Response of cowpea to integrated MYCOROOT™ inoculation, biochar-compost mixture and soil variation: drought tolerance and performance

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DECLARATION

I, Sezilungile Zenelile Coka declare that the dissertation, which I hereby submit for the degree of Master of Science in Agriculture at the University of Mpumalanga has not previously been submitted to any other university. I declare that this is my original work, conducted under the supervision of Professor F.R. Kutu. All other sources of data and information used are appropriately acknowledged.

Student's signature: Coka SZ Date: 01/02/2024

Supervisor's signature…………………… Date…01 February 2024…

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DEDICATION

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ABSTRACT

Cowpea (*Vigna unguiculata* L.) also known as black eye pea, is an annual heat-loving leguminous crops cultivated mostly in the semi-arid environments as grain and vegetable crop for human consumption and as animal feeds. It is a highly nutritious crop, rich in protein, fibre and other nutritional components such as vitamin, minerals and other secondary metabolites. Cowpea grain availability in the local market in South Africa remains very low due to poor soil fertility status, low soil moisture condition and low production level mainly by smallholder farmers. Adoption of improved production practices such as Mycoroot^{TM}, a locally produced biofertilizer containing natural *Arbuscular mycorrhizae fungi (AMF)* have been used to enhance crop growth due to their ability to facilitate water and nutrients uptake and facilitate osmotic adjustments under adverse conditions such as moisture stress. Hence, a greenhouse pot experiment was conducted to examine the combined effect of Mycoroots^{TM} AMF inoculation with varied biochar-compost mixtures as an agronomic package to enhance cowpea growth, phenological and yield attributes in two soil textural types with different moisture levels. Trial consisted 2 soil textural types (sandy loam and loamy sand), 4 soil amendments comprising different mix ratios of biochar (BC) and compost (C), 2 AMF levels (inoculated and uninoculated) and 2 soil moisture regimes (adequate soil moisture and moisture stressed) as main treatment factors. The soil amendments comprised of 50% biochar 50% compost (50:50 BC/C), 75% biochar 25% compost (75:25 BC/C), 25% biochar 75% compost (25:75 BC/C) and a control with no amendment. The treatment factors were combined and laid out in a 2x2x4x2 factorial design fitted into RCBD with each replicated 4 times. Data collected included growth, phenological, yield attributes, grain nutrients and secondary plant metabolites.

Results revealed a significant ($p<0.05$) soil textural types and moisture levels interaction effect on measured growth attributes while the effect of soil moisture stress on the measured growth attributes was less severe in sandy loam than loamy sand soil. AMF inoculation gave highest leaf length (13.8 cm) at reproductive stage than non-inoculated treatments. Integrated use of 75Bio25Comp as soil amendment gave the highest leaf length (14.12 cm). Variation in soil textural types exert significant ($p<0.05$) effect on all measured yield parameters with higher yields recorded under sandy loam than loamy sand soil. However, the measured cowpea growth and phenological attributes were higher in plants grown on loamy sand soil. Although AMF inoculation exerted inconsequential ($p>0.05$) effect on the measured yield parameters, it significantly ($p<0.05$) lowered the flavonoid content of cowpea grains. Application of 25:75

Biochar and Compost mix ratio as soil amendment resulted in increased anthocyanin contents in cowpea seeds. Interaction between soil moisture levels and AMF inoculation exerted a significant effect ($p<0.05$) on protein content. AMF inoculation increased cowpea seed protein (29.5%) and P content under moisture stressed condition. Integrated use of biochar, compost and Mycoroot^{TM} product as soil amendment represents an important nutrients management strategy to mitigate the adverse effect of soil nutrients and moisture stress condition and enhance cowpea yield.

Key words: Cowpeas, moisture stress, *Arbuscular mycorrhizal fungi*, biochar, compost, secondary metabolites, proteins, soil textural types.

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CHAPTER 1

Introduction

1.1 Background

Cowpea (*Vigna unguiculata* L.) is a leguminous crop that originated from the Southern part of Africa where it is still cultivated even today (Guimarães et al 2023; Gerrano at al 2020). It is cultivated mainly for human consumption as it consists of protein ranging from 23-32%, rich in amino acid essential for human health and useful as animal feeds (Ishikawa et al 2022; Jayathilake et al 2018; Machado et al 2017). The dual character of cowpea makes it a valuable and economic crop where land is becoming a limited resource (Alexandre et al 2016; Dube and Fanadzo 2013). It is a crop known to survive and thrive in a broad range of conditions due to its nitrogen fixing ability (Xu et al 2017; Carvalho et al 2017). Recent estimate of global cowpea production suggests over 14.5 million ha of planted area annually with at total annual production of 7.8 million tonnes (Kebede and Bekeko 2020). Over 95% of the global estimated production of cowpeas is in Africa with Nigeria regarded as the biggest producer and consumer in the world (Mohammed et al 2021; Osipitan et al 2021). The crop is regarded as a multipurpose food crop in many African countries including South Africa where its seeds, fresh leaves and green pods are consumed (Belay et al 2017; Alemu et al 2016; Moswatsi et al 2013).

Cowpea is a resilient crop known to survive and perform well under extreme agricultural conditions (Noort et al 2022; Sanda and Maina 2013). Notwithstanding its ability to adapt to extreme climatic conditions including drought, its production (yield) is heavily constrained (Alexandre et al 2016). Moreover, the unpredictability, uneven distribution and low amount of rainfall, soil moisture stress, and depleted soil nutrients content are factors that exert negative impact on the production and establishment of the crop (Lundqvist and Falkenmark 2010; Ahmed et al 2016). Among other abiotic factors, moisture stress and poor soils has been highlighted to be the major factors that contribute significantly to the declining cowpea yield and productivity which tends to threaten global food security especially in tropical Africa (Saka et al 2018; Boukar et al 2018). Therefore, integration of seed inoculation and soil amendments offer an agricultural mediation strategy that is feasible and sustainable to overcome constrains associated with drought (Njeru and Koskey 2021). Seed inoculation is described as the process of effectively inserting a large number of microbes into the surface of seeds before planting (Pedrini et al 2020). Seed inoculation involving the introduction of beneficial microbes on seeds either prior to or at seed sowing have been reported to aid germination and root colonisation (O'Callaghan 2016). Mycoroot^{TM} inoculant is a typical example of such microbial plant booster produced and sold to farmers in South Africa. It consists of Arbuscular Micorrorrhizal isolates that are vital for the process of facilitating the transportation of water and nutrients to host plants (Nyakane et al 2019; Mukhongo et al 2016). *Arbuscular Micorrhizae fungi* (AMF) are defined as beneficial fungi that form symbiotic relationships with their host plant root systems by providing access to large number of nutrients and water while plants provide fungus with sugars (Berruti et al 2016). The fungi create microbiomes that play a very important role in supporting the plant health and aiding adaptation to changing environment (Willis et al 2013; Verbruggen et al 2013).

Biochar is a carbon-rich compound generated from pyrolyzing biomass under very low oxygen levels or anaerobic conditions at temperatures between 300-900°C (Liang et al 2021; Yi et al 2017). One of many advantages of biochar include its high volume of pores that correlate well with improve water retention and increase water holding capacity (Osman et al 2022; Tan et al 2015). Due to the challenge of soil deterioration and environmental stresses, amending agricultural soils with biochar supports and promotes sustainable agriculture especially in tropical regions and has received huge attention as a strategy in improving crop production, hence it inclusion and relevance in the present study (Song et al 2022). For centuries, compost has been used as a soil enrichment material made up of plant nutrients and beneficial organisms (Aryafar et al 2021). It is a soil conditioner that improves the quality and fertility of the soil (Martínez-Blanco et al 2013). Various research works (Yeboah et al 2020; Tammeorg et al 2014; Lui et al 2012; Downie 2011) illustrated that the combination of biochar and compost have synergistic positive effects on water holding capacity as well plant available water content. Biochar and compost serve as a win-win strategy to fight against drought as opposed to when either compost or biochar is used individually (Fischer and Glaser 2012). Application of a combination of biochar and AMF is reported to alter soil microbial activity and community structure thereby promoting nutrient cycling (Hammer et al 2015). Furthermore, Pontes et al (2017) reported that AMF inoculation and the use of organic amendments enables agroecosystems to retain high AMF species richness, which facilitates water and nutrient acquirement for crops. Numerous studies have established how AMF inoculation together with organic amendments interacts in enhancing soil quality and crop performance when faced with environmental stressors and degraded or deteriorated soils (Alguacil et al 2011, Medina and Azcón 2010).

1.2 Problem statement

Erratic rainfall patterns which often lead to frequent drought is regarded as one of the most disastrous abiotic stresses that affect crop production and exert huge negative impact on crop yield (Razi and Muneer 2021). The sharp increase in both global population and anticipated life expectancy in developing countries suggest greater demand for foods (Rohr et al 2019). Drought is one of the major crop production constrains that lead to poor growth, development and yields (Fahad et al 2017; Bodner et al 2015). Although, drought can occur at all crop growth stages, seed germination is first and foremost affected whereby the crucial water entry into the seed is limited thus affecting the physiological and metabolic processes of germination hence, inhibiting proper seedling establishment, growth and agricultural productivity (Yu et al 2019; Yi et al 2019; Ali and Elozeiri 2017).

Though, cowpea is a drought tolerant crop, growth and yield can be constrained by drought as well as economic losses for farmers (Farooq et al 2021). Regrettably, future climate change predictions in South Africa paint a gloomy picture of possible increase in drought (moisture and heat stress) that could exacerbate future crop production challenges in many parts including possible shift in crop production areas (Carvalho et al 2019; Weepener et al 2014). Similarly, the arid and semi-arid areas are well-known to experience high temperatures resulting in constant and rapid drying of the soil thereby affecting not only seed germination but also plant population due to poor emergence (Omoyo et al 2015). Furthermore, sandy soils comprising of large soil particles warm up easily mostly during spring, and tend to dry out quickly leading to low water retention and as such causes severe damage on seed germination and growing plants on crop fields (Zhao et al 2011). In contrast, heavy clay soils experience restricted water use due to their inability to drain water properly and poor aeration (Obia et al 2018) leading to possible water logging and inhibited seed germination and seedlings emergence (Finch et al 2014). Additionally, clay-rich soils tend to compact easily when drying out which limit root penetration and development of plants decreasing production (Zewide 2021).

Notwithstanding the fact that agricultural soils are replete of numerous beneficial microbes including fungi that support the well-being of plants, drought stressed soils can lower the quantity and quality of these microbes causing malfunction in the plant life (Glick 2012). Various reports demonstrated that resource-poor people from rural areas have limited access to proteins and minerals from expensive sources such as meat and fish (Gondwe et al 2019), this leads to high prevalence of mineral deficiencies causing malnutrition particularly in children

(Bain et al 2013; Amarakoon et al 2012). Unlike in many other drought tolerant plants where the presence of secondary metabolites has been widely reported as one of the coping mechanisms under drought condition (Yadav et al 2021), research gap on such secondary metabolites and their function on cowpea plants has been reported (Carvalho et al 2021).

1.3 Justification

Seed quality is an essential factor in crop establishment for higher yields. Promoting the protection of seeds sown in soil to guarantee excellent seedlings emergence under adverse abiotic conditions such as drought is crucial for farmers and represents key components of agronomic practices to guarantee increase yield and sustainable food production. Adoption of strategies that can help mitigate the negative impacts of elevated drought on crops (e.g. cowpea) such as the selection and use of appropriate soil amendment is crucial (Abbott et al 2018). This is essential to respond quickly to changing physiological traits that often accompany moisture stress in plant which can also be regulated by microbes such as mycorrhizal fungi (Bardgett et al 2014). Inoculation of seeds with Mycoroot™ inoculant, provides AMF that rapidly increases in the root cortex of the crop and creating hyphae network spreading around the roots to absorb water and valuable nutrients (Berruti et al 2016).

Evidence from earlier research works revealed that AMF support the development of fine elongated root hair that increases the contact surface area of roots with soil particles thereby aiding water and nutrient absorption under drought conditions (Wasson et al 2012). Such qualities and abilities collectively maximise plant growth under drought stress (Sahoo et al 2014; Ortas 2012). Biological inoculation of cowpea as a drought tolerant crop combined with the use of soil ameliorant such as biochar and compost could provide added benefits to counter the excessive effects of drought and heat on the crop. With prolonged dry spell due to climate change, it is important to understand and appreciate appropriate measures that can be taken to mitigate the negative impact of drought in order to maximize cowpea production.

Cowpeas are excellent sources of proteins and valuable minerals that offer huge human health benefits (Carvalho et al 2017). Various studies reported that cowpeas consumption offers protective advantages against chronic diseases such as obesity, vascular disease and disorders (Jayathilake et al 2018). The secondary metabolites in cowpea have also been reported to play significant role in life cycles of plant such as protection against drought and other environmental stressors (Weidner et al 2018).

1.4 Aim and objectives of the study

The aim of the study was to examine the combined effects of Mycoroot™ inoculation with varied biochar-compost mixtures as agronomic package to enhance cowpea germination, nodulation, growth and yield in two soil types with different moisture levels. The specific objectives of the study include:

i. To evaluate the combined effect of Mycoroot™ inoculation with variable biochar compost mixtures on cowpea growth and yield attributes under moisture stress condition.

ii. To evaluate the sole and combine effect of Mycoroot™ inoculation and variable biochar-compost mixtures on cowpea mineral, protein and secondary metabolites content under variable soil moisture regimes.

1.5 Hypotheses

i. Combined Mycoroot™ inoculation and variable biochar-compost mixtures has no effect on cowpea growth and yield attributes under moisture stress condition.

ii. Combined Mycoroot™ inoculation and variable biochar-compost mixtures will not have a significant effect on cowpea mineral, protein and secondary metabolites content under variable soil moisture regimes.

1.6 Outline of dissertation

This dissertation consists of six chapters, three of which (chapter 3, 4 & 5) are constructed and presented as manuscripts to be submitted to journals. The summary of each chapter is as summarized below.

Chapter 1: *"Introduction*": The introductory chapter outlines the background information, problem statement, aim, objectives and hypothesis of the study.

Chapter 2: "*Literature review*": This chapter reviews the literature related to integrating Mycoroot™ inoculation with variable biochar-compost mixtures and soil variation effect on drought tolerance and performance of cowpeas.

Chapter 3: ***"****Response of growth and phenological attributes of cowpeas (Vigna unguilata Walp L.) to integrated MYCOROOT™ inoculation, biochar-compost mixtures and two moisture regimes"*: This chapter assesses and investigates growth responses of integrating cowpea seeds with Mycoroot™ inoculation, variable biochar-compost mixtures, and soil variation under two moisture regimes (Adequate moisture and moisture stress).

Chapter 4: *"Integrated MYCOROOT™ inoculation and biochar-compost mixture application enhanced cowpea (Vigna unguiculata L. Walp.) yield attributes under variable soil conditions"*: This chapter evaluates yield parameters of cowpeas exposed to two moisture levels (Adequate moisture and moisture stress) cultivated with biochar-compost mixture under two soil variable and Mycoroot™ inoculation.

Chapter 5: "*Integrated MYCOROOT™ inoculation and biochar-compost mixture application under variable soil moisture conditions enhance cowpea grain proteins, secondary metabolites and mineral composition"*: This chapter analyses mineral and nutritional concentration of cowpeas cultivated under integrating Mycoroot™ inoculation, biochar-compost mixtures, soil variation and moisture regimes.

Chapter 6: "*Conclusion*": Chapter six concludes the study and provides the summary, conclusions and recommendations.

References

Abbott, L.K., Macdonald, L.M., Wong, M.T.F., Webb, M.J., Jenkins, S.N., and Farrell, M., (2018). Potential roles of biological amendments for profitable grain production–A review. Agriculture, *Ecosystems and Environment* 256:34-50.

Alemu, M., Asfaw, Z., Woldu, Z., Fenta, B.A., and Medvecky B., (2016). Cowpea (*Vigna unguiculata (L.) Walp*.) (Fabaceae) landrace diversity in northern Ethiopia. *International Journal of Biodiversity and Conservation* 11:297-309.

Alexandre, G., Piebiep, G., Ana, B., Raúl, D.P., Henrique, T., Eduardo, A.S.R., Luis, F., and Miguel, R., (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* 96:2941-2951.

Alguacil, M.M., Torres, M.P., Torrecillas, E., Díaz, G., and Roldán, A., (2011). Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land. *Soil Biology and Biochemistry* 43:167-173.

Ali, A.S., and Elozeiri, A.A., (2017). Metabolic processes during seed germination. P.141-166. In: *Advances in Seed Biology*. InTech. DOI: 10.5772/intechopen.70653.

Amarakoon, D., Thavarajah, D., McPhee, K., and Tha-varajah, P., (2012). Iron, zinc and magnesium-richeld peas (*Pisum sativum L.)* with naturally low phytic acid: A potential foodbased solution to global micronutrient malnutrition. *Journal of Food Composition and Analysis* 27:8-13.

Aryafar, S., Sirousmehr, A., and Najafi, S., (2021). The Impact of Compost on Seed Yield and Essential Oil of Black Cumin under Drought Stress Conditions. *Agrotechniques in Industrial Crops* 1:139-148.

Bain, L.E., Awah, P.K., Awah, K.P., and Geraldine, N., (2013). Malnutrition in sub-Saharan Africa: burden causes and prospects. *The Pan African Medical Journal* 15:120.

Bardgett, R.D., Mommer, L., and De Vries, F.T., (2014). Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution* 12:692-699.

Belay, F., Gebreslasie, A., and Meresa, H., (2017). Agronomic performance evaluation of cowpea (*Vigna unguiculata (L.) Walp*) varieties in Abergelle District, Northern Ethiopia. *Journal of Plant Breeding and Crop Science* 8:139-143.

Berruti, A., Lumini, E., Balestrini, R., and Bianciotto, V., (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in Microbiology* 6:1559.

Bodner, G., Nakhforoosh, A., and Kaul, H.P., (2015). Management of crop water under drought: a review. *Agronomy for Sustainable Development* 35:401-442.

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., and Fatokun, C., (2019). Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding* 138:415-424.

Carvalho, M., Bebeli, P.J., Pereira, G., Castro, I., Egea-Gilabert, C., Matos, M., and Carnide, V., (2017). European cowpea landraces for a more sustainable agriculture system and novel foods. *Journal of the Science of Food and Agriculture* 13:4399-4407.

Carvalho, M., Matos, M., Castro, I., Monteiro, E., Rosa, E., Lino-Neto, T., and Carnide, V., (2019). Screening of worldwide cowpea collection to drought tolerant at a germination stage. *Scientia Horticulturae* 247:107-115.

Carvalho, M.D., and Halecki, W., (2021). Modeling of Cowpea (*Vigna unguiculata*) Yield and Control Insecticide Exposure in a Semi-Arid Region. *Plants* 10:1074.

Downie, A., (2011). Biochar production and use: environmental risks and rewards. *Applied Soil Ecology* 15:3-6.

