Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. *Molecular Plant-Microbe Interactions*, *24*(11), pp.1276-1288.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T. and Yermiyahu, U., 2015. The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy*, *130*, pp.141-174.

Haruna, I.M. (2011). Dry matter partitioning and grain yield potential in sesame (*Sesamum indicum* L.) as influenced by poultry manure, nitrogen, and phosphorus at Samaru, Nigeria*. Journal of Agricultural Technology*, 7(6), 1571-1577.

Hawkesford, M.J., Cakmak, I., Coskun, D., De Kok, L.J., Lambers, H., Schjoerring, J.K. and White, P.J. 2023. Functions of macronutrients☆. In Z. Rengel, I. Cakmak, & P. J. White, eds. *Marschner's Mineral Nutrition of Plants* (Fourth Edition). San Diego: Academic Press: 201–281. https://www.sciencedirect.com/science/article/pii/B9780128197738000198 5 June 2024.

Hedin, L.O., Brookshire, E.J., Menge, D.N. and Barron, A.R., 2009. The nitrogen paradox in tropical forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *40*, pp. 613-635.

Horn, L.N. and Shimelis, H., 2020. Production constraints and breeding approaches for cowpea improvement for drought prone agro-ecologies in Sub-Saharan Africa. *Annals of Agricultural Sciences*, *65*(1), pp.83-91.

Itelima, J.U., Bang, W.J., Onyimba, I.A., Sila, M.D. and Egbere, O.J., 2018. Bio-fertilizers as key player in enhancing soil fertility and crop productivity: A review. *Agricultural Reviews*, *39*(4), pp. 284-293.

Jalal, A., Filho, M.C.M.T., da Silva, E.C., da Silva Oliveira, C.E., Freitas, L.A. and do Nascimento, V., 2022. Plant growth-promoting bacteria and nitrogen fixing bacteria: sustainability of non-legume crops. In Nitrogen fixing bacteria: sustainable growth of non-legumes (pp. 233-275). Singapore: Springer Nature Singapore.

Janati, W., Benmrid, B., Elhaissoufi, W., Zeroual, Y., Nasielski, J. and Bargaz, A., 2021. Will phosphate bio-solubilization stimulate biological nitrogen fixation in grain legumes?. Frontiers in Agronomy, 3, p.637196.

Jansa, J., Finlay, R., Wallander, H., Smith, F.A. and Smith, S.E. 2011. Role of Mycorrhizal Symbioses in Phosphorus Cycling. In E. Bünemann, A. Oberson, & E. Frossard, eds. Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Berlin, Heidelberg: Springer: 137–168. https://doi.org/10.1007/978-3-642-15271-9_6 5 June 2024.

Javed, A., Ali, E., Afzal, K.B., Osman, A. and Riaz, S., 2022. Soil fertility: Factors affecting soil fertility, and biodiversity responsible for soil fertility. International Journal of Plant, Animal and Environmental Sciences, 12(1), pp.21-33.

Kalegore, N.K., Gavhane, M.A., Bhusari, S.A., Kasle, S.V. and Dhamane, R.S. 2018. Response of Cowpea (*Vigna unguiculata*) to Inorganic and Biofertilizers. *International Journal of Economic Plants*, 5(Nov 4), pp. 167–169.

Karapanos, I., Papandreou, A., Skouloudi, M., Makrogianni, D., Fernández, J.A., Rosa, E., Ntatsi, G., Bebeli, P.J. and Savvas, D., 2017. Cowpea fresh pods–a new legume for the market: assessment of their quality and dietary characteristics of 37 cowpea accessions grown in southern Europe. *Journal of the Science of Food and Agriculture*, *97*(13), pp.4343-4352.

Kebede, E. and Bekeko, Z. 2020. Expounding the production and importance of cowpea (*Vigna unguiculata* (L.) Walp.) in Ethiopia M. Tejada Moral, ed. Cogent Food & Agriculture, 6(1), p.1769805.

Khoury, C.K., Achicanoy, H.A., Bjorkman, A.D., Navarro-Racines, C., Guarino, L., Flores-Palacios, X., Engels, J.M., Wiersema, J.H., Dempewolf, H., Sotelo, S. and Ramírez-Villegas, J., 2016. Origins of food crops connect countries worldwide. Proceedings of the royal society B: biological sciences, 283(1832), p.20160792.