Dube, E., and Fanadzo, M., (2013). Maximising yield benefits from dual-purpose cowpea. *Food Security* 6:769-779.

Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., and Ihsan, M.Z., (2017). Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science* 8-2017
Farooq, M., Romdhane, L., Rehman, A., Al-Alawi, A.K., Al-Busaidi, W.M., Asad, S.A., and Lee, D.J., (2021). Integration of seed priming and biochar application improves drought tolerance in cowpea. *Journal of Plant Growth Regulation* 40:1972-1980.

Finch, H.J.S., Samuel, A.M., and Lane, G.P.F., (2014). Soils and soil management. Lockhart and Wiseman’s Crop Husbandry Including Grassland, 9th ed.; Elsevier: Amsterdam, The Netherlands 37-62.

Fischer, D., and Glaser, B., (2012). Synergisms between compost and biochar for sustainable soil amelioration. *Management of Organic Waste* 10:5772-5778.

Gerrano, A.S., Jansen van Rensburg, W.S., Mathew, I., Shayanowako, A.I., Bairu, M.W., Venter, S.L., Swart, W., Mofokeng, A., Mellem, J., and Labuschagne, M., (2020). Genotype and genotype x environment interaction effects on the grain yield performance of cowpea genotypes in dryland farming system in South Africa. *Euphytica* 216:1-11.

Glick, B.R., (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 2012.

Gondwe, T.M., Alamu, E.O., Mdziniso, P., and Maziya-Dixon, B., (2019). Cowpea (*Vigna unguiculata (L.) Walp*) for food security: an evaluation of end-user traits of improved varieties in Swaziland. *Scientific Reports* 1:1-6.

Guimarães, J.B., Nunes, C., Pereira, G., Gomes, A., Nhantumbo, N., Cabrita, P., Matos, J., Simões, F., and Veloso, M.M., (2023). Genetic Diversity and Population Structure of Cowpea (*Vigna unguiculata (L.) Walp*.) Landraces from Portugal and Mozambique. *Plants* 12:846.

Hammer, E.C., Forstreuter, M., Rillig, M.C., and Kohler, J., (2015). Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress. *Applied Soil Ecology* 96:114-121.

Ishikawa, H., Batieno, B.J., Fatokun, C., and Boukar, O., (2022). A high plant density and the split application of chemical fertilizer increased the grain and protein content of cowpea (*Vigna unguiculata*) in Burkina Faso, West Africa. *Agriculture* 12:199.

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B.C., Nammi, S., and Liyanage, R., (2018). Cowpea: An overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture* 13:4793-4806.

Kebede, E., and Bekeko, Z., (2020). Expounding the production and importance of cowpea (*Vigna unguiculata (L.) Walp.)* in Ethiopia. *Cogent Food and Agriculture* 1:1769805.

Liang, L., Xi, F., Tan, W., Meng, X., Hu, B., and Wang, X., (2021). Review of organic and inorganic pollutants removal by biochar and biochar-based composites. *Biochar* 3:255-281.

Lui, J.H., Schulz, S., Brandl, H,M., Miehtke, B., Huwe, B., and Glaser, B., (2012). Short-term effect of biochar and compost on soil fertility and water status of a Dystric cambisol in NE Germany under field conditions. *Journal of Plant Nutrition and Soil Science* 10:698-707.

Lundqvist, J., and Falkenmark, M., (2010). Adaptation to rainfall variability and unpredictability: New dimensions of old challenges and opportunities. *International Journal of Water Resources Development* 26:595-612.

Machado, N., Oppolzer, D., Ramos, A., Ferreira, L., Rosa, E.A.S., Rodrigues, M., and Barros, A.I.R.N.A., (2017). Evaluating the freezing impact on the proximate composition of immature cowpea (*Vigna unguiculata L.*) pods: Classical versus spectroscopic approaches. *Journal of the Science of Food and Agriculture* 13:4295-4305.

Martínez-Blanco, J., Lazcano, C., Christensen, T.H., Muñoz, P., Rieradevall, J., Møller, J., Antón, A., and Boldrin, A., (2013). Compost benefits for agriculture evaluated by life cycle assessment. A review. *Agronomy for Sustainable Development* 33:721-732.

Medina, A., and Azcón, R., (2010). Effectiveness of the application of arbuscular mycorrhiza fungi and organic amendments to improve soil quality and plant performance under stress conditions. *Journal of Soil Science and Plant Nutrition* 10:354-372.

Mohammed, S.B., Dzidzienyo, D.K., Umar, M.L., Ishiyaku, M.F., Tongoona, P.B., and Gracen, V., (2021). Appraisal of cowpea cropping systems and farmers' perceptions of production constraints and preferences in the dry savannah areas of Nigeria. *AgriRxiv* 2:25.

Moswatsi, M.S., Kutu, F.R., and Mafeo, T.P., (2013). Response of cowpea to variable rates and methods of zinc application under different field conditions. *African Crop Science Conference Proceedings* 11:757-762.

Mukhongo, R.W., Tumuhairwe, J.B., Ebanyat, P., AbdelGadir, A.H., Thuita, M., and Masso, C., (2016). Production and use of arbuscular mycorrhizal fungi inoculum in sub-Saharan Africa: challenges and ways of improving. *International Journal of Soil Science* 11:108-122.

Njeru, E.M., and Koskey, G., (2021). Using beneficial microorganisms to promote sustainable crop production and resilience of smallholder agroecosystems to changing climate. *Climate Change and Resilient Food Systems: Issues, Challenges, and Way Forward* 287-314.

Noort, M.W., Renzetti, S., Linderhof, V., Du Rand, G.E., Marx-Pienaar, N.J., De Kock, H.L., Magano, N., and Taylor, J.R., (2022). Towards sustainable shifts to healthy diets and food security in Sub-Saharan Africa with climate-resilient crops in bread-type products: *A Food System Analysis Foods* 2:135.

Nyakane, N.E., Sedibe, M.M., and Markus, E., (2019). Growth response of rose geranium (*Pelargonium graveolens L.*) to calcium: Magnesium ratio, magnetic field, and mycorrhizae. *HortScience* 10:1762-1768.

O’Callaghan, M., (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied microbiology and biotechnology* 100:5729-5746.

Obia, A., Mulder, J., Hale, S.E., Nurida, N.L., and Cornelissen, G., (2018). The potential of biochar in improving drainage, aeration and maize yields in heavy clay soils. *Plos One* 13:0196794.

Omoyo, N.N., Wakhungu, J., and Oteng’i, S., (2015). Effects of climate variability on maize yield in the arid and semi arid lands of lower eastern Kenya. *Agriculture and Food Security* 4:1-13.

Ortas, I., (2012). The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. *Field Crops Research* 125:35-48.

Osipitan, O.A., Fields, J.S., Lo, S. and Cuvaca, I., 2021. Production Systems and Prospects of Cowpea (*Vigna unguiculata (L.) Walp*.) in the United States. *Agronomy* 11:2312.

Osman, A.I., Fawzy, S., Farghali, M., El-Azazy, M., Elgarahy, A.M., Fahim, R.A., Maksoud, M.A., Ajlan, A.A., Yousry, M., Saleem, Y. and Rooney, D.W., (2022). Biochar for agronomy, animal farming, anaerobic digestion, composting, water treatment, soil remediation, construction, energy storage, and carbon sequestration: a review. *Environmental Chemistry Letters* 20:2385-2485.

Pedrini, S., Balestrazzi, A., Madsen, M.D., Bhalsing, K., Hardegree, S.P., Dixon, K.W., and Kildisheva, O.A., (2020). Seed enhancement: getting seeds restoration-ready. *Restoration Ecology* 28:266-275.

Pontes, J.S., Oehl, F., Marinho, F., Coyne, D., Silva, D.K.A.D., Yano-Melo, A.M., and Maia, L.C., (2017). Diversity of arbuscular mycorrhizal fungi in Brazil's Caatinga and experimental agroecosystems. *Biotropica* 49:413-427.

Razi, K., and Muneer, S., (2021). Drought stress-induced physiological mechanisms, signaling pathways and molecular response of chloroplasts in common vegetable crops. *Critical Reviews in Biotechnology* 4:669-691.

Rohr, J.R., Barrett, C.B., Civitello, D.J., Craft, M.E., Delius, B., DeLeo, G.A., Hudson, P.J., Jouanard, N., Nguyen, K.H., Ostfeld, R.S., and Remais, J.V., (2019). Emerging human infectious diseases and the links to global food production. *Nature Sustainability* 6:445-456.

Sahoo, R.K., Ansari, M.W., Pradhan, M., Dangar, T.K., Mohanty, S., and Tuteja, N., (2014). A novel Azotobacter vinellandii (SRI Az 3) functions in salinity stress tolerance in rice. *Plant Signaling and Behaviour* 7:511-23.

Saka, J.O., Agbeleye, O.A., Ayoola, O.T., Lawal, B.O., Adetumbi, J.A. and Oloyede-Kamiyo, Q.O., (2019). Assessment of varietal diversity and production systems of cowpea (Vigna unguiculata (L.) Walp.) in Southwest Nigeria. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 119:43-52.

Sanda, A.R.., and Maina, I.M., (2013). Effect of drought on the yields of different cowpea cultivars and their response to time of planting in Kano State. Nigeria. *International Journal of Environment and Bioenergy* 6:171-176.

Song, B., Almatrafi, E., Tan, X., Luo, S., Xiong, W., Zhou, C., Qin, M., Liu, Y., Cheng, M., Zeng, G., and Gong, J., (2022). Biochar-based agricultural soil management: An applicationdependent strategy for contributing to carbon neutrality. *Renewable and Sustainable Energy Reviews* 164:112529.

Tammeorg, P., Simojoki, A., Makela, P., Stoddard, F.L., Alakukku, L., and Helenius, J., (2014). Short-term effect of biochar on soil properties and wheat yield formation with meat bone meal and inorganic fertiliser on a boreal loamy sand. *Agriculture, Ecosystem and Environment* 1:2-7.

Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y. and Yang, Z., (2015). Application of biochar for the removal of pollutants from aqueous solutions. *Chemosphere* 125:70-85.

Verbruggen, E., Van der Heijden, M.G., Rillig, M.C., and Kiers, E.T., (2013). Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist* 4:1104-1109.

Wasson, A.P., Richards, R.A., Chatrath, R., Misra, S.C., Prasad, S.V., Rebetzke, G.J., Kirkegaard, J.A., Christopher, J., and Watt, M., (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal Experiments Botany* 63:3485-3498.

Weepener, H.L., Engelbrecht, C.J., and Carstens, J.P., (2014). Sensitivity of crop suitability to climate change in South Africa. Final report of DAFF/ARC-ISCW project submitted to the Department of Agriculture, Forestry and Fisheries, Directorate of Climate Change and Disaster Management.

Weidner, S., Król, A., Karamać, M., and Amarowicz, R., (2018). Phenolic compounds and the antioxidant properties in seeds of green and yellow-podded bean (*Phaseolus vulgaris L.*) varieties. *CYTA Journal of Food* 1:373-380.

Willis, A., Rodrigues, B.F., and Harris, P.J., (2013). The ecology of arbuscular mycorrhizal fungi. *Critical Reviews in Plant Sciences* 1:1-20.

Xu, P., Wu, X., Muñoz-Amatriaín, M., Wang, B., Wu, X., Hu, Y., Huynh, B.L., Close, T.J., Roberts, P.A., Zhou, W., and Lu, Z., (2017). Genomic regions, cellular components and gene regulatory basis underlying pod length variations in cowpea (*Vigna unguiculata L. Walp*). *Plant Biotechnology Journal* 5:547-557.

Yadav, B., Jogawat, A., Rahman, M.S., and Narayan, O.P., (2021). Secondary metabolites in the drought stress tolerance of crop plants: A review. *Gene Reports* 23:101040.

Yeboah, E., Asamoah, G., Ofori, P., Amoah, B., and Agyeman, K.O.A., (2020). Method of biochar application affects growth, yield and nutrient uptake of cowpea. *Open Agriculture* 1:352-360.

Yi, F., Wang, Z., Baskin, C.C., Baskin, J.M., Ye, R., Sun, H., Zhang, Y., Ye, X., Liu, G., Yang, X., and Huang, Z., (2019). Seed germination responses to seasonal temperature and drought stress are species-specific but not related to seed size in a desert steppe: Implications for effect of climate change on community structure. *Ecology and Evolution* 9:2149-2159.

Yi, SZ., Sun, Y.Y., Hu, X., Xu, H.X., Gao, B., and Wu, J.C., (2017). Porous nano-cerium oxide wood chip biochar composites for aqueous levofloxacin removal and sorption mechanism insights. *Environmental Science and Pollution Research* 25:25629-25637.

Yu, H., Wu, Y., Hu, B., You, D., and Wang, Y., (2019). Physiological regulation of peg-seed primings on sweet corn germination and seedling growth under drought stress. *Journal of Shanxi Agricultural University (Natural Science Edition)* 39:34.

Zewide, I., (2021). Review paper on effect of natural condition on soil infiltration. *Chemistry* 7:34-41.

Zhao, L.P., Wu, G.L., and Cheng, J.M., (2011). Seed mass and shape are related to persistence in a sandy soil in northern China. *Seed Science Research* 21:47-53.

CHAPTER 2

Literature Review

2.1 Overview and origin of cowpea production

Cowpea (*Vigna unguiculata* L) is an annual legume crop mostly grown in the semi-arid regions and used as both grains and vegetable, as well as animal feed (Carvalho and Halecki 2021; Mfeka 2019; Ba et al 2018; Fabunmi et al 2012). The crop belongs to the family Fabaceae and originated from the Southern Africa but later domesticated in the East and West of Africa and other parts of the world including Asia (Affrifah et al 2021). Cowpeas, also known as the black eye peas, are heat-loving crop that thrives well in a 30°C temperature for both growth and development (Barros et al 2020; Olalekan and Bosede 2010). Several research work have suggested that cowpeas contribute significantly in the livelihoods of humans as it plays a vital role of providing dietary proteins, fibre and other nutritional components such as vitamins and minerals (Horn et al 2022; Affrifah et al 2021; Manzeke et al 2017).

Cowpeas are widely cultivated in the African continent and particularly in Nigeria, which accounts for 66% of the world production but utilised widely as a dual-purpose crop for humans and animal as forage (Fabunmi et al 2012). Globally, there are 6.2 million tonnes of cowpeas produce annually on an estimated 14.5 million acres of planted land (Kebede and Bekeko 2020). Moreover, it is anticipated that cowpea yield potential will rise over the coming decade and possibly reaching a peak of 12.3 million tonnes by 2030 (Omomowo and Babalola 2021). However, a number of abiotic factors that hinder maximum yield potential including drought stress affects the production of this crop. Cowpeas are known to be drought tolerant crops (Stoilove et al 2022). Although cowpeas are tolerant to drought, it is still susceptible to drought stress particularly at seedling establishment, growth and development stages during the vegetative stage (Ravelombola et al 2020).

2.2 *Arbuscular Micorrhizae fungi* and its association with crop

Environmental stress mitigation programmes in plants against drought has been conducted throughout the world since the beginning of the 20th century. However, drought is predicted to escalate in intensity due to extreme weather patterns (Saharwardi and Kumar 2022; Li et al 2019a; Mathur et al 2018; Piao et al 2010). *Arbuscular Micorrhizae fungi* is a group of soil microbes that form symbiotic relationships with root system of crops (Brundrett and Tedersoo

2018). Their function is to facilitate the uptake of water and valuable nutrient during adverse moisture stress conditions (Bowles et al 2018; Baum et al 2015). Furthermore, Wang et al (2022) documented that these fungi display important roles in various ecological and biological processes such as organic matter that improves soil fertility in plant health and nutrition.

Various studies have revealed that AMF can improve plant resistance to moisture stress (Li et al 2019a). AMF are able to locate more soil pores by creating a hyphae network and release glomalin secretions that surrounds root thereby retrieving water sources during drought periods (Pagano 2014; Gong et al 2013). Furthermore, Chang et al (2018) revealed that the introduction of AMF to root systems can improve crop water use efficiency by enhancing stomatal conductance. Earlier research work from Bhardwaj et al (2014) and Dodd and Ruiz-Lozano (2012) revealed that AMF symbiosis consist of an important strategy in which the strategy enables cowpeas to overcome extended drought period. Moreover, soil microorganisms such as *Arbuscular Micorrhizae fungi* assist crops with overcoming water-limiting conditions by implementing osmotic adjustments, antioxidant metabolisms and phytohormone modulation (Rubin et al 2017; Vurukonda et al 2016).

2.3 Biochar and compost utilization and their effect on soil health

Soil nutrition and health are of great concern as these are directly linked with food security and sustainable agricultural land use. Biochar is a carbon-rich carbonaceous compound generated from pyrolyzing biomass under very low oxygen levels or anaerobic conditions at temperatures between 300-900°C (Lusiba et al 2021; Li et al 2019b). It can also be formed as a result of human activities such as fire or volcanic eruptions (Spokas et al 2012). There are numerous benefits reported that comes with the utilization of biochar one of which is water retention (Lentz et al 2019; Schnell et al 2012).

Biochar is extremely porous and resembles a sponge, which soak up and keep water and nutrients within the soil which is used by cultivated crops (Tan et al 2015; Fiaz et al 2014). Biochar utilization in tropical region has gained interest due to challenges around soil degradation and environmental stresses (Rashid et al 2020). Numerous researchers Diatta et al 2020; Ding et al 2016; Kammann et al 2011; Verheijen et al 2010 have reported that the application of biochar represents a win-win strategy improve soil fertility as it can also be used as a fertiliser due to its chemical composition carbon among other nutrients. Biochar is an organic substance that comes highly recommended due to its effectiveness over a very long time without decomposition when compared other organic materials (Verheijen et al 2010).

Contrarily, compost is defined as a mixture of decomposed organic matter that is used to condition the soil and as a fertiliser (Agegnehu et al 2015). Composting is a practise that originated from the Roman empire and was modernised from the beginning in the 1920's and has been used as a tool in organic farming (Gilbert 2017). Several research works have proven that compost is a resource filled with co-substrates and valuable nutrients that can enhance the soil's capacity to retain water by increasing macroporosity of the soil, ventilation and alter soil beneficial microorganism structure (Wu et al 2020). The use of compost in agricultural production is increasing daily as both soil amendment and fertiliser to enhance soil structure, increasing infiltration rate, organic matter and nutrients (Nguyen et al 2012).

2.4 Effects of compost and biochar application on crop growth and development

Biochar application enhance plant growth by improving the soil physical and chemical traits such as bulk density, cation exchange capacity, water status and permeability including biological properties which collectively increases crop productivity (Taiz and Zeiger 2013; Sohi et al 2010). Biochar is compost of organic carbon, which is an essential nutrient in plants as it serves as a primary building block and energy source (Yeboah et al 2020). Furthermore, biochar is resistant to decomposition, which enables it to stay in soil for long periods and provide necessary benefits seasons after seasons to cultivated crops (Major et al 2010). Biochar application rises nutritional contents and available of water to the crop which in turn improves and maximise growth and yield (Yeboah et al 2020; Lehmann and Joseph 2015).