Kimiti, J.M. and Odee, D.W., 2010. Integrated soil fertility management enhances population and effectiveness of indigenous cowpea rhizobia in semi-arid eastern Kenya. *Applied Soil Ecology*, *45*(3), pp.304-309.

Kumar, A. J. Prakash and Arora, N. K. 2015. Biofertilizers: an alternative source of chemical fertilizer for sustainable crops in 21st century. Pp: 28 – 31. *Microbiology World Issue* 10 Mar – Apr 2015, ISSN. pp. 2350 – 8774.

Kumar, A., 2023. Role of biotechnology in creating sustainable agriculture. *PLOS Sustainability and Transformation*. Available

Kumar, K., Shukla, U.N., Kumar, D., Pant, A.K., Prasad, S.K. and Prasad, K., 2013. Bio-Fertilizers for organic agriculture. *Popular Kheti*, *1*(4), pp.91-96.

Kumar, S., Diksha, Sindhu, S.S. & Kumar, R. 2022. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Current Research in Microbial Sciences, 3, p.100094.

Kwon, S.-J., Kim, H.-R., Roy, S.K., Kim, H.-J., Boo, H.-O., Woo, S.-H. and Kim, H.-H., 2019. Effects of Nitrogen, Phosphorus and Potassium Fertilizers on Growth Characteristics of Two Species of Bellflower (*Platycodon grandiflorum*). *Journal of Crop Science and Biotechnology*, 22(5), pp. 481–487.

Kyei-Boahen, S., Savala, C.E., Chikoye, D. and Abaidoo, R., 2017. Growth and yield responses of cowpea to inoculation and phosphorus fertilization in different environments. *Frontiers in Plant Science*, *8*, p.238588.

Lambot, C., 2002. Industrial potential of cowpea. *Challenges and opportunities for enhancing sustainable cowpea production*, pp.367-375.

Lazaridi, E., Ntatsi, G., Savvas, D. and Bebeli, P.J. 2017. Diversity in cowpea (*Vigna unguiculata* (L.) Walp.) local populations from Greece. Genetic Resources and Crop Evolution, 64(7), pp. 1529–1551.

Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D.C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K. and Connell, S., 2016. Genomic insights into the origin of farming in the ancient Near East. *Nature*, *536*(7617), pp. 419-424.

Liu, D., 2021. Root developmental responses to phosphorus nutrition. *Journal of Integrative Plant Biology*, *63*(6), pp.1065-1090.

Madzivhandila, V., 2020. *The effect of phosphorus fertilizer and bradyrhizobium innoculation on grain yield and nutrients accumulation in two chickpea (Cicer aritienum L.) genotypes* (Doctoral dissertation).

Mahanta, D., Rai, R.K., Mishra, S.D., Raja, A., Purakayastha, T.J. and Varghese, E., 2014. Influence of phosphorus and biofertilizers on soybean and wheat root growth and properties. Field Crops Research, 166, p. 1–9.

Makoi, J.H.J.R., Belane, A.K., Chimphango, S.B.M. and Dakora, F.D., 2010. Seed flavonoids and anthocyanins as markers of enhanced plant defence in nodulated cowpea (*Vigna unguiculata* L. Walp.). Field Crops Research, 118(1), pp.21–27.

Malhotra, H., Vandana, Sharma, S. and Pandey, R., 2018. Phosphorus nutrition: plant growth in response to deficiency and excess. In Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K. & Hawrylak-Nowak, B. (eds.), *Plant Nutrients and Abiotic Stress Tolerance*, Springer, Singapore, pp. 171-190.

Malusa, E. and Vassilev, N., 2014. A contribution to set a legal framework for biofertilisers. *Applied Microbiology and Biotechnology*, *98*, pp.6599-6607.

Mamiro, P.S., Mbwaga, A.M., Mamiro, D.P., Mwanri, A.W. and Kinabo, J.L. 2011. Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. African Journal of Food, Agriculture, Nutrition and Development, 11(1). https://www.ajol.info/index.php/ajfand/article/view/65876 29 May 2024.

Mapfumo, P. 2011. Comparative Analysis of the Current and Potential Role of Legumes in Integrated Soil Fertility Management in Southern Africa. In A. Bationo, B. Waswa, J. M. Okeyo, F. Maina, J. Kihara, and U. Mokwunye, eds. Fighting Poverty in Sub-Saharan Africa: The Multiple Roles of

Legumes in Integrated Soil Fertility Management. Dordrecht: Springer Netherlands: 175–200. https://doi.org/10.1007/978-94-007-1536-3_8 5 June 2024.

Mapope, N. and Dakora, F.D., 2016. N_2 fixation, carbon accumulation, and plant uptake relations in soybean (*Glycine max* L. Merrill) varieties sampled from farmers' fields in South Africa, measured using ^{15}N and ^{13}C natural abundance. *Agriculture, Ecosystems and Environment*. 221, pp. 174-186.

Margenot, A.J., Singh, B.R., Rao, I.M. and Sommer, R. 2016. Phosphorus Fertilization and Management in Soils of Sub-Saharan Africa. In Soil Phosphorus. CRC Press.

Martins, L.M.V., Xavier, G.R., Rangel, F.W., Ribeiro, J.R.A., Neves, M.C.P., Morgado, L.B. and Rumjanek, N.G., 2003. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. *Biology and Fertility of Soils*, *38*, pp.333-339.

Maseko, M.S., Zungu, M.M., Ehlers Smith, D.A., Ehlers Smith, Y.C. and Downs, C.T., 2020. Effects of habitat-patch size and patch isolation on the diversity of forest birds in the urban-forest mosaic of Durban, South Africa. *Urban Ecosystems*, *23*(3), pp.533-542.

Maxted, N., Mabuza-Diamini, P., Moss, H., Padulosi, S., Jarvis, A. and Guarino, L. 2004. An ecogeographic study African *vigna*. https://hdl.handle.net/10568/105017 26 May 2024.

Mazid, M. and Khan, T.A., 2015. Future of biofertilizers in overview. Indian International Agriculture: *Journal of Agricultural and Food Research* 3(3), pp.10-23.

Mensah, K.H., 2021. Evaluation Of Yield And Sub-Yield Components Of Cowpea (Vigna Unguiculata (L) Walp) Accessions (Doctoral dissertation, University of Ghana).

Mitra, A., Kataki, S., Singh, A.N., Gaur, A., Razafindrabe, B.H.N., Kumar, P., Chatterjee, S. and Gupta, D.K., 2021. Plant stress, acclimation, and adaptation: A review. In Gupta, D.K. and Walther, C. (eds.), *Plant growth and stress physiology*, Springer, Cham, pp. 1-22.

Mndzebele, B., Ncube, B., Fessehazion, M., Mabhaudhi, T., Amoo, S., du Plooy, C., Venter, S. and Modi, A., 2020. Effects of cowpea-amaranth intercropping and fertiliser application on soil phosphatase activities, available soil phosphorus, and crop growth response. *Agronomy*, *10*(1), p.79.

Mndzebele, B., Ncube, B., Nyathi, M., Kanu, S.A., Fessehazion, M., Mabhaudhi, T., Amoo, S. and Modi, A.T., 2020. Nitrogen fixation and nutritional yield of cowpea-amaranth intercrop. Agronomy, 10(4), p.565.

Mohale, K.C., Belane, A.K. and Dakora, F.D., 2014. Symbiotic N nutrition, C assimilation, and plant water use efficiency in Bambara groundnut (*Vigna subterranea* L. Verdc) grown in farmers' fields in South Africa, measured using ^{15}N and ^{13}C natural abundance. Biology and Fertility of Soils, 50(2), pp. 307–319.

Mohammadi, K. and Sohrabi, Y., 2012. Bacterial biofertilizers for sustainable crop production: a review. I SSN, 7(5).

Mokwunye and Bationo, 2002. Meeting the phosphorus needs of the soils and crops of West Africa: the role of indigenous phosphate rocks. In *Integrated plant nutrient management in Sub-Saharan Africa: from concept to practice* (pp. 209-224). Wallingford UK: CABI Publishing.

Moseley, J. and Grossman, A.R., 2009. Chapter 6 - Phosphate Metabolism and Responses to Phosphorus Deficiency. In E. H. Harris, D. B. Stern, & G. B. Witman, eds. The Chlamydomonas Sourcebook (Second Edition). London: Academic Press: 189–215. https://www.sciencedirect.com/science/article/pii/B9780123708731000149 5 June 2024.