Organic matter from compost constitutes huge capabilities to hold on to moisture and nutrients that are the fundamentals in crop productivity (Usharani et al 2019). Compost is an organic material that replenish soil organic matter and provide nutrients to plants, which enhances crop enhancement (Martínez-Blanco et al 2013). Kranz et al (2020) revealed that compost increases plant water availability which plays a significant role in crops thus, providing great benefits to crops. Marschner (2012) found that the addition of compost increases shoot and root growth under drought stress conditions while Duong et al (2012) showed that compost incorporation into the soil increases plant height, roots and shoot growth when compared with unamended soils.

2.5 Effect of biochar-compost mixture on crop and soil health

Organic soil amendments application such as biochar and compost on agricultural soils assist with maintaining crop productivity by restoring nutrient recycling and altering soil structure thus representing a crucial strategy to improve crop growth, performance and sustainable land use (Ullah et al 2021; Sánchez-Monedero et al 2019). Nadeem et al (2017) reported that the combination of biochar and compost have a positive synergistic impact on the soil nutrient content and water-holding capacity. The use of biochar and compost as a mixture is of suitable use as they stabilise soil structure, improve soil water holding capacity and allowing limited use of inorganic fertilisers as inputs (Agegnehu et al 2015). Mensah and Frimpong (2018) documented that the application of compost-biochar mixture promotes an increase in plant height, stem girth and dry matter accumulation in maize.

2.6 The effects of moisture stress on cowpea growth, development and yield attributes

Cowpea as a leguminous crop, widely reported to be drought-tolerant (Ravelombola et al 2020). Drought stress due to uneven rainfall and higher temperatures are reported to exert significant impact on cowpea growth, development and yield (Gray and Brady 2016). Many physiological interaction processes that occur during crop growth and development influence cowpea yields (Anjum et al 2011). Exposure to limited water supply have been reported to often lead to disruption of leaf gaseous exchange which negatively affect the quality and quantity of crop development and crop yield leading to reduced source and tissue sink (Vessal et al 2020; Anjum et al 2011).

Tiwari et al (2016) reported that moisture stress reduces the production and expansion of leaves, which ultimately results in leaf senescence and abscission. This phenomenon occurs during moisture stress where macromolecules relocate nutrients from leaves to other organs to improve plants fitness that causes stunted growth (Guo et al 2021). Moisture stress does not only affect dry matter accumulation it also affects light interception which hinders crop growth as light is one of the most fundamental factors needed for growth (Pan et al 2020). A study reported by Yahaya et al (2019) comparing cowpeas exposed to drought stress and non-stress observed that biomass accumulation and yields dropped significantly, biomass reduction reflected a decrease of 33.8% in terms of dry weight.

Cowpeas are more sensitive to moisture stress before and during flowering stage however, seed or grain filling is the most sensitive phase (Anjum et al 2011). According to Anuradha et al

(2017), grain yield that experienced moisture stress show poor carbohydrates partitioning and photosynthate assimilation during seed development. According to Ishiyaku and Aliyu (2013), seed yield is very sensitive to moisture stress and causes noticeable reduction in yield by producing seeds with deformities and reduce seed components. Yield components are excellent indicators of insufficient water, moisture stress negatively affects the number of pods per plant and pod size causing major reduction in grain yield (Rehman et al 2015).

2.7 Effect of moisture stress on the content of primary and secondary metabolites

Secondary metabolites are described as the compounds produced by crops, which are not utilised for growth, development and reproduction but they are essential for adaptive roles such as competition, symbiosis, defence, ecological interactions (Tiwari and Rana 2015). The secondary metabolites in plants are harvested by humans to enhance their diets through healthy nutrition while serving as growth promoters and other beneficial effects in animals (Franzoni et al 2019). Secondary metabolites contribute vastly to food additives, flavours and pharmaceuticals industries (Ramakrishna and Ravishankar 2011). Notwithstanding the extent of research works done on cowpea as a leguminous crop globally, information on the secondary metabolites content of cowpea seeds in response to moisture stress are scanty (Abebe and Alemayehu 2022). Yet, it contributes greatly to nutritional security by being heavily associated with significant amounts of proteins, vitamins and minerals elements that support millions of livelihood in developing countries particularly those in tropic and subtropics regions (Enyiukwu et al 2018; Afiukwa et al 2013; Animasaun et al 2015; Hall 2012). Broader research spectra across wide range of environment are required to provide in-depth understanding of the effects of moisture stress on cowpea metabolites such as vitamins and minerals to address the current research gap in this regards.

2.7.1 Protein

Primary metabolites are organic compounds that directly participate in organism's metabolic processes essentially for growth, development and reproduction (Fernie and Pichersky 2015). Protein fall under primary metabolites, they are directly involved in living organism's development, repair, maintenance and regeneration of tissues (Pereira 2018; Yiğit 2015). Cowpeas are deemed to be the most high-quality plant protein source consumed in various part of the world as food stuff for humans and part of fodder for animals (Yahaya et al 2019). The grains of cowpeas consist of about 25% of protein (Hall 2012) that serves as the most important component in human consumption financial gain through sales (Machado et al 2017). However, moisture stress limits the quantity and quality of seeds due to drought-induced suppression of the production of proteins (Farooq et al 2017).

Reduction of protein accumulation in legumes seeds is caused when the processes of partitioning and fixing of atmospheric nitrogen are hindered and suppressed by scarcity of water (Kumari et al 2022). Furthermore, moisture stress does not only reduced protein content in seeds, it also significantly affects the mineral composition of the seed (Ghanbari et al 2013). Results of a study by Bellaloui et al (2013) revealed that moisture stress doubles the level of free amino acid pool in soybean whilst preventing amino acid inclusion into the protein chains during flower and pod filling stage. Similarly, Reis et al (2016) reported that maintaining high levels of soil moisture is paramount during the vegetative stage in legumes as moisture stress exposure during the vegetative stage tend to decreases the level of protein content in seeds. Furthermore, Chavoshi et al (2018) documented that moisture stress imposed during flowering stage limits grain protein content accumulation in red beans (*Phaseolus vulgaris cv. Goli.*)

2.7.2 Total soluble sugar

Total soluble sugars are crucial constituents of plant cells essential for sustaining and maintaining the general structure and developments of plants (Gao et al 2018). In cowpeas, total soluble sugars influence seed production and cooking quality by desirable taste (Baptiste et al 2011). Previous research work by Dien et al (2019) suggests that soluble sugars play a fundamental role in maintaining water content and osmotic adjustment in plants under adverse moisture stress conditions. It has been reported that moisture stress increases soluble sugar accumulation in plants (Mabudi-Bilasvar et al 2022; Xu et al 2015). Cowpea seeds are highly sensitive to moisture stress as it inhibits the process of germination. However, the presence of soluble sugars in seeds enhances storability capabilities and mitigate against abiotic stress or drought tolerance (Teixera et al 2012).

2.7.3 Flavonoids

Flavonoids are one of the secondary metabolites found in various crops that mainly form part of human diets in edible plants (Kaur et al 2021; Luthra et al 2021). They are mainly found in such crops as cowpeas, citrus fruits and other agricultural crops responsible for producing colour particularly in flowers where they have responsible as a source of attraction to pollinators (Palma-Tenango et al 2017). Flavonoids form part of the two major classes of biologically active secondary metabolites that are crucial in seedling development as well as promotion of plant growth (Shen et al 2022). In addition, it is reported that production and accumulation of flavonoids in plant tissues and seeds play crucial role in legume symbiosis together with rhizobia in leguminous crops (Lui and Murray 2016).

The presence of flavonoids in cowpea seeds during adequate moisture levels releases molecules that act as chemical inhibitors against pest attack and pathogens during seed germination but moistures stress tends to inhibit that process (Makoi et al 2010). Furthermore, in a study done by Li et al (2021) revealed that flavonoids can facilitate moisture stress tolerance in some crops by regulating stomatal movement in leaves. Jaafar et al (2012) stated that flavonoids content is enhanced under moisture limited conditions. In contrast, researchers Mundim and Pringle (2018) and Basu et al (2010) argues that moisture stress can induce flavonoids that function to regulate metabolic process. In addition, several research authors have revealed that flavonoids protect cardiovascular systems and has antioxidant and anti-neuroinflammatory capacities which is of great benefit to human health (Li et al 2020; Singh et al 2018).

2.7.4 Anthocyanin

Nassour et al (2020) defined anthocyanin as pigments that dissolves in water which contributes to plant development and their interaction with the environment. Anthocyanin accumulates vastly in crops upon exposure to moisture stress (Landi et al 2015). Also, Ramakrishna and Ravishnkar (2011) reported that anthocyanin content increases during the presence of drought and cold temperatures. Cirillo et al (2021) documented that plants containing anthocyanin in their tissues are usually tolerant to moisture stress. Anthocyanin is essential not only for assisting plants with their physical environmental interaction but serves as excellent natural food colorant (Geera et al 2012; Ojwang and Awika 2010); and possesses diverse human health benefits that include anti-inflammatory, antidiabetic, anticancer effects (Khoo et al 2017; He et al 2011).

2.8 Effect of moisture stress and poor soil fertility status on cowpea mineral composition

Moisture availability and soil fertility status are the most crucial limiting factors in crop production (Tittonell and Giller 2013). Agricultural productivity is restricted by a number of poor soil fertility related issues aggravated by a variety of factors such as unreliable rainfall, continuous cultivation nutrient mining without replenishment, farmers’ mismanagement practices, soil types, soil erosion (Aleminew and Melkame 2020; Vanlauwe et al 2019; Lal 2015; Tekalign and Tegbaro 2015). These factors collectively result in poor soil fertility status that leads to a serious withdrawal of plant nutrients from the non-renewable resource known as soil (Bogale 2014). For instance, according to Aleminew and Melkame (2020), continuous cultivation significantly reduces the levels of phosphorus and calcium that become limiting factors in legume production. Furthermore, some soil types are reported to cause a significant decrease in potassium, calcium and phosphorus levels due to low organic matter content and acidity, which then hinders productivity. Salinity in soils cause growth reduction and metabolic changes that lessen nutrient accumulation (Mekonnen et al 2022). Grain filling is known as the critical stage, moisture possess a negative impact during this stage as it affects absorption and partitioning of minerals to the grain resulting in poor seed nutrient quality impaired grain mineral quality (Namatsheve et al 2021). Although leguminous crops like cowpeas contain high concentration of important minerals including zinc and iron (Snapp et al 2018; Gonçalves et al 2016), poor soil fertility status and nutrients deficiency could negatively impact mineral constituents of the grains and the other edible plant parts (Mohammed et al 2021).

Nutrients movement and distribution within plants and grain is moisture dependent (Fischer et al 2019). It has been reported that pre and post exposure of moisture levels has an effect on grain nutrient quality (Xia et al 2013). Numerous research works have suggested that moisture stress causes interruption in the flow of vital minerals in the soil causing an imbalance in the nutritional status of the plants (Kapoor et al 2020; Kumawat and Sharma 2018; Kheradmand et al 2014). Reduced nutrient availability in plants exposed to moistures stress may be driven by poor nutrient absorption and reduced transpiration flow (Tadayyon et al 2018). Furthermore, Sehgal et al (2019) stated that moisture stress plays a significant role in the reduction of important nutrients such as potassium, calcium, iron, phosphorus and zinc on legumes leading to poor nutritional quality of seeds. The reduction of these nutrients is attributed to weaken transpiration, stomatal conductance and decrease in root functioning upon drought exposure (De Bang et al 2021; Resco de Dios et al 2019; Qi et al 2019). Permanent or temporary moisture deficit stress inhibits various internal processes in plants such as potassium gene encoding and channel transportation in grape vines (Cuéllar et al 2010). In addition, some minerals such as iron and manganese use xylem as a mode of transport across various part of the plant however, the xylem is affected by moisture stress thus hindering the allocation of those nutrients to seeds

(Fischer et al 2019; Sevanto 2018). To improve food security, it is imperative to explore sustainable solutions in order to increase grain yields with balanced mineral composition.

2.9 Summary

Moisture stress is one of the widespread global challenges faced by farmers that limit agricultural production. It affects crops' physiological, morphological and biochemical pathways, and ultimately reduces crop productivity. Plant metabolites and valuable nutrients that play important roles in growth and development of plants, and human health are however, limited by moisture stress. The above review revealed that though various studies have been conducted, agronomic strategies to overcome moisture stress and soil fertility challenges through combined biochar and compost use together with seed inoculation are yet to be fully investigated. Therefore, this study examined the benefits of inoculating cowpeas seeds with Mycoroot^{TM} sown in two different soils (loam and sandy) combined with different biochar-compost mixtures to mitigate problems associated with drought in cowpeas production. Moreover, there is knowledge gap on the potential of optimum integrated compost-biochar mixture to increase soil water retention and mitigate the impact of moisture (drought) stress condition in soils on cowpea performance. Such information is critical in promoting soil moisture retention to achieve field crop establishment particularly in drought prone and water scarce areas.

References

Abebe, B.K., and Alemayehu, M.T., (2022). A review of the nutritional use of cowpea (*Vigna unguiculata L. Walp*) for human and animal diets. *Journal of Agriculture and Food Research* 10:100383.

Affrifah, N.S., Phillips, R.D., and Saalia, F.K., (2021). Cowpeas: Nutritional profile, processing methods and products: a review. *Legume Science* 131.

Afiukwa, C.A., Ubi, B.E., Kunert, K.J., Titus, E.J., and Akusu, J.O., (2013). Seed protein content variation in cowpea genotypes. *World Journal of Agricultural Research* 1:94-99.

Agegnehu, G.M.I., Bird, P.N.N., and Bass, A.M., (2015). The ameliorating effects of biochar and compost on soil quality and plant growth on Ferralsol. *Soil Research* 1:1-12.

Aleminew, A., and Alemayehu, M., (2020). Soil fertility depletion and its management options under crop production perspectives in Ethiopia: A review. *Agricultural Reviews* 41:91-105.

Animasaun, D.A., Oyedeji, S., Azeez, Y.K., Mustapha, O.T., and Azeez MA., (2015). Genetic variability study among ten cultivars of cowpea (*Vigna unguiculata L. Walp*) using morphoagronomic traits and nutritional composition. *Journal of Agricultural Science* 10:119-130.

Anjum, S,A., Wang, L.C., Farooq, M., Xue, L.L., and Zou, C.M., (2011). Brassinolide application improves the drought tolerance in Maize through modulation of enzymatic antioxidants and leaf gas exchange. *Journal of Agronomy Crop Science* 197:177-185.

Anuradha., Goyal, R.K., and Bishnoi, C., (2017). Assimilate partitioning and distribution in fruit crops. *Journal of Pharmacognosy and Phytochemistry* 6:479-484.

Ba, M.N., Huesing, J.E., Tamò, M., Higgins, T.J., Pittendrigh, B.R., and Murdock, L.L., (2018). An assessment of the risk of Bt-cowpea to non-target organisms in West Africa. *Journal of Pest Science* 91:1165-1179.

Baptiste, N.T.J., Youmbi, E., Njintang, N.Y., and Youmbi, E., (2011) Generation Means Analysis of Seed Sucrose Content in Cowpea (*Vigna unguiculata L. Walp*.). *Asian Journal of Agricultural Sciences* 3:4795-4805.

Barros, J.R.A., Angelotti, F., Santos, J.D.O., Silva, R., Dantas, B.F. and De Melo, N.F., (2020). Optimal temperature for germination and seedling development of cowpea seeds. In Embrapa Semiárido-Artigo em anais de congresso (ALICE). *Revista Colombiana de Ciencias Hortícolas* 14:1-19.

Basu, S., Roychoudhury, A., Saha, P.P., and Sengupta, D.N., (2010). Differential antioxidative responses of indica rice cultivars to drought stress. *Plant Growth Regulation* 60:51-59.

Baum, C., El-Tohamy, W., and Gruda, N., (2015). Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: a review. *Scientia Horticulturae* 187:131-141.

Bellaloui, N., Mengistu, A., and Kassem, M.A., (2013). Effects of genetics and environment on fatty acid stability in soybean seed. *Food Nutrition Science* 4:165-175.

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* 13:66.

Bogale, G., (2014). Resource and nutrient flows in smallholders farming systems of Kumbursa village, Ada'a district of central Ethiopia. MA thesis paper presented for Addis Ababa University, Addis Ababa, Ethiopia.

Bowles, T.M., Jackson, L.E., and Cavagnaro, T.R., (2018). Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes. *Global Change Biology* 24:171-182.

Brundrett, M.C., and Tedersoo, L., (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* 220:1108-1115.

Carvalho, M.D., and Halecki, W., (2021). Modeling of Cowpea (*Vigna unguiculata*) Yield and Control Insecticide Exposure in a Semi-Arid Region. *Plants* 10:1074.

Chang, W., Sui, X., Fan, X., Jia, T., and Song, F., (2018). Arbuscular mycorrhizal symbiosis modulates antioxidant response and ion distribution in salt-stressed *Elaeagnus angustifolia* seedlings. *Frontiers in Microbiology* 9:652.

Chavoshi, S., Nourmohamadi, G., Madani, H., Heidari Sharif Abad, H., and Alavi Fazel, M., (2018). The effects of biofertilizers on physiological traits and biomass accumulation of red beans (*Phaseolus vulgaris cv. Goli*) under water stress. *Iranian Journal of Plant Physiology* 8:2555-2562.

Cirillo, V., D'Amelia, V., Esposito, M., Amitrano, C., Carillo, P., Carputo, D., and Maggio, A., (2021). Anthocyanins are key regulators of drought stress tolerance in tobacco. *Biology* 10:139.

Cuéllar, T., Pascaud, F., Verdeil, J.L., Torregrosa, L., Adam-Blondon, A.F., Thibaud, J.B., and Sentenac, H., (2010). Gaillard, I. A grapevine Shaker inward K(+) channel activated by the calcineurin B-like calcium sensor 1-protein kinase CIPK23 network is expressed in grape berries under drought stress conditions. *Plant Journal* 61:58-69.

De Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., and Schjoerring, J.K., (2021). The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. *New Phytologist* 229:2446-2469.

Diatta, A.A., Fike, J.H., Battaglia, M.L., Galbraith, J.M., and Baig, M.B., (2020). Effects of biochar on soil fertility and crop productivity in arid regions: a review. *Arabian Journal of Geosciences* 13:1-17.

Dien, D.C., Mochizuki, T., and Yamakawa, T., (2019). Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in Rice (*Oryza sativa L.*) varieties. *Plant Production Science* 22:530-545.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., and Zheng, B., (2016). Biochar to improve soil fertility. A review. *Agronomy for Sustainable Development* 36:1-18.

Dodd, I.C., and Ruiz-Lozano, J.M. (2012). Microbial enhancement of crop resource use efficiency. *Current Opinion in Biotechnology* 23:236-242.

Duong, T.T., Penfold, C., and Marschner, P., (2012). Amending soils of different texture with six compost types: impact on soil nutrient availability, plant growth and nutrient uptake. *Plant and Soil* 354:197-209.

Enyiukwu, D.N., Ononuju, C.C., and Maranzu, J.O., (2018). Mycotoxins in foods and indoor air: their attendant diseases and modes of injury on biological and human systems. *Greener Journal of Epidemiology Public Health* 6:034-051.

Fabunmi, T.O., Gbadamosi, B.K., and Adigbo, S.O., (2012). Seed hydro-priming and early moisture stress impact on biomass production and grain yield of cowpea. *International Journal of Applied Science and Technology* 10:2-10.

Farooq, M., Gogoi, N., Barthakur, S., Baroowa, B., Bharadwaj, N., Alghamdi, S.S., and Siddique, K.H., (2017). Drought stress in grain legumes during reproduction and grain filling. *Journal of Agronomy and Crop Science* 203:81-102.

Fernie, A.R., and Pichersky, E., (2015). Focus issue on metabolism: Metabolites, metabolites everywhere. *Plant Physiology* 169:1421-1423.

Fiaz, K., Malik, S,A., Younis, U., Danish, S., Raza Shah, M.H., and Naiz, S., (2014). Drought impact on Pb/Cd toxicity remediated by biochar in (*Brassica campestris)*. *Journal of Soil Science and Plant Nutrition* 4:845-854.

Fischer, S., Hilger, T., Piepho, H.P., Jordan, I., and Cadisch, G., (2019). Do we need more drought for better nutrition? The effect of precipitation on nutrient concentration in East African food crops. *Science of the Total Environment* 658:405-415.

Franzoni, G., Trivellini, A., Bulgari, R., Cocetta, G., and Ferrante, A., (2019). Bioactive molecules as regulatory signals in plant responses to abiotic stresses. In Plant signaling molecules Woodhead Publishing 169-182.

Gao, L., Zhao, S., Lu, X., He, N., Zhu, H., Dou, J., and Liu, W., (2018). Comparative transcriptome analysis reveals key genes potentially related to soluble sugar and organic acid accumulation in watermelon. *Plos One* 13:0190096.

Geera, B., Ojwang, L.O., and Awika, M.J., (2012). New highly stable dimeric 3deoxyanthocyanidin pigments from sorghum bicolor leaf Sheath. *Journal of Food Science* 5:77.

Ghanbari, A.A., Shakiba, M.R., Toorchi, M., and Choukan, R., (2013). Nitrogen changes in the leaves and accumulation of some minerals in the seeds of red, white and chitti beans (*Phaseolus vulgaris*) under water deficit conditions. *Crop Sciences* 7:706-712.

Gilbert, M., (2017). Introduction to environmental engineering and science. Prentice Hall.

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* 96:2941-2951.

Gong, M., Tang, M., Chen, H., Zhang, Q., and Feng, X., (2013). Effects of two Glomus species on the growth and physiological performance of Sophora davidii seedlings under water stress. *New Forests* 44:399-408.

Gray, S.B., and Brady, S.M., (2016). Plant developmental responses to climate change. *Developmental Biology* 419:64-77.

Guo, Y., Ren, G., Zhang, K., Li, Z., Miao, Y., and Guo, H., (2021). Leaf senescence: Progression, regulation, and application. *Molecular Horticulture* 1:1-25.

Hall, E.A., (2012). Phenotyping cowpeas for adaptation to drought. *Frontiers in Physiology* 3:1-8.

He, K., Li, X., Chen, X., Ye, X., Huang, J., Jin, Y., Li, P., Deng, Y., Jin, Q., Shi, Q., and Shu, H., (2011). Evaluation of antidiabetic potential of selected traditional Chinese medicines in STZ-induced diabetic mice. *Journal of Ethnopharmacology* 137:1135-1142.

Horn, L.N., Nghituwamata, S.N., and Isabella, U., (2022). Cowpea Production Challenges and Contribution to Livelihood in Sub-Saharan Region. *Agricultural Sciences* 13:25-32.

Ishiyaku, M.F., and Aliyu, H., (2013). Field evaluation of cowpea genotypes for drought tolerance and Striga resistance in the dry Savanna of the North-West Nigeria responsive. *International Journal of Plant Breeding and Genetics* 7:47-56.

Jaafar, H.Z., Ibrahim, M.H., Fakri, M., and Farhana, N., (2012). Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and photosynthetic responses of Malaysian Kacip Fatimah (*Labisia pumila Benth*). *Molecules* 17:7305-7322.

Kammann, C.I., Linsel, S., Gobling, W.J., and Koyro, H., (2011). Influence of biochar on drought tolerance of Chenopodium quinoa willd and on soil-plant relations. *Plant Soil* 345:195-210.

Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., and Sharma, A., (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. *Applied Sciences* 10:5692.

Kaur, S., and Roy, A., (2021). A Review on the nutritional aspects of wild edible plants. *Current Traditional Medicine* 7:552-563.

Kebede, E., and Bekeko, Z., (2020). Expounding the production and importance of cowpea (*Vigna unguiculata (L.) Walp*) in Ethiopia. *Cogent Food and Agriculture* 1:1769805.

Kheradmand, M.A., Fahraji, S.S., Fatahi, E., and Raoofi, M.M., (2014). Effect of water stress on oil yield and some characteristics of *Brassica napus*. *International Journal of Applied and Basic Medical Research* 8:1447-1453.

Khoo, H.E., Azlan, A., Tang, S.T., and Lim, S.M., (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food and Nutrition Research* 61:1361779.

Kranz, C.N., McLaughlin, R.A., Johnson, A., Miller, G., and Heitman, J.L., (2020). The effects of compost incorporation on soil physical properties in urban soils-A concise review. *Journal of Environmental Management* 261:110209.

Kumari, V.V., Banerjee, P., Verma, V.C., Sukumaran, S., Chandran, M.A.S., Gopinath, K.A., Venkatesh, G., Yadav, S.K., Singh, V.K., and Awasthi, N.K., (2022). Plant Nutrition: An Effective Way to Alleviate Abiotic Stress in Agricultural Crops. *International Journal of Molecular Sciences* 23:8519.

Kumawat, K.R., and Sharma, N.K., (2018). Effect of Drought Stress on Plants Growth. *Popular Kheti* 6:239-241.

Lal, R., (2015). Restoring soil quality to mitigate soil degradation. *Sustainability* 7:5875-5895.

Landi, M., Tatttini, M., and Gould, K.A., (2015). Multiple functional roles of anthocyanins in plant-environment interactions. *Environmental and Experimental Botany* 119:4-17.

Lehmann, J., and Joseph, S., (2015). Biochar for environmental management: an introduction. In: Lehmann J, Joseph S (eds) Biochar for environmental management: science, technology and implementation, Routledge, New York 1-12.

Lentz, R.D., Ippolito, J.A., and Lehrsch, G.A., (2019). Biochar, Manure, and Sawdust Alter Long-Term Water Retention Dynamics in Degraded Soil. *Soil Science Society of America Journal* 83:1491-1501.

Li, J., Meng, B., Chai, H., Yang, X., Song, W., Li, S., Lu, A., Zhang, T., and Sun, W., (2019a). Arbuscular mycorrhizal fungi alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses via altering antioxidant enzyme activities and photosynthesis. *Frontiers in Plant Science* 10:499.

Li, J., Meng, Y., Zhang, K., Li, Q., Li, S., Xu, B., and Georgiev, M.I., (2021). Jasmonic acidresponsive RRTF1 transcription factor controls DTX18 gene expression in hydroxycinnamic acid amide secretion. *Plant Physiology* 185:369-384.

Li, Q., Wang, Y., Mai, Y., Li, H., Wang, Z., Xu, J., and He, X., (2020). Health benefits of the flavonoids from onion: Constituents and their pronounced antioxidant and antineuroinflammatory capacities. *Journal of Agricultural and Food Chemistry* 68:799-807.

Li, S., Harris, S., Anandhi, A., and Chen, G., (2019b). Predicting biochar properties and functions based on feedstock and pyrolysis temperature: A review and data syntheses. *Journal of Cleaner Production* 215:890-902.

Liu, C.W., and Murray, J.D., (2016). The role of flavonoids in nodulation host-range specificity: an update. *Plants* 5:33.

Lusiba, S.G., Odhiambo, J.J.O., Adeleke, R., and Maseko, S.T., (2021). The potential of biochar to enhance concentration and utilization of selected macro and micro nutrients for chickpea (*Cicer arietinum*) grown in three contrasting soils. *Rhizosphere* 17:100289.

Luthra, R., Roy, A., Pandit, S., and Prasad, R., (2021). Biotechnological methods for the production of ginsenosides. *South African Journal of Botany* 141:25-36.

Mabudi-Bilasvar, H., Ghassemi-Golezani, K., and Mohammadi Nassab, A.D., (2022). Seed development, oil accumulation and fatty acid composition of drought stressed rapeseed plants affected by salicylic acid and putrescine. *Gesunde Pflanzen* 74:333-345.

Machado, N., Oppolzer, D., Ramos, A., Ferreira, L., Rosa, E.A.S., Rodrigues, M., and hhrros, A.I.R.N.A., (2017). Evaluating the freezing impact on the proximate composition of immature cowpea (*Vigna unguiculata L.*) pods: Classical versus spectroscopic approaches. *Journal of the Science of Food and Agriculture* 13:4295-4305.

Major, J., Rondon, M., Molina, D., Riha, S.J., and Lehmann, J., (2010). Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant and Soil* 333:117-128.

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

Manzeke, MG.., Mtambanegwe, F., Nezomba, H., Watts, M.J., Broadley, M.R., and Mapfumo, P., (2017). Zinc fertilization increases productivity and grain nutritional quality of cowpea (*Vigna unguiculata [L.] Walp.*) under integrated soil fertility management. *Field Crops Research* 213:231-244.

Marschner, P., (2012). Marschner's mineral nutrition of higher plants. Academic Press: London. UK 17:8-189.

Martínez-Blanco, J., Lazcano, C., Christensen, T.H., Muñoz, P., Rieradevall, J., Møller, J., Antón, A. and Boldrin, A., (2013). Compost benefits for agriculture evaluated by life cycle assessment. A review. *Agronomy for Sustainable Development* 33:721-732.

Mathur, S., Tomar, R.S., and Jajoo, A., (2018). Arbuscular mycorrhizal fungi (AMF) protects photosynthetic apparatus of wheat under drought stress. *Photosynthesis Research* 139:227-238.

Mekonnen, T.W., Gerrano, A.S., Mbuma, N.W., and Labuschagne, M.T., (2022). Breeding of vegetable cowpea for nutrition and climate resilience in Sub-Saharan Africa: progress, opportunities, and challenges. *Plants* 11:1583.

Mensah, A.K., and Frimpong, K.A., (2018). Biochar and or compost applications improve soil properties, growth, and yield of maize grown in acidic rainforest and coastal savannah soils in Ghana. *International Journal of Agronomy* 8:683-740.

Mfeka, N., Mulidzi, R.A., and Lewu, F.B., (2019). Growth and yield parameters of three cowpeas (*Vigna unguiculata L. Walp*) lines as affected by planting date and zinc application rate. *South African Journal of Science* 115:27-34.

Mohammed, S.B., Dzidzienyo, D.K., Yahaya, A., L., Umar, M., Ishiyaku, M.F., Tongoona, P.B., and Gracen, V., (2021). High soil phosphorus application significantly increased grain yield, phosphorus content but not zinc content of cowpea grains. *Agronomy* 11:802.

Mundim, F,M., and Pringle, E.G., (2018). Whole-plant metabolic allocation under water stress. *Frontier Scientific* 9:852.

Nadeem, S.M., Imran, M., Naveed, M., Khan, M.Y., Ahmad, M., Zahir, Z.A., and Crowly, D.E., (2017). Synergistic use of biochar, compost and plant growth-promoting rhizobacteria for enhancing cucumber growth under water deficit conditions. *Journal Science Food Agriculture* 97:5139-5145.

Namatsheve, T., Chikowo, R., Corbeels, M., Mouquet-Rivier, C., Icard-Vernière, C., and Cardinael, R., (2021). Maize-cowpea intercropping as an ecological intensification option for low input systems in sub-humid Zimbabwe: Productivity, biological N2-fixation and grain mineral content. *Field Crops Research* 263:108052.

Nassour, R., Ayash, A., and Al-Tameemi, K., (2020). Anthocyanin pigments: Structure and biological importance. *Journal of Chemical and Pharmaceutical Sciences* 13:45-57.

Nguyen, T.T., Fuentes, S., and Maeschener, M., (2012). Effects of compost on water availability and gas exchange in tomato during drought and recovery. *Plant Soil and Environment* 11:58-495.

Ojwang, L.O., and Awika, J.M., (2010). Stability of apigeninidin and its methoxylated derivatives in the presence of sulphites. *Journal of Agricultural and Food Chemistry* 58:90779082.

Olalekan, A.J., and Bosede, B.F., (2010). Comparative study on chemical composition and functional properties of three Nigerian legumes (Jack beans, Pigeon pea and Cowpea). *Journal of Emerging Trends in Engineering and Applied Science* 1:89-95.

Omomowo, O.I., and Babalola, O.O., (2021). Constraints and Prospects of Improving Cowpea Productivity to Ensure Food, Nutritional Security and Environmental Sustainability. *Frontiers Plant Science* 12.

Pagano, M.C., (2014). Drought stress and mycorrhizal plant. In Use of Microbes for the Alleviation of Soil Stresses, Springer, New York, NY 97-110.

Palma-Tenango, M., Soto-Hernández, M., and Aguirre-Hernández, E., (2017). Flavonoids in agriculture. *Flavonoids from Biosynthesis to Human Health* 189-201.

Pan, J., Huang, C., Peng, F., Zhang, W., Luo, J., Ma, S., and Xue, X., (2020). Effect of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR) inoculations on *Elaeagnus angustifolia L.* in saline soil. *Applied Sciences* 10:945.

Pereira, L., (2018). Seaweeds as source of bioactive substances and skin care therapycosmeceuticals, algotheraphy, and thalassotherapy. *Cosmetics* 5:68.

Piao, S., Ciais, P., Huang, Y., Shen, Z., Peng, S., Li, J., Zhou, L., Liu, H., Ma, Y., Ding, Y., and Friedlingstein, P., (2010). The impacts of climate change on water resources and agriculture in China. *Nature* 467:43-51.

Qi, J., Sun, S., Yang, L., Li, M., Ma, F., and Zou, Y., (2019). Potassium uptake and transport in apple roots under drought stress. *Horticultural Plant Journal* 5:10-16.

Ramakrishna, A., and Ravishankar, G.A., (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behaviour* 6:1720-1731.

Rashid, M., Hussain, Q., Khan, K.S., Al-Wabel, M.I., Afeng, Z., Akmal, M., Ijaz, S.S., Aziz, R., Shah, G.A., Mehdi, S.M., and Alvi, S., (2020). Prospects of biochar in alkaline soils to mitigate climate change. In Environment, climate, plant and vegetation growth. Springer, Cham 133-149.

Ravelombola, W., Shi, A., Chen, S., Xiong, H., Yang, Y., Cui, Q., Olaoye, D., and Mou, B., (2020). Evaluation of cowpea for drought tolerance at seedling stage. *Euphytica* 216:1-19.

Rehman, A.U., Rather, G.H., Gull, Y., Mir, M.R., Mir, M.M., Waida, U.I., and Hakeem, K.R., (2015). Effect of climate change on Horticultural. In: Hakeem, K.R., Ed., Crop production and global environment issues, Springer, Berlin 211-239.

Reis, P.A.B., Carpinetti, P.A., Freitas, P.P.J., Santos, E.G.D., Camargos, L.F., Oliveira, I.H.T., and Fontes, E.P.B., (2016). Functional and regulatory conservation of the soybean ER stressinduced DCD/NRP-mediated cell death signaling in plants. *BMC Plant Biology* 16:156.

Resco de Dios, V., Chowdhury, F.I., Granda, E., Yao, Y., and Tissue, D.T., (2019). Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. *New Phytologist* 223:1696-1706.

Rubin, R.L., van Groenigen, K. J., and Hungate, B.A., (2017). Plant growth promoting rhizobacteria are more effective under drought: A metaanalysis. *Plant and Soil* 416:309-323.

Saharwardi, M.S., and Kumar, P., (2022). Future drought changes and associated uncertainty over the homogenous regions of India: a multimodel approach. *International Journal of Climatology* 42:652-670.

Sánchez-Monedero, M.A., Cayuela, M.L., Sánchez-García, M., Vandecasteele, B., D'Hose, T., López, G., Martínez-Gaitán, C., Kuikman, P.J., Sinicco, T., and Mondini, C., (2019). Agronomic evaluation of biochar, compost and biochar-blended compost across different cropping systems: Perspective from the European project FERTIPLUS. *Agronomy* 9:225.

Schnell, R.W., Vietor, D.M., Provin, T.L., Munster, C.L., and Capareda, S., (2012) Capacity of biochar application to maintain energy crop productivity: Soil chemistry, sorghum growth, and runoff water quality effects. *Journal of Environmental Quality* 4:1044-1049.

Sehgal, A., Sita, K., Bhandari, K., Kumar, S., Kumar, J., Vara Prasad, P.V., Siddique, K.H., and Nayyar, H., (2019). Influence of drought and heat stress, applied independently or in combination during seed development, on qualitative and quantitative aspects of seeds of lentil (*Lens culinaris Medikus*) genotypes, differing in drought sensitivity. *Plant, cell and Environment* 42:198-211.

Sevanto, S., (2018). Drought impacts on phloem transport. *Current Opinion in Plant Biology* 43:76-81.

Shen, N., Wang, T., Gan, Q., Liu, S., Wang, L., and Jin, B., 2022. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry* 132531.

Singh, J., Jayaprakasha, G.K., and Patil, B.S., (2018). Extraction, identification, and potential health benefits of spinach flavonoids: a review. *Advances in Plant Phenolics: From Chemistry to Human Health* 107-136.

Snapp, S., Rahmanian, M., and Batello, C., (2018). Pulse crops for sustainable farms in subSaharan Africa. United Nations.

Sohi, S.P., Krull, E., Lopez-Capel, E., and Bol, R., (2010). A review of biochar and its use and function in soil. *Advances in Agronomy* 105:47-82.

Spokas, K.A., Cantrell, K.B., Novak, J.M., Archer, D.W., Ippolito, J.A., Collins, H.P., Boateng, A.A., (2012). Biochar: A synthesis of its agronomic impact beyond carbon sequestration. *Journal of Environmental Quality* 4:973-981.

Stoilova, T., Chavdarov, P., Velcheva, N. and Petrova, S., (2022). Cowpea (*Vigna unguiculata L.*) an alternative crop for dry areas. *Agricultural Sciences* 14:32.

Tadayyon, A., Nikneshan, P., and Pessarakli, M., (2018). Effects of drought stress on concentration of macro-and micro-nutrients in Castor (*Ricinus communis L.*) plant. *Journal of Plant Nutrition* 41:304-310.