Mouhamad, R., Atiyah, A., Mohammad, R. and Iqbal, M., 2015. Decomposition of organic matter under different soil textures. Current Science, 1(1), pp.22-25.

Mtangadura, T.J., Mtambanengwe, F., Nezomba, H., Rurinda, J. and Mapfumo, P., 2017. Why organic resources and current fertilizer formulations in Southern Africa cannot sustain maize productivity: Evidence from a long-term experiment in Zimbabwe. PLoS One, 12(8), p.e0182840.

Muindi, E.D.M., 2019. Understanding soil phosphorus. *International Journal of Plant & Soil Science*, 31(2), pp.1-18.

Müller, F.L., Raitt, L.M., Chimphango, S.B., Samuels, M.I., Cupido, C.F., Boatwright, J.S., Knight, R. and Trytsman, M., 2017. Prioritisation of native legume species for further evaluation as potential forage crops in water-limited agricultural systems in South Africa. *Environmental Monitoring and Assessment*, *189*(10), p.512.

Muranaka, S., Shono, M., Myoda, T., Takeuchi, J., Franco, J., Nakazawa, Y., Boukar, O. and Takagi, H., 2016. Genetic diversity of physical, nutritional and functional properties of cowpea grain and relationships among the traits. *Plant Genetic Resources*, *14*(1), pp.67-76.

Musa, E.M., Elsheikh, E.A., Mohamed Ahmed, I.A. and Babiker, E.E., 2011. Effect of intercropping, Bradyrhizobium inoculation and N, P fertilizers on yields, physical and chemical quality of cowpea seeds. *Frontiers of Agriculture in China*, *5*, pp. 543-551.

Namlı, A., Mahmood, A., Sevilir, B. and Özkır, E., 2017. Effect of phosphorus solubilizing bacteria on some soil properties, wheat yield and nutrient contents. *Eurasian Journal of Soil Science*, *6*(3), pp. 249-258.

Njonjo, M.W., 2018. Quality of cowpea seed used by farmers in Makueni and Taita Taveta Counties and its effect on crop performance (Doctoral dissertation, University of Nairobi).

Nounagnon, M., Roko, G., Agbodjato, N.A., Dah-Nouvlessounon, D., Babalola, O.O. and Baba-Moussa, L., 2024. Cowpea (Vigna unguiculata). Potential Pulses: Genetic and Genomic Resources, pp.58-77.

Nuruzzaman, M., Lambers, H., Bolland, M.D. and Veneklaas, E.J., 2005. Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. *Australian Journal of Agricultural Research*, *56*(10), pp.1041-1047.

Nwagboso, C., Andam, K.S., Amare, M., Bamiwuye, T. and Fasoranti, A., 2024. The economic importance of cowpea in Nigeria trends and Implications for achieving agri-food system transformation. Intl Food Policy Res Inst.

Nwagboso, C., Andam, K.S., Amare, M., Bamiwuye, T. and Fasoranti, A., 2024. The Economic Importance of Cowpea in Nigeria. Trends and Implications for Achieving Agri-food System Transformation. IFPRI Discussion paper 02241, February.

Nwokwu, G.N., 2020. Effect of NPK (12: 12: 17) Fertilizer Rates on the Growth and Yield of Cowpea (*Vigna unguiculata*) Varieties. *Journal of Biology, Agriculture and Healthcare.*

Nyoki, D. and Ndakidemi, P.A. 2018. Rhizobium inoculation reduces P and K fertilization requirement in corn-soybean intercropping. *Rhizosphere*, 5, pp.51–56.

Ogbazghi, Z.M., Tesfamariam, E.H. and Annandale, J.G., 2016. Modelling N mineralisation from sludge-amended soils across agro-ecological zones: A case study from South Africa. Ecological modelling, 322, pp.19-30.

Ogola, J.B., Madzivhandila, V., Maseko, S.T. and Leboho, T.M., 2023. P-fertiliser and rhizobial inoculation increased the concentration of mineral nutrients in the rhizosphere of two chickpea genotypes. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *73*(1), pp. 94-101.

Okalebo, J.R., Gathua, K.W. and Woomer, P.L., 2002. Laboratory methods of soil and plant analysis: a working manual second edition. *Sacred Africa, Nairobi*, *21*, pp. 25-26.

Okereke, C.N., Iroka, C.F., Doris, C.N., Mediatrix, N.E., Christiana, N.I. and Ukpaka, C.G., 2017. Evaluation of the Differences in Morphometry and Biomass of *Abelmoschus Esculentus* (L.) Moench Cultivated under Three Conditions of Manuring in Abakaliki Peri-Urban Area, South-Eastern Nigeria. *SM Journal of Medicinal Plant Studies*, *1*(1), p.1003

Okereke, G.U., Egwu, S.E. and Nnabude, P., 2006. Effect of cowpea organic residues and fertilizer N on soil fertility, growth and yield of upland rice. *Proceedings of the Eighteenth World Congress of Soil Science. Philadelphia, Pennsylvania*.

Olaleye, O., Fagbola, O., Abaidoo, R. C., and Ikeorah, N., 2011. Phosphorus response efficiency in cowpea genotypes. IITA, Ibadan. Ublished by Canadian Centre of Sciences and Education. Pp.1-10.

Omundi, J.M., 2020. *Effects of Intercropping Cowpeas with Maize and Phosphorous Levels on Growth and Yields of Cowpeas in Meru County* (Doctoral dissertation, KeMU).

Opitz, I., Berges, R., Piorr, A. and Krikser, T., 2016. Contributing to food security in urban areas: differences between urban agriculture and peri-urban agriculture in the Global North. Agriculture and Human Values, 33(2), pp.341-358.

Ossom, E.M. and Rhykerd, R.L., 2007. Effects of corn (*Zea mays* L.) and grain legume associations on soil mineral nutrient concentration, soil temperature, crop yield, land equivalent ratio and gross income in Swaziland. In *8th African Crop Science Society Conference, El-Minia, Egypt, 27-31 October 2007* (pp. 225-230). *African Crop Science Society*.

Oyewale, R.O. and Bamaiyi, L.J. 2013. Management of Cowpea Insect Pests. http://repository.futminna.edu.ng:8080/jspui/handle/123456789/4151 24 May 2024.

Pandey, R.K., Herrera, W. A. T., Villegas, A.N. and Pendleton, J.W. 1984. Drought Response of Grain Legumes Under Irrigation Gradient: III. Plant Growth1. *Agronomy Journal*, 76(4), pp.557–560.

Panhwar, Q.A., Othman, R., Rahman, Z.A., Meon, S. and Ismail, M.R. 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. African Journal of Biotechnology, 11(11), pp. 2711–2719.

Patel, H.F., Maheriya, V.D., Attar, S.K. and Patel, H.R., 2018. Nutrient uptake and yield of Kharif green gram as influenced by levels of sulphur, phosphorus and PSB inoculation. *Legume Research-An International Journal*, *41*(3), pp. 405-409.

Phares, C.A., Atiah, K., Frimpong, K.A., Danquah, A., Asare, A.T. and Aggor-Woananu, S., 2020. Application of biochar and inorganic phosphorus fertilizer influenced rhizosphere soil characteristics, nodule formation and phytoconstituents of cowpea grown on tropical soil. *Heliyon*, *6*(10).

Quin, F.M., 1997. Importance of cowpea. *Advances in cowpea research. BB Singh, KE Dashiell, DR Mohan Raj and LEN Jackai (Eds.)*, pp.10-12.

Raimi, A., Adeleke, R. and Roopnarain, A., 2017. Soil fertility challenges and Biofertiliser as a viable alternative for increasing smallholder farmer crop productivity in sub-Saharan Africa. *Cogent Food & and Agriculture*, *3*(1), p.1400933.

Raspor, M., Motyka, V., Kaleri, A.R., Ninković, S., Tubić, L., Cingel, A. and Ćosić, T., 2021. Integrating the roles for cytokinin and auxin in de novo shoot organogenesis: from hormone uptake to signaling outputs. *International Journal of Molecular Sciences*, *22*(16), p. 8554.

Regassa, A., Kibret, K., Selassie, Y.G., Kiflu, A. and Tena, W., 2023. Soil properties. In The Soils of Ethiopia (pp. 111-156). Cham: Springer International Publishing.