Taiz, L., and Zeiger, E., (2013). Plant physiology. 3rd edition. The Banjamin Cummings publishing company, Redwood Califorlia.

Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y., and Yang, Z., (2015). Application of biochar for the removal of pollutants from aqueous solutions. *Chemosphere* 125:70-85.

Teixeira, A.I., Ribeiro, L.F., Rezende, S.T., Barros, E.G., and Moreira, M.A., (2012). Development of a Method to Quantify Sucrose in Soybean Grains. *Food Chemistry* 130:11341136.

Tekalign, M., and Tegbaru, B., (2015). Ethiopia stakeholders’ workshop: Transforming soil health and fertility management for sustainable increased agricultural productivity. Soil Workshop Proceedings 5 and 6 November 2015 Addis Ababa, Ethiopia 36.

Tittonell, P., and Giller, K.E., (2013). When yield gaps are poverty traps: The paradigm of ecological intensification in African smallholder agriculture. *Field Crops Research* 143:76-90.

Tiwari, R., and Rana, C.S., (2015). Plant secondary metabolites. *International Journal of Engineering Research and General Science* 3:2091-2730.

Tiwari, S., Lata, C., Chauhan, P.S., and Nautiyal, C.S., (2016). *Pseudomonas putida* attunes morphophyisiological, biochemical and molecular responses in *Cicer arietinim* L. during drought stress and recovery. *Plant Physiology* 99:108-117.

Ullah, N., Ditta, A., Imtiaz, M., Li, X., Jan, A.U., Mehmood, S., Rizwan, M.S., and Rizwan, M., (2021). Appraisal for organic amendments and plant growth-promoting rhizobacteria to enhance crop productivity under drought stress: A review. *Journal of Agronomy and Crop Science* 207:783-802.

Usharani, K.V., Roopashree, K.M., and Naik, D., (2019). Role of soil physical, chemical and biological properties for soil health improvement and sustainable agriculture. *Journal of Pharmacognosy and Phytochemistry* 8:1256-1267.

Vanlauwe, B., Hungria, M., Kanampiu, F., and Giller, K.E., (2019). The role of legumes in the sustainable intensification of African smallholder agriculture: Lessons learnt and challenges for the future. *Agriculture, Ecosystems and Environment* 284:106583.

Verheijen, F., Jeffery, S., Bastos, A.C., Van der Velde, M., and Diafas, F., (2010). Biochar application to soils. A critical scientific review of effects on soil properties, processes, and functions. 1st edition. EUR 24099 EN Office for the Official Publications of the European communities, Luxembourgp.

Vessal, S., Arefian, M., and Siddique, K.H., (2020). Proteomic responses to progressive dehydration stress in leaves of chickpea seedlings. *BMC Gegomics* 21:523.

Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M., and Skz, A., (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research* 184:13-24.

Wang, F., Zhang, L., Zhou, J., Rengel, Z., George, T.S., and Feng, G., (2022). Exploring the secrets of hyphosphere of arbuscular mycorrhizal fungi: processes and ecological functions. *Plant and Soil* 1-22.

Wu, M., Guo, X., Wu, J., and Chen, K., (2020). Effect of compost amendment and bioaugmentation on PAH degradation and microbial community shifting in petroleumcontaminated soil. *Chemospher*e 256:126998.

Xia, H., Zhao, J., Sun, J., Xue, Y., Eagling, T., Bao, X., Zhang, F., and Li, L., (2013). Maize grain concentrations and above-ground shoot acquisition of micronutrients as affected by intercropping with turnip, faba bean, chickpea, and soybean. *Science China Life Sciences* 56:823-834.

Xu, W., Cui, K., Xu, A., Nie, L., Huang, J., and Peng, S., (2015). Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. *Acta Physiologiae Plantarum* 37:1-11.

Yahaya, D., Denwar, N., and Blair, M.W., (2019). Effects of moisture deficit on the yield of cowpea genotypes in the Guinea Savannah of Northern Ghana. *Agricultural Sciences* 4:577595.

Yeboah, E., Asamoah, G., Ofori, P., Amoah, B., and Agyeman, K.O.A., (2020). Method of biochar application affects growth, yield and nutrient uptake of cowpea. *Open Agriculture* 5:352-360.

Yiğit, A., (2015). Determination of protein, amino acid distribution and antioxidant activity of widely grown bread wheat (*Triticum aestivum L.*) varieties in Turkey (Doctoral dissertation, M.Sc. Thesis, Adnan Menderes University, Turkey.

CHAPTER 3

Response of growth and phenological attributes of cowpeas (*Vigna unguilata Walp L.*) to integrated MYCOROOT™ inoculation, biochar-compost mixtures and two moisture regimes

Abstract

Cowpeas (*Vigna unguilata L.*) is a food legume that provides food for millions of people, mainly in developing countries. The crop is consumed for its high protein content and other nutritional benefits such vitamins and minerals. Incorporating more efficient approaches of production such as Mycoroot^{TM} which is a biofertiliser that consist of natural *Arbuscular mycorrhizae fungi* (AMF) has been shown to improve growth in crops as these fungi can assist crops with water and nutrients absorption, and control osmotic adjustment in adverse conditions such as moisture stress. Therefore, a pot experiment was conducted under Greenhouse condition at the University of Mpumalanga to examine the combined effect of Mycoroots^{TM} AMF inoculation with varied biochar-compost mixtures as an agronomic package to enhance cowpea growth and phenological attributes in two soil textural types with different moisture levels. The experiment consisted of 2 soil textural types (sandy loam and loamy sand), 4 soil amendments comprising different mix ratios of biochar (BC) and compost (C), 2 AMF levels (inoculated and uninoculated) and 2 soil moisture regimes (adequate soil moisture and moisture stressed) as main treatment factors. The soil amendments comprised of 50%biochar50%compost (50:50 BC/C), 75%biochar25%compost (75:25 BC/C), 25%biochar75%compost (25:75 BC/C) and a control without amendment. Treatment factors were combined and laid out in a 2x2x4x2 factorial design fitted into RCBD; with each replicated 4 times. Data collected included growth data measured at 3 weeks interval and phenological data monitored daily in the reproduction stage and weekly for stomatal conductance. Results revealed a significant ($p<0.05$) interaction between soil textural types and moisture levels on the assessed growth attributes while the number of days to flowering was less severe in sandy loam than in loamy sand. AMF inoculation resulted in the highest leaf length (13.8 cm) at reproductive stage in comparison with non-inoculated treatments. Integrated use of 75BC:25C as soil amendment gave the highest leaf length (14.12 cm). Integrated use of biochar, compost and Mycoroot^{TM} product as a soil amendment represents a valuable approach to lessen the deleterious effects of soil nutrients and moisture stress condition and improve cowpea growth.

Keywords: Cowpeas**,** moisture stress**,** *arbuscular mycorrhizal fungi*, biochar, compost

3.1 Introduction

As the world population is exponentially increasing so does the requirement for food to feed the population (Dinar et al 2019). Agricultural farmers particularly based in semi-arid and arid areas have been facing challenges of constant reduction of crop yields (Ndhleve et al 2017). These reductions often influenced by climate change limits the amount and distribution rainfall leading to adverse moisture stress being introduced to crops (Corwin 2021). Moisture stress limits crop production thereby aiding food insecurity especially in rural underprivileged settlements (Wheeler and Von Braun 2013).

Cowpeas are widely consumed vegetables that are essential component in the human diet due to their high protein content, carbohydrates and valuable amino acids (Jayathilake et al 2018; Beebe et al 2013). The crop is mostly produced and consumed by subsistence farmers situated in semi-arid and arid regions whereby they are not grown only for their dry seeds, but their leaves and pods as vegetables (Gerrano et al 2022; Wabwayi et al 2020; Dube and Fanadzo 2013). The leaves and pods provide ordinary households with valuable protein and mineral sources particularly those from underprivileged communities as compared to other leguminous grains (Alemu et al 2016; Bvenura and Afolayan 2015). Cowpeas is a drought tolerant legume crop (Nkomo et al 2021). Despite the ability of cowpeas to adapt in low moisture levels, there is enough documented information illustrating that growth and productivity is hindered under extent levels of moisture stress, threatening the genetic potential of cultivated varieties (Ritte et al 2022; Rathore et al 2015).

Mycoroot^{TM} is a locally produced commercial granular product comprised of indigenous strains of *Arbuscular mycorrhizae fungi* (AMF) applied in agricultural soils (Dames 2011). In natural environments, *Arbuscular mycorrhizae fungi* (AMF) play a significant role in plant abiotic alleviation stresses (Augé et al 2015). They form symbiotic relationships between the roots of higher plants (Kafle et al 2019). AMF form hyphae networks which are important components in the development of stable soil aggregates and the establishment of microspores, assisting plants with the utilisation and absorption of water and nutrients under moisture stress conditions (Rush et al 2021; Symanczik et al 2020; Soka and Ritchie 2014). A number of studies have shown that the presence of AMF can improve water transport, nutrient acquisition and osmotic adjustment, which is of benefit for the survival of plants under stress (Cheng et al 2022; Jongen et al 2022; Fattahi et al 2021).

Biochar is a carbon-rich material composed from heating biomass under anaerobic conditions through the pyloric process (Wang and Wang 2019; Meyer et al 2017). This material is highly porous in nature which enables it to soak up and store in water and nutrients within the soil which is used by cultivated crops (Tan et al 2015). This improves physical properties of soil such as water holding capacity (Zhou et al 2021). Additionally, biochar surfaces provides a cation exchange surfaces that support valuable nutrient absorption which leads to improved nutrition in more biochar amended soils (Hollister et al 2013).

Compost refers to a type of decomposed organic material that is utilised as a fertiliser and a conditioner for the soil to enhance fertility (Ding et al 2021). Compost in numerous studies, has been shown to be a valuable resource that contains co-substrates and important nutrients that can improve the soil's ability to hold onto water by increasing soil macro porosity, improving soil ventilation, and changing the structure of the soil's beneficial microorganisms (Wu et al 2020). Soil texture is probably the most crucial of these properties, which has an impact on soil water dynamics and nutrient. Sandy loam is distinguished by a weak structure, poor water and nutrient retention, which can cause the development of various adverse effects on crop establishment due to its high permeability and poor drainage (You et al 2019). While loamy sand allows efficient drainage and is filled with nutrients. There are various reported benefits associated with soil amendments and *Arbuscular mycorrhizae fungi* that contributes significantly to agricultural sustainability. However, not a lot of studies have been done on cowpea production. Hence, it is hypothesized that sole and combined Mycoroot™ inoculation and variable biochar-compost mixtures have no effect on cowpea growth and phenological attributes with and without moisture stress, they will equally perform the same. Identifying the use of appropriate recommended integrated biochar and compost rates in combination with AMF inoculation will assist resource poor small-scale farmers to be able to improve cowpeas growth and development.

3.2 Materials and Methods

3.2.1 Description of the study site

The study involved a pot trail, conducted under greenhouse conditions at the Teaching and Research farm (25°43˚65˚S; 30˚98˚18˚E) of University of Mpumalanga, Mbombela Campus.

3.2.2 Cowpeas seed inoculation

A locally produced and marketed commercial inoculant Mycoroot™ (Figure 1) obtained from Mycoroot™ (Pty) Ltd, South Africa based at Grahamstown, Eastern Cape Province was used as seed inoculant for the study. It comprises of indigenous Mycorrhiza fungi *(Arbuscular mycorrhizae fungi)* was used for the inoculation of the cowpea seeds.

Figure 1: Mycoroot™ inoculant grannules

3.2.3 Description of experiment, treatments, research design and layout

The experiment consisted of four different factors namely: two soil textural types (sandy loam and loamy sand), soil amendments based on complementary application of different mix ratios of biochar (BC) and compost (C) including a control without amendments as a standard check, Moisture levels (adequate moisture and moisture stressed) and cowpea seed inoculation (AMF inoculated and AMF non-inoculated seeds). The three soil amendments comprised of 50% biochar and 50% compost (50:50 BC/C), 75% biochar + 25% compost (75:25 BC/C) and 25% biochar + 75% compost (25:75 BC/C). The various factors resulted in 2x2x4x2 factorial experiment with a total of thirty-two (32) treatment combinations (Table 3.1) replicated 4 times. All these resulted in a total of 128 pots used for the trial.

Table 3. 1: Treatment combinations

Treatment combination	With inoculation	Without inoculation
S1M1SA1	S1M1SA1 $^{N+}$	S1M1SA1 $^{N-}$
S1M1SA2	S1M1SA2 $^{N+}$	S1M1SA2 $^{N-}$
S1M1SA3	S1M1SA3 $^{N+}$	S1M1SA3 $^{N-}$
S1M1SA4	S1M1SA4 $^{N+}$	S1M1SA4 $^{N-}$
S1M2SA1	S1M2SA1 $^{N+}$	S1M2SA1 $^{N-}$
S1M2SA2	S1M2SA2 $^{N+}$	S1M2SA2 $^{N-}$
S1M2SA3	S1M2SA3 $^{N+}$	S1M2SA3 $^{N-}$
S1M2SA4	S1M2SA4 $^{N+}$	S1M2SA4 $^{N-}$
S2M1SA1	S2M1SA1 $^{N+}$	S2M1SA1 $^{N-}$
S2M1SA2	S2M1SA2 $^{N+}$	S2M1SA2 $^{N-}$
S2M1SA3	S2M1SA3 $^{N+}$	S2M1SA3 $^{N-}$
S2M1SA4	S2M1SA4 $^{N+}$	S2M1SA4 $^{N-}$
S2M2SA1	S2M2SA1 $^{N+}$	S2M2SA1 $^{N-}$
S2M2SA2	S2M2SA2 $^{N+}$	S2M2SA3^{N-}
S2M2SA3	S2M2SA3 $^{N+}$	S2M2SA3^{N-}
S2M2SA4	S2M2SA4 $^{N+}$	S2M2SA4 $^{N-}$

3.2.4 Agronomic Practices

Two soils with distinct textural types (sandy loam and loamy sand) collected from uncultivated areas at the University of Mpumalanga experimental farm were used for the study. The soils collected from 0-20 cm soil depth were subjected to air-drying, sieving to remove plant roots and stones and homogenization before weighing into 30-cm diameter pots used for planting the trial. Commercially produced biochar used was purchased from Organic Matter SA (Pty)

Limited, Garsfontein, Pretoria while Earth 2 Earth compost was obtained from Plasgrow Whiteriver, which is approximately 18 km away from the University campus. Filling of each planting pot with 10 kg of the homogenized soil was followed by the application of the various compost-biochar mix ratios based on the treatments as soil amendments in each of well-labelled pot. The rate of 12 t ha^{-1} for biochar based on the recommendation by Berihun et al (2017) supported by Neyton et al (2020) and 10 t ha^{-1} according to Adejumo et al (2017) for compost was adopted for this study. Each soil-filled pot containing soil amendments (i.e. biochar-compost mixtures) were thoroughly mixed with 800 ml tap water subsequently added, allowed for equilibration and left for a period of 7 days to allow for soil reaction and mineralization before planting was undertaken. Prior to seed sowing, each pot received further 500 ml water and left overnight for equilibration. Four seeds were sown at a depth of 2-3 cm per pot, 1 g of Mycoroot™ inoculant granules (figure 1) were placed next to the seeds at 2 cm distance, thinned to two plants per pot 12 days after seedling emergence. All pots containing treatments were arranged in a completely randomized design and each replicated four times. The pots containing growing plants continued to receive regular irrigation for a further period of 6 weeks (42 days) after which moisture stress was imposed in the specified pots by withholding watering for a period of 12 days. Thereafter, irrigation with 250 ml water resumed to keep and maintain the plants alive until physiological maturity as described by Mwela et al (2017).

3.2.5 Data collection on cowpea growth and phenology attributes

Plant height, number of trifoliate leaves per plant, length and width of trifoliate leaves and chlorophyll content were measured on the two plants per pot at vegetative stage, reproductive stage and physiology maturity. Plant height was measured from ground level to the tip of the plant using a measuring tape. The number of trifoliate leaves were counted per plant and recorded. Length (cm) and width (cm) were measured using a ruler. To measure leaf length, a ruler was placed at the stem base of each trifoliate leaf to the growing apex. Leaf width was measured by placing the ruler across the broadest part of the trifoliate leaf. Chlorophyll content was measured using a chlorophyll meter. Each reading was made by placing the chlorophyll meter at the adaxial surface of the top most fully expanded leaf (Chimonyo et al 2015).

3.2.6 Phenological attributes

Regular daily visit to the greenhouse throughout the period of study to monitor and maintain the trial. Observation and recording of flower initiation and the number of days to 100%

flowering was recorded. Stomatal conductance was measured between 8 am and 12 noon during vegetative, reproductive and physiological using a leaf porometer where sensor head is attached to the abaxial surface of the most fully developed leaf.

3.3 Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) using Statistic 10.0. The significant treatment means were separated using Tukey's test at 5% level of significance. The statistical model used for data analysis is as follow:

$Y_{ijkl} = \mu + ST_i + ML_j + SA_k + IN_l + (S_i \times ML_j) + \ldots\ldots.. + (ST_i \times ML_j \times SA_k \times IN_l) + E_{jikl}$ where Y_{ijkl} = yield (measure parameters). μ = population mean. ST = effect of soil textural types. ML = effect of moisture levels. SA = effect of soil amendments. IN = effect of inoculation. $ST_i \times ML_j \times SA_k \times IN_l$ = effects of interactions. E_{jikl} = random experimental errors.

Growth data from the experient was subjected to Pearson correlation analysis to determine the relationship between the measured growth attributes.

3.4 Results

3.4.1 Sampled soil analysis results

The results of soil sample analysis for this study are presented in Table 3.2. The measured pH value of 6.80 for loamy sand and 6.27 for sandy loam suggest that the soils were slightly acidic. Available P measured in sandy loam soil is adequate based on 13.51 mg kg^{-1} is higher than the 7.0 mg kg^{-1} described optimum (FSSA 2016) required for growth and development while in loamy sand soil, the measure available P is 5.86 mg kg^{-1} is lower than the critical soil available P hence, deficient and inadequate for growth. Furthermore, the exchangeable Ca, Mg and K contents in the studied loamy sand soil were 446, 60 and 22 mg kg^{-1}, respectively that are higher than the critical level suggested by Hazelton and Murphy (2007). Similarly, the observed Ca, Mg and K contents of 456, 117 and 96 mg kg^{-1}, respectively in sandy loam soil were also higher than the critical levels described by Hazelton and Murphy (2007). The K:(Ca+Mg) of 23 and 6.0 measured in loamy sand and sandy loam soil, respectively are above the range of 1.8-2.2 prescribed for legumes by Grzegorczyk et al (2013).

3.4.2 Characteristics of the soils and amendments used in the trial

The results of chemical analysis of the two soil amendments (biochar and compost) used for this study are presented in Table 3.3. The pH of biochar indicates that it is alkaline while that of the compost indicates acidity, which suggests that the compost was possibly, produced from materials that are acidic in nature.