Rowe, H., Withers, P.J.A., Baas, P., Chan, N.I., Doody, D., Holiman, J., Jacobs, B., Li, H., MacDonald, G.K., McDowell, R., Sharpley, A.N., Shen, J., Taheri, W., Wallenstein, M. and Weintraub, M.N., 2016. Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security. Nutrient Cycling in Agroecosystems, 104(3), pp. 393–412

Rychter, A. and Rao, I. 2005. Role of Phosphorus in Photosynthetic Carbon Metabolism. In M. Pessarakli, ed. Handbook of Photosynthesis, Second Edition. Books in Soils, Plants, and the Environment. CRC Press. http://www.crcnetbase.com/doi/abs/10.1201/9781420027877.ch7 24 May 2024.

Sansinenea, E., 2021. Chapter 14 - Application of biofertilizers: Current worldwide status. In A. Rakshit, V. S. Meena, M. Parihar, H. B. Singh, and A. K. Singh, eds. Biofertilizers. Woodhead Publishing: 183–190. https://www.sciencedirect.com/science/article/pii/B978012821667500004X 24 May 2024.

Sariah, J.E., 2010. *Enhancing cowpea (Vigna unguiculata L.) production through insect pest resistant line in East Africa* (Doctoral dissertation, University of Copenhagen, Faculty of Life Sciences [Department of Agriculture and Ecology]). University of Copenhagen. security and gender empowerment into the African Green Revolution. *Food Security*, *1*, pp. 351-360.

Selvakumar, G., Reetha, S. and Thamizhiniyan, P., 2012. Response of biofertilizers on growth, yield attributes and associated protein profiling changes of blackgram (*Vigna mungo L. Hepper*). *World Applied Sciences Journal*, *16*(10), pp.1368-1374.

Shah, F. and Wu, W., 2019. Soil and crop management strategies ensure higher crop productivity within sustainable environments. *Sustainability*, *11*(5), p.1485.

Sheahan, C.M., 2012. Plant guide for cowpea (*Vigna unguiculata*). *USDA-Natural Resources Conservation Service, Cape May Plant Materials Center, Cape May, NJ*. Singh, B.B. ed., 1997. *Advances in cowpea research*. IITA.

Shegro, G.A., Sternberg, J.V.R.W., Isack, M., IT, S.A., Wolday, B.M., Venter, S.L., Wijnand, S., Alina, M., Mellem, J. and Maryke, L., 2020. Genotype and genotype× environment interaction effects on the grain yield performance of cowpea genotypes in dryland farming system in South Africa. *Euphytica*, *216*(5).

Singh, D., Agusti, A., Anzueto, A., Barnes, P.J., Bourbeau, J., Celli, B.R., Criner, G.J., Frith, P., Halpin, D.M.G., Han, M., Varela, M.V.L., Martinez, F., Oca, M.M. de, Papi, A., Pavord, I.D., Roche, N., Sin, D.D., Stockley, R., Vestbo, J., Wedzicha, J.A. and Vogelmeier, C., 2019. Global Strategy for Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019. *European Respiratory Journal,* 53(5). https://erj.ersjournals.com/content/53/5/1900164 24 May 2024.

Singh, N., Singh, G., Aggarwal, N. and Khanna, V., 2018. Yield enhancement and phosphorus economy in lentil (*Lens culinaris Medikus*) with integrated use of phosphorus, Rhizobium and plant growth promoting rhizobacteria. *Journal of Plant Nutrition*, 41(6), pp. 737-748.

Soil Classification Working Group (1991). Soil Classification. A Taxonomic System for South Africa, 2nd (revised) edition, Memoirs on the Agricultural Natural Resources of South Africa no 15, Department of Agricultural Development, Pretoria, 257pp

Sommer, F. and Bäckhed, F., 2013. The gut microbiota—masters of host development and physiology. *Nature Reviews Microbiology*, *11*(4), pp. 227-238.

Srinivasamurthy, R. and Dayamani, K.J., 2014. Influence of Mineral Phosphate Solubilizing Gram-positive Bacteria on Growth and Yield of Cowpea (*Vigna unguiculata*). *International Journal of Scientific and Research Publications*, p. 565.

Stamford, N.P., Silva Junior, S.D., Rosália, C.E.D., Santos, S., Freitas, A.D.S.D., Lira Júnior, M.D.A. and Barros, M.D.F.C., 2013. Cowpea nodulation, biomass yield and nutrient uptake, as affected by biofertilizers and rhizobia, in a sodic soil amended with Acidithiobacillus. *Acta Scientiarum. Agronomy*, *35*, pp. 453-459.