Table 3.2: Physical and chemical characteristics of the two soils

Soil properties	**Soil 1**	**Soil 2**
Silt	0.4%	19.4%
Sand	82%	65.2%
Clay	17.4%	15.4%
Textural class	Loamy sand	Sandy loam
pH(water)	6.80	6.27
Resistance (ohms)	580	600
Calcium, Ca (mg/kg)	446	459
Magnesium, Mg (mg/kg)	60	117
Potassium, K (mg/kg)	22	96
Sodium, Na (mg/kg)	19	8
Phosphorus, P-Bray1 (mg/kg)	5.86	13.51
Aluminium, Al (mg/kg)	5	9
Ca/Mg ratio	7.43	3.92
(Ca+Mg)/K ratio	23	6.00

Table 3.3: Chemical characteristics of the two soil amendments

Biochar		**Compost**	
Parameters	Values	Parameters	Values
Ph	10	pH	4.34
Fixed carbon content (%)	90	Moisture content (%)	8.63
Ash content	3	Ca (mg/l)	5.25

Moisture content (%)	5	Mg (mg/l)	1.19
Iodine number (mg/g)	471	K (mg/l)	17.84
Bulk density (kg/m^3)	100	Na (mg/l)	3.43
Average pore diameter	18	Cl (mg/l)	13.39
Total pore volume (cm^3/kg)	254	Zn (mg/l)	0.09
Micro-pore volume (cm^3/kg)	182	Cu (mg/l)	0.05
Total surface area (m^2/g)	551	Fe (mg/l)	2.31
Micro-pore area (m^2/g)	471	P (mg/l)	1.72
		N (mg/l)	5.39
		B (mg/l)	0.48
		Air filled porosity (%)	18.00
		Water holding capacity (%)	30.54

Source: ARC-TSC Chemical Leaf and Soil Laboratory, Nelspruit

Ca=Calcium; Mg=Magnesium; K=Potassium; Na=Sodium; P=Phosphorus; Cu=Copper; Fe=Iron; N=Nitrogen; Cl= Chloride

3.4.3 Treatment effect on measured growth parameters

3.4.3.1 Plant height

Inoculation and soil amendments had no significant effects ($p>0.05$) on plant height across all growth stages while all the measured growth parameters at the vegetative growth stage differed significantly ($p<0.05$) across the two soil textural (Table 3.4 and 3.5). However, differences in soil moisture levels exerted a significant ($p<0.05$) effect on plant height only at reproductive stage (Table 3.6). Furthermore, the interaction between soil amendments and soil textural types exerted significant ($p<0.05$) effect on plant height at vegetative stage (Table 3.6). Similarly, the interaction between inoculation and moisture levels had a significant effect on plant height at reproductive stage (Table 3.6).

3.4.3.2 Number of trifoliate leaves

Among the main treatment factors, inoculation and soil amendments had no significant effect ($p>0.05$) on the mean number of trifoliate leaves measured across all growth stages while soil textural types showed a significant effect ($p<0.05$) at both vegetative and reproductive stages (Table and 3.6). The interaction between inoculation and soil amendments had a significant

($p<0.05$) effect on the number of trifoliate at reproductive stage (Table 3.6). Similarly, soil amendments and soil textural types interaction had a significant ($p<0.05$) effect on the mean number of trifoliate per plant only at reproductive stage (Table 3.6).

3.4.2.3 Leaf length

Soil textural types were significant ($p<0.05$) on leaf length across all growth stages (Table 3.4 and 3.6). The application of Mycoroot^{TM} as inoculant had a significant effect ($p<0.05$) on leaf length at reproductive stage but insignificant at the other two growth stages (Table 3.4 and 3.6). Interestingly, moisture levels did not significantly influence ($p>0.05$) leaf length across all growth stages (Table 3.4 and 3.6). Leaf length showed a positive response ($p<0.05$) to soil amendments at reproductive and physiological maturity (Table 3.6). Moreover, a significant ($p<0.05$) interaction effect was observed between soil amendments and soil textural types on leaf length at reproductive stage while moisture levels and soil amendments interaction on leaf length was also significant at physiological maturity stage (Table 3.6).

3.4.3.4 Leaf width

Inoculation, moisture levels and soil amendments were insignificant ($p>0.05$) on leaf width across all growth stages (Table 3.4 and 3.6). However, soil textural types were significant on leaf width at reproductive and physiological maturity stage (Table 3.6). Moreover, interaction between soil amendments and soil textural types had a significant effect ($p<0.05$) on leaf width at reproductive stage (Table 3.6). Similarly, inoculation and soil textural types interaction was also significant ($p<0.05$) on leaf width at physiological maturity stage (Table 3.6).

3.4.3.5 Chlorophyll

Inoculation exerted insignificant ($p>0.05$) effect on chlorophyll content across all growth stages while soil textural types had a significant effect ($p<0.05$) across all growth stages (Table 3.4 and 3.6). However, variation in soil moisture levels had significant ($p<0.05$) effect on chlorophyll content only at the physiological stage (Table 3.6). Interaction between inoculation and soil amendments as well as between soil moisture levels and soil amendments were significant ($p<0.05$) on chlorophyll only at the physiological maturity (Table 3.6).

Table 3.4: p-values of ANOVA for the measured growth parameters at vegetative stage after seedling emergence

Factor	Plant height (cm)	No trifoliate leaves	Leaf length (cm)	Leaf width (cm)	Chlorophyll ($\mu mol\ m^{-2}$)

Inoculation (IN)	0.187	0.399	0.170	0.144	0.302
Moisture levels (ML)	0.136	1.000	0.336	0.334	0.236
Soil amendments (SA)	0.524	0.981	0.592	0.227	0.704
Soil textural types (ST)	0.000***	0.000***	0.000***	0.000***	0.000***
SA*ST	0.035*	0.363	0.450	0.267	0.371

*= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

Table 3.5: Effects of inoculation, soil moisture levels, soil amendments and soil textural types on cowpea growth parameters at vegetative stage after planting

Factor	Plant height (cm)	No of trifoliate leaves	Leaf length (cm)	Leaf width (cm)	Chlorophyll (µmol m^{-2})
Seed Inoculation					
Inoculated	39.62a	2.36a	11.92a	19.9a	15.47a
Non-inoculated	38.72a	2.30a	11.47a	19.3a	14.48a
!Critical value	1.3514	0.1464	0.6565	0.8163	1.8973
Moisture levels					
Moisture stressed	39.68a	2.33a	11.86a	19.80a	15.55a
Adequate moisture	38.66a	2.33a	11.53a	19.40a	14.41a
!Critical value	1.3514	0.1464	0.6565	0.8163	1.8973
Soil amendments					
75Bio25Comp	39.78a	2.34a	11.88a	19.46a	14.5a
25Bio75Comp	39.42a	2.32a	11.95a	19.95a	15.48a
Control	39.09a	2.34a	11.40a	18.95a	14.33a
50Bio50Comp	38.39a	2.31a	11.54a	20.3a	15.60a
!Critical value	2.5164	0.2726	1.2224	1.52	3.5329
Soil Textural types					
Sandy loam	44.95a	2.95a	14.53a	24.45a	21.64a
Loamy sand	33.39b	1.70b	8.86b	14.75b	8.31b
!Critical value	1.3514	0.1464	0.6565	0.8163	1.8973

*! Implies critical value for comparison (Tukey s HSD)

Table 3.6: p-values of ANOVA for the measured parameters at reproductive and physiological maturity stage

Factor	Plant height (cm)	No trifoliate (cm)	Leaf length	Leaf leaves width (cm)	Chlorophyll content (µmol m^{-2})	Stomatal conductance ($mmol^{-2}$ s^{-1})
		Reproductive stage				
Inoculation (IN)	0.125	0.715	0.028*	0.856	0.980	0.827
Moisture levels (ML)	0.002**	0.146	0.634	0.105	0.295	0.898

Soil amendments (SA)	0.107	0.737	0.001***	0.101	0.924	0.563
Soil textural types (ST)	0.000***	0.000***	0.000***	0.000***	0.000**	0.664
IN*ML	0.053*	0.070	0.644	0.603	0.948	0.165
IN*SA	0.285	0.033*	0.626	0.091	0.199	0.274
SA*ST	0.020**	0.021*	0.003**	0.016*	0.594	0.392
		Physiological maturity				
Inoculation (IN)	0.896	0.640	0.171	0.204	0.610	0.896
Moisture levels (ML)	0.785	0.000***	0.966	0.586	0.058*	0.034*
Soil amendments (SA)	0.727	0.749	0.018*	0.350	0.113	0.350
Soil textural types(ST)	0.000***	0.305	0.000***	0.000***	0.000***	0.578
IN*SA	0.399	0.683	0.739	0.925	0.008**	0.538
IN*ST	0.776	0.640	0.080	0.027*	0.441	0.690
ML*SA	0.820	0.636	0.051*	0.101	0.051*	0.141
ML*ST	0.132	0.001***	0.330	0.145	0.075	0.039*

*= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

Table 3.

7: Effects of inoculation, soil moisture levels, soil amendments and soil textural types on cowpea growth parameters at reproductive and physiological maturity stage

Factor	Plant height (cm)	No trifoliate leaves	Leaf length (cm)	Leaf width (cm)	Chlorophyll content (μmol m^{-2})
			Reproductive stage		
Seed Inoculation					
Inoculated	54.79a	4.16a	13.81a	22.06a	39.59a
Non-inoculated	51.59a	4.22a	13.29b	21.97a	39.53a
!Critical value	4.09	0.34	0.46	1.02	4.70
Moisture levels					
Moisture stressed	54.45a	4.06a	13.61a	22.43a	40.81a
Adequate moisture	49.94b	4.31a	13.5a	21.59a	38.31a
!Critical value	4.0911	0.3387	0.461	1.0195	4.6954
Soil amendments					
75Bio25Comp	55.87a	4.19a	14.16a	22.81a	40.25a
25Bio75Comp	51.46a	4.03a	13.54ab	21.95a	40.34a
Control	49.89a	4.25a	12.77b	21.04a	38.33a
50Bio50Comp	55.55a	4.28a	13.76a	22.29a	39.32a
!Critical value	7.6179	0.6306	0.6306	1.8984	8.7432
Soil Textural types					
Sandy loam	60.53a	4.66a	15.06a	24.64a	49.10a
Loamy sand	45.86b	3.72b	12.05b	19.39b	30.02b
!Critical value	4.0911	0.3387	0.461	1.0195	4.6954
			Physiological maturity		
Seed Inoculation					
Inoculated	63.43a	3.69a	12.69a	20.51a	37.83a
Non-inoculated	63.69a	3.77a	13.20a	21.51a	36.51a
!Critical value	3.9571	0.3305	0.7282	1.1349	5.1332
Moisture levels					
Moisture stressed	63.83a	3.23b	12.95a	20.99a	34.68a
Adequate moisture	63.29a	4.22a	12.94a	21.30a	39.66a
!Critical value	3.9571	0.3305	0.7282	1.1349	5.1332
Soil amendments					
75Bio25Comp	64.27a	3.69a	13.94a	22.04a	34.85a
25Bio75Comp	63.29a	3.59a	12.74ab	20.85a	39.76a
Control	61.83a	3.81a	12.72ab	20.96a	40.83a
50Bio50Comp	64.86a	3.81a	13.37b	20.74a	33.23a

Table 3.

!Critical value	7.3684	0.6155	1.3559	2.1132	9.5585
Soil Textural types					
Sandy loam	68.67a	3.81a	14.00a	23.37a	30b
Loamy sand	58.45b	3.64a	11.88b	18.93b	44.34a
!Critical value	3.9571	0.3305	0.7282	1.1349	5.1332

*! Implies critical value for comparison (Tukey s HSD)

3.4.3.6 Stomatal conductance

None of Mycoroot^{TM} inoculation, soil amendments nor the variation in soil textural types had no significant ($p>0.05$) effect on stomatal conductance at both vegetative and physiological maturity (Table 3.8). However, the variation in soil moisture as well as the interaction between soil moisture levels and soil textural types had significant ($p<0.05$) effect on stomatal conductance of cowpea plants at physiological maturity stage.

Table 3. 8: p-values of ANOVA for the measured stomatal conductance across all growth stages

Factors	**Stomatal conductance (mmol^{-2} s^{-1})**
Vegetative stage	
Inoculation (IN)	0.156
Moisture levels (MLs)	0.105
Soil amendments (SAs)	0.704
Soil textural types (STs)	0.200
Reproduction stage	
Inoculation (IN)	0.827
Moisture levels (MLs)	0.898
Soil amendments (SAs)	0.563
Soil textural types (STs)	0.664
Physiological maturity	
Inoculation (IN)	0.896
Moisture levels (ML)	0.034*
Soil amendments (SA)	0.350
Soil textural types (ST)	0.578
ML*ST	0.039*

*= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

Table 3.

9. Effects of inoculation, soil moisture levels, soil amendments and soil textural types on stomatal conductance (mmol^{-2} s^{-1}) across all growth stages

Factor	Vegetative stage	Reproductive stage	Physiological maturity
Inoculation (IN)			
Inoculated	1353.3a	1473.7a	616.61a
Non-inoculated	1018.5a	1526.0a	602.58a
!Critical value	464.01	472.14	211.75
Moisture levels (MLs)			
Moisture stressed	1377.4a	1484.5a	494.99b
Adequate moisture	994.4a	1515.2a	724.19a
!Critical value	464.01	472.14	211.75
Soil amendments (SAs)			
75Bio25Comp	1003.9a	1712.2a	485.39a
25Bio75Comp	1120.1a	1622.5a	742.91a
Control	1242.1a	1347a	653.1a
50Bio50Comp	1377.4	1317.8a	556.97a
!Critical value	864.02	879.16	394.29
Soil Textural types			
Sandy loam	1336.8a	1448a	579.75
Loamy sand	1034.9a	1551.7a	639.44a
!Critical value	464.01	472.14	211.75

*! Implies critical value for comparison (Tukey s HSD)

3.4.3.7 Days to flowering

Variation in soil moisture levels and soil textural types had significant ($p<0.05$) effect on the mean number of days to flowering while none of Mycoroot^{TM} inoculation nor soil amendments

Table 3.

exerted any significant (p>0.05) effect on the mean number of days to flowering (Table 3.10). Nonetheless, the interaction between Mycoroot^{TM} inoculation and soil amendments as well as variation in soil moisture levels and soil amendments interaction had significant effect on the mean number of days to flowering of the cowpea plant (Table 3.10).

Table 3. 10: p-values of ANOVA for the measured parameter days to flowering

Factor	Days to flowering
Inoculation (IN)	0.573
Moisture levels (MLs)	0.016*
Soil Amendments (SAs)	0.326
Soil textural types (STs)	0.000***
IN*SA	0.012*
MLs*SAs	0.002**

*= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

11: Effects of inoculation, soil moisture levels, soil amendments and soil textural types on the number of days to flowering on selected cowpea

Factor	Days to flowering
Inoculation (IN)	
Inoculated	42.77a
Non-inoculated	42.95a
!Critical value	0.658
Moisture levels (MLs)	
Moisture stressed	42.43b
Adequate moisture	43.27a
!Critical value	0.658
Soil amendments (SAs)	
75Bio25Comp	42.44a
25Bio75Comp	42.69a
Control	43.13a

Table 3.

50Bio50Comp	43.13a
!Critical value	1.2252
Soil Textural types (STs)	
Sandy loam	40.86b
Loamy sand	44.86a
!Critical value	0.658

*! Implies critical value for comparison (Tukey s HSD)

3.4.3.8 Pearson correlation analysis

Correlation analysis revealed that plant height was significantly and positively correlated with all four plant growth attributes (number of trifoliate leaves, leaf length, leaf width and chlorophyll) across all three growth stages (Table 3.12 to 3.15). Likewise, there was a strong and positive correlation between the mean number of trifoliate leaves per plant and leaf length, leaf width and Chlorophyll across all three growth stages. A significant and positive correlation was obtained between leaf length, leaf width and chlorophyll as well as leaf width stomatal conductance in all three stages of growth (Table 3.12 to 3.15).

Table 3.

12: Correlation coefficients among different growth attributes in cowpeas at vegetative stage

	Plant height (cm)	No of trifoliate leaves	Leaf length (cm)	Leaf width (cm)	Chlorophyll (μmol m^{-2})
Plant height	1				
No of trifoliate leaves	0.980***	1			
Leaf length	0.985***	0.981***	1		
Leaf width	0.989***	0.983***	0.992***	1	
Chlorophyll	0.910***	0.935***	0.936***	0.941***	1
Stomatal conductance	0.647***	0.647***	0.651***	0.648***	0.565***

*** indicates significance

Table 3. 13: Correlation coefficients among different growth attributes in cowpeas at reproductive stage

	Plant height	No of trifoliate leaves	Leaf length	Leaf width	Chlorophyll (μmol m^{-2})
Plant height	1				
No of trifoliate leaves	0.964***	1			
Leaf length	0.978***	0.973***	1		
Leaf width	0.978***	0.969***	0.995***	1	
Chlorophyll	0.930***	0.911***	0.940***	0.938***	1
Stomatal conductance	0.704***	0.727***	0.738***	0.739***	0.661***

*** indicates significance

14: Correlation coefficients among different growth attributes in cowpeas at physiological maturity

	Plant height (cm)	No of trifoliate leaves	Leaf length (cm)	Leaf width (cm)	Chlorophyll (μmol m^{-2})
Plant height	1				

Table 3.

No of trifoliate leaves	0.946***	1			
Leaf length	0.973***	0.962***	1		
Leaf width	0.974***	0.964***	0.995***	1	
Chlorophyll	0.873***	0.861***	0.886***	0.881***	1
Stomatal conductance	0.674***	0.770***	0.701***	0.704***	0.608***

*** indicates significance

3.5 Discussion

3.5.1 Growth and physiological parameters

Plant height is a crucial morphological and development plant attribute that determines the overall plant growth and predict yield (Wang et al 2018). The application of *Arbuscular mycorrhizae fungi* inoculation in the form of Mycoroot^{TM} did not significantly influence plant height. Our results are consistent with the findings by Ibiremo et al (2012) and Long et al (2010). These earlier authors revealed that inoculation of *Arbuscular mycorrhizae fungus* may not always yield favourable results leading to ineffectiveness of the AMF, which may possibly be affected by the presence of native AMF microbes that readily colonized the roots in understudied soils. Contrarily, Abeer et al (2015) reported that inoculation of AMF improves plant height by 49.52%. The findings from the current study that soil amendments had no positive effect on plant height across all measured growth stages concur with previous work by Trupiano et al (2017) who reported that integration of biochar and compost was negligible to growth of Lettuce (*Lactuca sativa*). Similarly, Cobb et al (2018) reported that integrating biochar and compost as soil amendments did not significantly increase the aboveground cowpea production. Furthermore, the observed significant effect of soil moisture variation on plant height during the reproductive growth stage, which was taller under moisture stress conditions than in well-watered pot may be attributed to drought escape and avoidance mechanism that involved rapid plant growth and development facilitated by the prioritised completion of the lifespan (Nadeem et al 2019). These findings contradict various previous research works reported (Santo et al 2020; Dong et al 2019; Olajide and Ilori 2017). Additionally, the significant interaction between inoculation and soil moisture levels on plant height in the current study is in agreement with earlier study by Aganchich et al (2022).