Stamford, N.P., Silva Junior, S.D., Santos, C.E.D.R.E.S., Freitas, A.D.S.D., Lira Junior, M.D.A. and Barros, M.D.F.C., 2013. Cowpea nodulation, biomass yield and nutrient uptake, as affected by biofertilizers and rhizobia, in a sodic soil amended with Acidithiobacillus - doi: 10.4025/actasciagron.v35i4.16994. *Acta Scientiarum. Agronomy*, 35(4), pp. 453–459.

Tarawali, S.A., Singh, B.B., Fernández Rivera, S., Peters, M., Smith, J.W., Schultze-Kraft, R. and Ajeigbe, H.A., 1997. Optimizing the contribution of cowpea to food and fodder production in crop-livestock systems in West Africa. In *Singh, B.B., Mohan Raj, D.R., Dashiell, K.E., & Jackai, L.E.N. (eds.), Advances in Cowpea Research*. IITA, Ibadan, Nigeria & Japan International Research Center for Agricultural Sciences, Tsukuba, Japan, pp. 313-325.

Tarawali, S.A., Singh, B.B., Gupta, S.S., Tabo, R., Harris, F., Nokoe, S., Fernandez-Rivera, S., Bationo, A., Manyong, V.M., Makinde, K. and Odion, E.C., 2003. Cowpea as a key factor for a new approach to integrated crop–livestock systems research in the dry savannas of West Africa. In *Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. & Tamo, M. (eds.), Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. IITA, Ibadan, Nigeria, pp. 233-251.

Thomas, G.W., 1996. Soil pH and soil acidity. *Methods of soil analysis: part 3 chemical methods*, *5*, pp. 475-490.

Ugwu, I.M. and Igbokwe, O.A., 2019. Sorption of heavy metals on clay minerals and oxides: a review. Advanced sorption process applications, 2019, pp.1-23.

Unkovich, M. J., Baldock, J., and Forbes, M. (2010). Variability in harvest index of grain crops and potential significance for carbon accounting: Examples from Australian agriculture. *Advances in Agronomy*, 105, pp. 173-219.

Vacu, M.V., Nzama, P.S. and Adegbaju, M.S., 2025. Insights on the utilisation of tissue culture to aid new breeding techniques for cowpea (*Vigna unguiculata* L.) improvement. *Frontiers in Horticulture*, *4*, p.1520119.

Van Niekerk, J. and Wynberg, R., 2017. Traditional seed and exchange systems cement social relations and provide a safety net: A case study from KwaZulu-Natal, South Africa. *Agroecology and Sustainable Food Systems*, *41*(9-10), pp. 1099-1123.

Vanlauwe, B. and Zingore, S., 2011. Integrated soil fertility management: an operational definition and consequences for implementation and dissemination. Better Crops, 95(3), pp. 4-7

Vidigal, P., Romeiras, M.M. and Monteiro, F., 2019. Crops diversification and the role of orphan legumes to improve the Sub-Saharan Africa farming systems. In Sustainable crop production. IntechOpen.

Wei, C., Adamowski, J.F., Liu, Y., Zhang, Y., Liu, C., Zhou, J., Wang, X., Zhang, X. and Cao, J., 2020. A comparative study of rotation patterns on soil organic carbon in China's arid and semi-arid regions. Agronomy, 10(2), p.160.

White, P.J. and Brown, P., 2010. Plant nutrition for sustainable development and global health. *Annals of Botany*, *105*(7), pp. 1073-1080.

Xiao-Ping, S. and Xi-Gui, S., 2006. Cytokinin-and auxin-induced stomatal opening is related to the change of nitric oxide levels in guard cells in broad bean. *Physiologia Plantarum*, *128*(3), pp. 569-579.

Yoseph, T., Baraso, B. and Ayalew, T. 2017. Influence of Bradyrhizobia inoculation on growth, nodulation and yield performance of cowpea varieties. *African Journal of Agricultural Research*, 12(22): pp. 1906–1913.

Zaghloul, R. A., El-Husseiny, T. M., Ehsan, A., Hanafy, A., Rahal, G. H. and Abdelrahman, H. M. (2009): Interaction effect between biofertilization and organic manuring on some enzymes activity macronutrients and essential oil content of marjoram. *Microbiology Conference*., Cairo, Egypt. pp. 1-29.