Table 3.

Soil textural types significantly influenced cowpea plant height. Taller plants were obtained in sandy loam textured treatments than in loamy sand textured treatments, supporting the findings of Al-Tawaha et al (2018). According to Shahid et al (2012) sandy loam increase water retention, which is beneficial to plant growth. Furthermore, the presence of abundant nutrients and moisture retention in sandy loam soil may have been the cause of the higher plant height. Moreover, loamy sand soils, which is similar to sandy soil, are often associated with low nutrient content (Mulcahy et al 2013). Plant height was higher in cowpea plants inoculated with Mycoroot^{TM} compare to the non-inoculated under moisture stress level. This could be attributed to the hyphae created by the Mycoroot^{TM} AMF that enhance root functionality to acquire moisture and nutrients under moisture deficits conditions (Chitarra et al 2016). In addition, plant height had a positive association with the mean number of trifoliate leaves, leaf width, leaf length and chlorophyll suggesting that an increase in plant height leads to an increase in an increase in the number of trifoliate leaves, leaf width, leaf length and chlorophyll content (Table 3.12 to 3.14).

Trifoliate leaves are excellent physical traits that indicate the plant proper development characterised by a leaf divided into three leaflets (Paixão et al 2019). The finding that the application of AMF inoculation in the form of Mycoroot^{TM} had no significant effect on the number of trifoliate leaves across all sampling days agrees with earlier findings by Aprahamian et al (2016) who reported a reduction in plant performance under inoculation compared to controls. Contrarily, several studies have shown a significant increase in leaf number following AMF inoculation (Sebastin et al 2021; Adeyemi et al 2020; Liu et al 2018). The significant effect of variation in soil moisture level on the number of trifoliate leaves reported in the current study is consistent with the findings of Olorunwa et al (2021) and Ndiso et al (2016). Under moisture stress, the mean number of trifoliate leaves were less compared to adequately watered plants. Moisture stress has been reported to cause a significant increase in leaf drop and/or leaf senescence (Santos et al 2020; Hayatu et al 2014; Okon 2013). The reduction in the number of trifoliate leaves during moisture stress is believed to be caused by reduced node emergence and cellular expansion (Wijewardana et al 2019). Moreover, Riaz et al (2013) revealed that the reduction of leaf number is associated with moisture preservation mechanism under moisture stress conditions. Moisture stress proves to be the most detrimental environmental stress on leaves development. Leaf senescence occurred during drought stress imposition period where leaves gradually changed in colour. This phenomenon occurs during moisture stress where macromolecules relocates nutrients from leaves to other organs to improve plants fitness (Guo et al 2021). This therefore caused drying out and eventually leaf drop. Leaves from adequate moisture regime performed better until physical maturity.

Application of different soil amendments rates showed no statistical effect on the number of trifoliate leaves. In the current study, leaf number was almost similar among all treatments. Response of soil amendment rates is likely depended on the soil characteristics such as pH and cation exchange capacity, which might have lessened the impact of the soil amendment rates on plant development (Agegnehu et al 2017; Schulz et al 2013). These results were contrary to those of Mensah and Frimpong (2018) who observed an increase in the number of leaves under intergrated application of biochar and compost. Numerous research works have documented that biochar and compost integration may result in an oversupply of toxic elements such as lead, cadmium, aluminium and manganese or micronutrients that are only required in small quantity, which hinder crop physical performance (Domene 2016; Kloss et al 2012; Beesley and Marmiroli 2011). As expected, soil textural types had a significant effect on the number of trifoliate leaves. Sandy loam textured soil recorded a higher leaf number in comparison to loamy sand. Soil classified as loamy sand are faced with low nutrient and moisture retention and thus limit crop performance (Uzoma et al 2011). Furthermore, current results showed a significant inoculation and soil amendments interaction effect on the mean number of trifoliate leaves, which is consistent with earlier findings by Pineiro et al (2013) who reported a significant effect of inoculation on the number of trifoliate leaves. The combined application of mycorrhizal inoculation and soil amendments resulted in increased growth and survival of the crop under moisture stress condition rather than when either is used alone. Similarly, the number of trifoliate leaves showed a positive response to the interaction between soil amendments and soil textural types. Sandy loam soil under different soil amendments rates recorded the highest number of trifoliate leaves compared to loamy sand.

Inoculation showed no significant effect on leaf width across all measured days. However, inoculation showed a significant effect on leaf length at vegetation stage after planting. The results collaborate with the findings of Long et al (2010). The AMF association maximise the use soil nutrients to benefits the host plant (Olawuyi et al 2012). However, the lack of inoculation could be attributed to the duration of the inoculation process. Previous studies have indicated that the efficiency depends on early colonization (Rahimzadeh and Pirzad 2017; Majewska et al 2016). Interestingly, both leaf attributes were not affected by moisture levels in the current experiment. contrary to the results of Ravelombola et al (2018) and Kunert et al (2016). Exposure to limited moisture conditions disrupt gaseous exchange in the leaves, which negatively affect crop development (Verma et al 2020).

Soil amendments had no significant effect on leaf width in all sampling dates supporting the findings of Trupiano et al (2017). However, a significant effect exerted on leaf length at reproductive and physiological stage. Treatments with soil amendments rates 75bio:25Comp produced the highest leaf length as compared to other rates and the control. The presence of more biochar and less compost promoted leaf length elongation. A possible explanation is that biochar surfaces provides a cation exchange sites that aid valuable nutrient absorption, which leads to improved nutrition in more biochar amendment soil pots (Hollister et al 2013; Jones et al 2012; Sohi et al 2010). Soil textural types were significant in both leaf attributes across all sampling dates. Leaf length and width developed the highest on sandy loam soil substrate than in loamy sand substrates. Chamizo et al (2018) reported similar results. Interaction between inoculation and soil textural types were significant in both leaf attributes. Inoculated treatments with sandy loam soil produced the highest leaf length and width in comparison with inoculated treatments with loamy sand textures soil.

Chlorophyll content in leaves is a practical indicators of plant vigour and photosynthetic productivity (Hokmaliour and Darbandi 2011). Likewise, AMF inoculation did not significantly influence chlorophyll content across all growth stages. Contrary to the findings of Saboor et al (2021), who reported an 8% significant increase following AMF inoculation in chlorophyll contents under stress. A possible reason could be that AMF colonisation from the Mycoroot^{TM} adversely affected by growing conditions that might have inhibited hyphal growth and expansion in the soil.

The effects of different soil amendments were not significant on chlorophyll content with considerably higher chlorophyll contents in leaves from soil amended soils particularly (75bio:25Comp) compared to un-amended soil. This may have been as result of improved uptake of water, nitrogen and other beneficial nutrients provided by the soil amendments (Agegnehu et al 2015a). In spite of the inconsequential effect of soil amendments on chlorophyll content, our results confirm those of Agegnehu et al (2015b). Nonetheless, variation in soil moisture levels exerted significant effect on chlorophyll content with decline in chlorophyll contents obtained under moisture stress as compared to pots that received adequate moisture. Previous research works (Xu et al 2020; Moaveni 2011) reported similar results. A decreased in chlorophyll content under moisture stress conditions are mainly a result of chloroplasts damage that are caused by active oxygen species and turgor pressure loss (Chaghakaboodi et al 2021). variation in soil moisture levels and soil amendments had significant ($p<0.05$) effect on chlorophyll content only at the physiological stage Soil textural variation exerted significant effect on chlorophyll content. The sandy loam soil treatment recorded the highest chlorophyll levels while loamy sand soil pots recorded the lower. Our results coincide with the findings of Sebetha et al (2018). Loamy sand soils possess low penetration resistance and nutrient holding capacity, and contribute to the poor performances of the plants which loam soil provides adequate nutrients (You et al 2019).

3.5.2 Phenological parameters

Days to flowering is a good measure of earliness in cowpeas production. Flower development marks the start of the reproductive stage after planting. However, the flowering process in this current study occurred fairly uniformly across all treatments. Application of AMF inoculation did not significantly affect the number of days to flowering. These results are in line with the findings of Othman et al (2022) who reported an insignificant effect of AMF inoculation in the mean number of days to flowering in both inoculated and non-inoculated plants. Contrary to our findings, Yaseen et al (2011) revealed that early flowering as well as maximum flowering were observed on inoculated treatments compared to non-inoculated treatments in cowpeas variates. The contradiction in the current study may be attributed to the fact that only one cowpea variety was studied. The beneficial impact presented by AMF colonisation is associated with improved nutrition and water (Navarro et al 2014). On the other hand, moisture levels significantly influence the number of days to flowering. Moisture stress imposed during the cause of flowering in current study resulted in delayed flowering and caused severe flower drop in already flowered plants. Research work from Islam et al (2011) reported similar results. Thus, it is apparent from the observations from the current study that cowpea flowering is sensitive to moisture deficit conditions. Likewise, soil textural types were significant on the days to flowering. Plants from loamy sand soil had the highest number of days to flowering in comparison with plants from sandy loam soil treatments. This means that sandy loam soil treatments will fill their seeds very fast compared to loamy sand treatments. Soil amendments were not significant on the number of days to flowering. Our results contradict those of Musa et al (2020). who reported a maximum flowering under soil incorporated with amendments in tomatoes (*Solanum lycopersicum*).

Stomatal conductance is an indicator of plant water use and can be used as a determinant of crop yield (Faralli et al 2019). Interestingly, inoculation, soil amendments and soil textural types were not significant on stomatal conductance. According to Tyagi et al (2017) the lack of effectiveness by AMF inoculation is the reduced supply of carbohydrates by the host plant. However, variation in soil moisture levels exerted significant effect at the physiological maturity stage. A reduction in stomatal conductance was observed under moisture stress as reported by Olorunwa et al (2021). Moisture stress mobilises the production of phytohormones and abscisic acid, which has the capacity stimulate stomatal closure in response to moisture stress (Agurla et al 2018). Numerous authors revealed similar findings (Zhao et al 2017; Huang et al 2019). Furthermore, interaction between moisture levels and soil textural types were significant. Under moisture stress, loamy sand treatments produced had a highest stomatal conductance than in sandy loam.

3.6. Conclusion

The current study rejects the hypothesis that sole and combined Mycoroot™ inoculation and variable biochar-compost mixtures exerted no effect on cowpea growth and phenological attributes with and without moisture stress, they will equally perform the same. Soil textural types and moisture levels showed significant effect on plant height, number of trifoliate leaves, leaf length, leaf width, chlorophyll content and stomatal conductance. Sole application of AMF showed significant variation on leaf length except other growth attributes. The interaction effect between Mycoroot^{TM} inoculation, soil amendments and moisture levels exerted a significant difference on plant height, no trifoliate leaves, leaf length and leaf width. Mycoroot^{TM} inoculation under moisture stress conditions enhanced plant height in cowpea. Under low soil moisture condition or erratic rainfall condition the use of Mycoroot^{TM} can increase crop production including cowpea. Selection of sandy loam soil managed to improve cowpea growth and development. The integration application of organic ameliorants such as biochar and compost combined with Mycoroot^{TM} inoculation can present a significant strategy in counteract against adverse effects of cowpea production under moistures stress.

References

Abeer, H., Abd Allah, E.F., Alqarawi, A.A., and Egamberdieva, D., (2015). Induction of salt stress tolerance in cowpea (*Vigna unguiculata (L.) Walp*.) by arbuscular mycorrhizal fungi. *Legume Research* 38:579-588.

Adejumo, S.A., Orimolade, D.A., and Olaniyan, A.B., (2017). Morpho-physiological Responses of Cowpea to Different Time and Rates of Compost Application under Water Stress. *Ibadan Journal of Agricultural Research* 2:1-13.

Adeyemi, N.O., Atayese, M.O., Olubode, A.A., and Akan, M.E., (2020). Effect of commercial arbuscular mycorrhizal fungi inoculant on growth and yield of soybean under controlled and natural field conditions. *Journal of Plant Nutrition* 43:487-499.

Aganchich, B., Wahbi, S., Yaakoubi, A., El-Aououad, H., and Bota, J., (2022). Effect of arbuscular mycorrhizal fungi inoculation on growth and physiology performance of olive tree under regulated deficit irrigation and partial rootzone drying. *South African Journal of Botany* 148:1-10.

Agegnehu, G., Bass, A.M., Nelson, P.N., Muirhead, B., Wright, G., and Bird, M.I., (2015a). Biochar and biochar-compost as soil amendments: effects on peanut yield. soil properties and greenhouse gas emissions in tropical North Queensland. Australia. *Agriculture, Ecosystems and Environment* 213:72-85.

Agegnehu, G., Bird, M.I., Nelson, P.N., and Bass, A.M., (2015b). The ameliorating effects of biochar and compost on soil quality and plant growth on a Ferralsol. *Soil Research* 53:1-12.

Agegnehu, G., Srivastava, A.K., and Bird, M.I., (2017). The role of biochar and biocharcompost in improving soil quality and crop performance: A review. *Applied Soil Ecology* 119:156-170.

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., and Raghavendra, A.S., (2018). Mechanism of stomatal closure in plants exposed to drought and cold stress. *Survival Strategies in Extreme Cold and Desiccation* 215-232.

Alemu, M., Asfaw, Z., Woldu, Z., Fenta, B.A., and Medvecky, B., (2016). Cowpeas (*Vigna unguiculata (L) Walp.)* (Fabaceae) landrance diversity in northern Ethopia. *International Journal of Biodiversity and Conversation* 11:297-309.

Al-Tawaha, A.R., Alu'datt, M., Al-Ghzawi, A.L., Wedyan, M., Al-Obaidy, S.D.A., and AlRamamneh, E.A.D., (2018). Effects of soil type and rainwater harvesting treatments in the growth, productivity and morphological trains of barley plants cultivated in semi-arid environment. *Australian Journal of Crop Science* 12:975-979.

Aprahamian, A.M., Lulow, M.E., Major, M.R., Balazs, K.R., Treseder, K.K., and Maltz, M.R., (2016). Arbuscular mycorrhizal inoculation in coastal sage scrub restoration. *Botany* 94:493499.

Augé, R.M., Toler, H.D., and Saxton, A.M., (2015). Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza* 25:13-24.

Beebe, S.E., Rao, I.M., Blair, M.W., and Acosta-Gallegos, J.A., (2013). Phenotyping common beans for adaptation to drought. *Frontiers in Physiology* 4:35.

Beesley, L., and M., Marmiroli., (2011). Th e immobilisation and retention of soluble aresenic, cadmium and zinc by biochar. *Enviromental Pollution* 159:474-480.

Berihun, T., Tolosa, S., Tadele, M., and Kebede, F., (2017). Effects of Biochar on growth of Garden Pea (*Pisum sativum L*) in acidic soils of Bule Woreda Gedeo Zone Southern Ethiopia. *International Journal of Agronomy* 2017:1-8.

Bvenura, C., and Afolayan, A.J., (2015). The role of wild vegetables in household food security in South Africa: A review. *Food Research International* 76:1001-1011.

Chaghakaboodi, Z., Kakaei, M., and Zebarjadi, A., (2021). Study of relationship between some agro-physiological traits with drought tolerance in rapeseed (*Brassica napus L.*) genotypes. *Central Asian Journal of Plant Science Innovation* 1:1-9.

Chamizo, S., Mugnai, G., Rossi, F., Certini, G., and De Philippis, R., (2018). Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. *Frontiers in Environmental Science* 6:49.

Cheng, H.Q., Giri, B., Wu, Q.S., Zou, Y.N., and Kuča, K., (2022). Arbuscular mycorrhizal fungi mitigate drought stress in citrus by modulating root microenvironment. *Archives of Agronomy and Soil Science* 68:1217-1228.

Chimonyo, V.G.P., Modi, A.T., and Mabhaudhi, T., (2015). Water use and productivity of a Sorghum-cowpeas-bottle gourd intercrop system. *Agricultural water management* 165:82-96.

Chitarra, W., Pagliarani, C., Maserti, B., Lumini, E., Siciliano, I., Cascone, P., Schubert, A., Gambino, G., Balestrini, R., and Guerrieri, E., (2016). Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. *Plant Physiology* 171:1009-1023.

Cobb, A.B., Wilson, G.W., Goad, C.L., and Grusak, M.A., (2018). Influence of alternative soil amendments on mycorrhizal fungi and cowpea production. *Heliyon* 7:00704.

Corwin, D.L., (2021). Climate change impacts on soil salinity in agricultural areas. *European Journal of Soil Science* 72:842-862.

Dames, J., (2011). Mycoroot (PTY) LTD. Department of microbiology. Rhodes university.

Dinar, A., Tieu, A., and Huynh, H., (2019). Water scarcity impacts on global food production. *Global Food Security* 23:212-226.

Ding, Z., Ali, E.F., Elmahdy, A.M., Ragab, K.E., Seleiman, M.F., and Kheir, A.M., (2021). Modeling the combined impacts of deficit irrigation. rising temperature and compost application on wheat yield and water productivity. *Agricultural Water Management* 244:106626.

Domene, X., (2016). A critical analysis of meso-and macrofauna effects following biochar supplementation. In Biochar Application, Elsevier 268-292.

Dong, D., Wang, C., Van Zwieten, L., Wang, H., Jiang, P., Zhou, M., and Wu, W., (2020). An effective biochar-based slow-release fertilizer for reducing nitrogen loss in paddy fields. *Journal of Soils and Sediments* 20:3027-3040.

Dube, E., and Fanadzo, M., (2013). Maximising yield benefits from dual-purpose cowpea. *Food Security* 5:769-779.

Faralli, M., Matthews, J., and Lawson, T., (2019). Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement. *Current Opinion in Plant Biology* 49:1-7.

Fattahi, M., Mohammadkhani, A., Shiran, B., Baninasab, B., Ravash, R., and Gogorcena, Y., (2021). Beneficial effect of mycorrhiza on nutritional uptake and oxidative balance in pistachio (Pistacia spp.) rootstocks submitted to drought and salinity stress. *Scientia Horticulturae* 281:109937.

Fertilizer Association of Southern Africa Handbook (FSSA)., (2016). Seventh revised edition. ISBN0-909071-86-1 471.

Gerrano, A.S., Lubinga, M.H., and Bairu, M.W., (2022). Genetic resources management. seed production constraints and trade performance of orphan crops in Southern Africa: A case of Cowpea. *South African Journal of Botany* 146:340-347.

Grzegorczyk, S., Alberski, J., and Olszewska, M., (2013). Accumulation of potassium, calcium and magnesium by selected species of grassland legumes and herbs. *Journal of Elementology* 1:18.

Guo, Y., Ren, G., Zhang, K., Li, Z., Miao, Y., and Guo, H., (2021). Leaf senescence: Progression. regulation. and application. *Molecular Horticulture* 1:1-25.

Hayatu, M., Muhammad, S.Y., Habibu, U.A., (2014). Effect of water stress on the leaf relative water content and yield of some cowpea (*Vigna unguiculata (L) Walp*.) genotype. *International Journal Scientific and Technology Research* 3:2277-8616.

Hazelton, P., and Murphy, B., (2007). Interpreting soil test results: What do all the numbers mean? 2nd ed. CSIRO.

Hokmalipour, S., and Darbandi, M.H., (2011) Effects of Nitrogen Fertilizer on Chlorophyll Content and Other Leaf Indicate in Three Cultivars of Maize (*Zea mays L.). World Applied Sciences Journal* 15:1780-1785.

Hollister, C.C., Bisogni, J.J., and Lehmann, J., (2013). Ammonium. nitrate. and phosphate sorption to and solute leaching from biochars prepared from corn stover (*Zea mays L.*) and oak wood (*Quercus spp.*). *Journal of Environmental Quality* 42:137-144.

Huang, B., Chen, Y.E., Zhao, Y.Q., Ding, C.B., Liao, J.Q., Hu, C., Zhou, L.J., Zhang, Z.W., Yuan, S., and Yuan, M., (2019). Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. *Frontiers in Plant Science* 10:677.

Ibiremo, O.S., Olubamiwa, O., Agbeniyi, S.O., and Akanbi, O.S.O., (2012). Response of cashew seedlings from different nut sizes to phosphate fertilizer and Arbuscular mycorrhizal inoculation in two soils in Nigeria. *International Journal of Plant. Animal and Environmental* 2:147-158.

Islam, S., Calvin, K., and Willy, V., (2011). Physiological and morphological characteristics of cowpea genotypes to drought stress. *Arkansas Environmental. Agricultural and Consumer Sciences Journal* 42-45.

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B.C., Nammi, S., and Liyanage, R., (2018). Cowpea: an overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture* 98:4793-4806.

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., and Murphy, D.V., (2012). Biocharmediated changes in soil quality and plant growth in a three-year field trial. *Soil Biology and Biochemistry* 45:113-124.

Jongen, M., Albadran, B., Beyschlag, W., and Unger, S., (2022). Can arbuscular mycorrhizal fungi mitigate drought stress in annual pasture legumes. *Plant and Soil* 472:295-310.

Kafle, A., Cope, K.R., Raths, R., Krishna Yakha, J., Subramanian, S., Bücking, H., and Garcia, K., (2019). Harnessing soil microbes to improve plant phosphate efficiency in cropping systems. *Agronomy* 9:127.

Kloss, S., Zehetner, F., Dellantonio, A., Hamid, R., Ottner, F., Liedtke, V., Schwanninger, M., Gerzabek, M.H., and Soja, G., (2012). Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties. *Journal of Environmental Quality* 41:990-1000.

Kunert, K.J., Vorster, B.J., Fenta, B.A., Kibido, T., Dionisio, G., and Foyer, C.H., (2016). Drought stress responses in soybean roots and nodules. *Frontiers in Plant Science* 7:1015.

Liu, C., Ravnskov, S., Liu, F., Rubæk, G.H., and Andersen, M.N., (2018). Arbuscular mycorrhizal fungi alleviate abiotic stresses in potato plants caused by low phosphorus and deficit irrigation/partial root-zone drying. *The Journal of Agricultural Science* 156:46-58.

Long, L.K. Yao, Q., Huang, Y.H., Yang, R.H., Guo, J., and Zhu, H.H., (2010). Effects of arbuscular mycorrhizal fungi on zinnia and the different colonization between Gigaspora and Glomus. *World Journal of Microbiology and Biotechnology* 26:1527-1531.

Majewska, M.L., Rola, K., and Zubek, S., (2016). The growth and phosphorus acquisition of invasive plants Rudbeckia laciniata and Solidago gigantea enhanced by arbuscular mycorrhizal fungi. *Mycorrhiza* 27:83-94.

Mensah, A.K., and Frimpong, K.A., (2018). Biochar and/or compost applications improve soil properties. growth. and yield of maize grown in acidic rainforest and coastal savannah soils in Ghana. *International Journal of Agronomy* 2018:6837404.

Meyer, S., Genesio, L., Vogel, I., Schmidt, H.P., Soja, G., Someus, E., Shackley, S., Verheijen, F.G., and Glaser, B., (2017). Biochar standardization and legislation harmonization. *Journal of Environmental Engineering and Landscape Management* 25:175-191.

Moaveni, P., (2011). Effect of water deficit stress on some physiological traits of wheat (*Triticum aestivum*). *Agricultural Science Research Journal* 1:64-68.

Mulcahy, D.N., Mulcahy, D.L., and Dietz, D., (2013). Biochar soil amendment increases tomato seedling resistance to drought in sandy soils. *Journal of Arid Environments* 8:222-225.

Musa, F.B., Abiodun, F.O., Falana, A.R., Ugege, B.H., Oyewumi, R.V., and Olorode, E.M., (2020). Growth and Yield of Tomato (*Lycopersicon esculentum Mill*.) as Influenced by Poultry Manure and Biochar in Two (2) Soil Depths. *Journal of Experimental Agriculture International* l42:55-63.

Mwela, S.E., Ochwo-Ssemakula, M., Sadik, K., Achola, E., Okul, V., Ginson, P., Edema, R., Singini, W., and Rubaihayo, P., (2017). Response of cowpea genotypes of drought stress in Uganda. *American Journal of Plant Sciences* 8:720-733.

Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., and Qiu, L., (2019). Research progress and perspective on drought stress in legumes: A review. *International Journal of Molecular Sciences* 20:2541.

Navarro, J.M., Pérez-Tornero, O., and Morte, A., (2014). Alleviation of salt stress in citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the rootstock salt tolerance. *Journal of Plant Physiology* 171:76-85.

Ndhleve, S., Nakin, M., Longo-Mbenza, B., (2017). Impacts of supplemental irrigation as a climate change adaptation strategy for maize production: a case of the Eastern Cape Province of South Africa. *Water SA* 43:222-228.

Ndiso, J.B., Chemining'wa, G.N., Olubayo, F.M., and Saha, H.M., (2016). Effect of drought stress on canopy temperature. growth and yield performance of cowpea varieties. *International Journal of Plant and Soil Science* 9:1-12.

Neyton, O.M., Isabel, G.M., Alexandre, S.P., Gleidson, B.D., Gulter, G.C.S., and Ermelinda, M.M.O., (2020). Effects of biochar application on production parameters of two cowpea cultivars planted in succession in five soils from the Brazilian semiarid region. *Arabian Journal of Geosdences* 13:506-511.

Nkomo, G.V., Sedibe, M.M., and Mofokeng, M.A., (2021). Production constraints and improvement strategies of cowpea (*Vigna unguiculata L. Walp.)* genotypes for drought tolerance. *International Journal of Agronomy* 2021:1-9.

Okon, J.E., (2013). Effect of water stress on some growth aspects of two varieties of cowpea. *Vigna unguiculata (L.) Walp* and Fabaceae. *Bulletin of Environment. Pharmacology and Life Sciences* 2:69-74.

Olajide, A.A., and Ilori, C.O., (2017). Effects of drought on morphological traits in some cowpea genotypes by evaluating their combining abilities. *Advances in Agriculture* 2017.

Olawuyi, O.J., Ezekiel-Adewoyin, D.T., Odebode, A.C., Aina, D.A., and Esenbamen, G.E., (2012). Effect ofarbuscular mycorrhizal (*Glomus clarum*) and organomineral fertilizer on growth and yield performance of okra (*Abelmoschus esculentus*). *African Journal of Plant Science* 6:84-88.

Olorunwa, O.J., Shi, A., and Barickman, T.C., (2021). Varying drought stress induces morphophysiological changes in cowpea (*Vigna unguiculata L.*) genotypes inoculated with *Bradyrhizobium japonicum*. *Plant Stress* 2:100033.

Othman, Y.A., Tahat, M., Alananbeh, K.M., and Al-Ajlouni, M., (2022). Arbuscular Mycorrhizal Fungi Inoculation Improves Flower Yield and Postharvest Quality Component of Gerbera Grown under Different Salinity Levels. *Agriculture* 12:978.

Paixão, J.S., Da Silva, J.R., Ruas, K.F., Rodrigues, W.P., Filho, J.A.M., Bernado, W.D.P., Abreu, D.P., Ferreira, L.S., Gonzalez, J.C., and Griffin, K.L., (2019). Photosynthetic capacity. leaf respiration and growth in two papayas (*Carica papaya*) genotypes with different leaf chlorophyll concentrations. *Annals of Botany Plants* 11:13-13.

Pineiro, J., Maestre, F.T., Bartolomé, L., and Valdecantos, A., (2013). Ecotechnology as a tool for restoring degraded drylands: a meta-analysis of field experiments. *Ecological Engineering* 61:133-144.

Rahimzadeh, S., and Pirzad, A., (2017). Arbuscular mycorrhizal fungi and Pseudomonas in reduce drought stress damage in flax (Linum usitatissimum L.) a field study. *Mycorrhiza* 27:537-552.

Rathore, D.K., Kumar, R., Meena, V., Kumar, U., Gupta, P.S., Yadav, T., and Makarana, G., (2015). Phosphorus and zinc fertilization in fodder cowpea-A review. *Indian Journal of Animal Research* 32: 388-392.

Ravelombola, W., Shi, A., Qin, J., Weng, Y., Bhattarai, G., Zia, B., Zhou, W., and Mou, B., (2018). Investigation on various aboveground traits to identify drought tolerance in cowpea seedlings. *HortScience* 53:1757-1765.

Riaz, A., Younis, A., Taj, A.R., Karim, A., Tariq, U., Munir, S., and Riaz, S., (2013). Effect of drought stress on growth and flowering of marigold (*Tagetes erecta L*.). *Pakistan Journal of Botany* 45:123-13.

Ritte, I.P., Egnin, M., Idehen, O., Mortley, D., Bernard, G.C., Binagwa, P.H., Brown, A.P., and Bonsi, C.K., (2022). Evaluation of Cowpea Morpho-physiological and Yield Responses to Vegetative and Pre-Anthesis Water-Deficit Stress Tolerance under Greenhouse Conditions. *European Journal of Applied Sciences* 10:2.

Rush, T.A., Shrestha, H.K., Gopalakrishnan Meena, M., Spangler, M.K., Ellis, J.C., Labbé, J.L., and Abraham, P.E., (2021). Bioprospecting Trichoderma: A systematic roadmap to screen genomes and natural products for biocontrol applications. *Frontiers in Fungal Biology* 2:716511.

Saboor, A., Ali, M.A., Danish, S., Ahmed, N., Fahad, S., Datta, R., Ansari, M.J., Nasif, O., and Glick, B.R., (2021). Effect of arbuscular mycorrhizal fungi on the physiological functioning of maize under zinc-deficient soils. *Scientific Reports* 11:1-11.

Santos, R., Carvalho, M., Rosa, E.V., and Carnide, I., (2020). Castro Root and agromorphological traits performance in cowpea under drought stress. *Agronomy* 10:1604.

Schulz, H., Dunst, G., and Glaser, B., (2013). Positive effects of composted biochar on plant growth and soil fertility. *Agronomy for Sustainable Development* 33:817-827.

Sebastin, Z.I., Maur, I.R.P., Heriberto, V.R., Mara, C.N.G., Irina, R.M., Reynaldo, G.P., and Carmelo, D.A.P., (2021). Coinoculation of rhizobia and mycorrhiza in cowpea beans (*Vigna unguiculata (L.) Walp*) in Calixto GarcÃa Municipality. Cuba. *African Journal of Agricultural Research* 17:1016-1023.

Sebetha, E.T., Mfanta, A., and Adebayo, R., (2018). Effect of different phosphorus fertilizer sources. cultivar and soil type on growth performance of soybean. *Research on Crops* 19:4.

Shahid, S.A., Qidwai, A.A., Anwar, F., Ullah, I., and Rashid, U., (2012). Improvement in the water retention characteristics of sandy loam soil using a newly synthesized poly (acrylamideco-acrylic acid)/AlZnFe2O4 superabsorbent hydrogel nanocomposite material. *Molecules* 17:9397-9412.

Sohi, S.P., Krull, E., Lopez-Capel, E., and Bol, R., (2010). A review of biochar and its use and function in soil. *Advances in Agronomy* 105 47-82.

Symanczik, S., Krützmann, J., Nehls, U., Boller, T., and Courty, P.E., (2020). Expression of major intrinsic protein genes in Sorghum bicolor roots under water deficit depends on arbuscular mycorrhizal fungal species. *Soil Biology and Biochemistry* 140:107643.

Trupiano, D., Cocozza, C., Baronti, S., Amendola, C., Vaccari, F.P., Lustrato, G., Di Lonardo S., Fantasma, F., Tognetti, R., and Scippa, G.S., (2017). The effects of biochar and its combination with compost on lettuce (*Lactuca sativa L.)* growth. soil properties. and soil microbial activity and abundance. *International Journal of Agronomy* 2017.

Tyagi, J., Varma, A., and Pudake, R.N., (2017). Evaluation of comparative effects of arbuscular mycorrhiza (*Rhizophagus intraradices*) and endophyte (*Piriformospora indica*) association with finger millet (*Eleusine coracana*) under drought stress. *European Journal of Soil Biology* 81:1-10.

Uzoma, K.C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., and Nishihara, E., (2011). Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use and Management* 27:205-212.

Verma, K.K., Anas, M., Chen, Z., Rajput, V.D., Malviya, M.K., Verma, C.L., Singh, R.K., Singh, P., Song, X.P., and Li, Y.R., (2020). Silicon supply improves leaf gas exchange. antioxidant defense system and growth in Saccharum officinarum responsive to water limitation. *Plants* 9:1032.

Wabwayi, N., Odhiambo, J.A., and Basweti, E.A., (2020). Evaluation of cowpea rust disease incidence and severity on selected cowpea genotypes in Western Kenya. *African Journal of Agricultural Research* 16:1015-1024.

Wang, J., and Wang, S., (2019). Preparation, modification and environmental application of biochar: a review. *Journal of Cleaner Production* 227:1002-1022.

Wang, X., Singh, D., Marla, S., Morris, G., and Poland, J., (2018). Field-based high-throughput phenotyping of plant height in sorghum using different sensing technologies. *Plant Methods* 14:1-16.

Wheeler, T., and Von Braun. J., (2013). Climate change: Impacts on global food security. *Science* 341:508-513.

Wijewardana, C., Alsajri, F.A., Irby, J.T., Krutz, L.J., Golden, B.R., Henry, W.B., and Reddy, K.R., (2019). Water deficit effects on soybean root morphology and early-season vigor. *Agronomy* 9:836.

Wu, M., Guo, X., Wu, J., and Chen, K., (2020). Effect of compost amendment and bioaugmentation on PAH degradation and microbial community shifting in petroleumcontaminated soil. *Chemospher*e 256:126998.

Xu, Q., Ma, X., Lv, T., Bai, M., Wang, Z., and Niu, J., (2020). Effects of water stress on fluorescence parameters and photosynthetic characteristics of drip irrigation in rice. *Water* 12:289.

Yaseen, T., Burni. T., and Hussain, F., (2011). Effect of arbuscular mycorrhizal inoculation on nutrient uptake. growth and productivity of cowpea (*Vigna unguiculata*) varieties. *African Journal of Biotechnology* 3:8593-8598.

You, J., Sun, L., Liu, X., Hu, X., and Xu, Q., (2019). Effects of sewage sludge biochar on soil characteristics and crop yield in loamy sand soil. *Polish Journal of Environmental Studies* 28:2973-2980.

Zhao, G., Xu, H., Zhang, P., Su, X., and Zhao, H., (2017). Effects of 2. 4-epibrassinolide on photosynthesis and Rubisco activase gene expression in Triticum aestivum L. seedlings under a combination of drought and heat stress. *Plant Growth Regulation* 81:377-384.

Zhou, Y., Qin, S., Verma, S., Sar, T., Sarsaiya, S., Ravindran, B., Liu, T., Sindhu, R., Patel, A.K., Binod, P., and Varjani, S., (2021). Production and beneficial impact of biochar for environmental application: A comprehensive review. *Bioresource Technology* 337:125451.

CHAPTER 4

Integrated MYCOROOT^{TM} inoculation and biochar-compost mixture application enhanced cowpea (*Vigna unguiculata L. Walp.*) yield attributes under variable soil conditions

Abstract

Cowpea, often identified as the most crucial legumes in human diet, provides inexpensive proteins and other beneficial nutrients as a grain legume. The use of Mycoroot^{TM}, a locally manufactured biofertilser, as one of the innovative approaches to enhancing crop production and yield. A greenhouse pot experiment was conducted to examine the combined effect of Mycoroots^{TM} inoculation with varied biochar-compost mixtures as an agronomic package to enhance the yield attributes of grain cowpea on two soils with distinct textural characteristics and moisture regimes. Treatment factors comprised of 2 soil textural types (sandy loam and loamy sand), 4 soil amendments comprising different mix ratios of biochar (BC) and compost (C), 2 AMF levels (inoculated and uninoculated) and 2 soil moisture regimes (adequate soil moisture and moisture stressed) as main treatment factors. The soil amendments comprised of 50:50 BC/C, 75:25 BC/C, 25:75 BC/C and a control with no amendment. The trial was laid out in a factorial design and fitted into RCBD with four replications. Results revealed that soil textural types had a significant effect ($p<0.05$) on measured yield parameters. Cowpea performance was higher in sandy loam soil across all measured yield attributes than in loamy sand soil. Moreover, the interaction between moisture levels and soil textural types exerted substantial ($p<0.05$) effect on mean pod dry weight and length, number of seeds, fodder, seed and haulm weight. Under moisture stress, increased pod dry weight, number of seeds, fodder weight, seed weight, haulm weight and pod length were obtained sandy loam suggesting that the symbiotic relationship from mycoroot^{TM} inoculation enhanced plant tolerance and relieve the negative effect of moisture stress. The findings underscore the importance of selection of

appropriate agronomic practices as key strategy for improving cowpea production under variable soil condition.

Keywords: *Arbuscular mycorrhizal fungi,* moisture stress, cowpeas, biochar, compost, soil textural types

4.1 Introduction

Commonly known as black eye peas, cowpeas (*Vigna unguiculata L. Walp*.) is a heat-loving legume crop that is often cultivated in the semi-arid and arid regions and utilised for human consumption as grain legume and vegetable (Barros et al 2020; Mfeka 2019). Among several legumes, cowpeas have the advantages of being an inexpensive source of protein, fiber and other nutritional components such as vitamins and minerals (Mekonnen et al 2022; Horn et al 2022). Cowpeas does not only provide a significant contribution to human diets, but it also displays the ability to enhance soil fertility through atmospheric nitrogen (N) conversion (Mndzebele et al 2020; Simunji et al 2019). Unfortunately, the yield of cowpea has remained below its genetic potentials due to various abiotic factors including soil nutrients and moisture stress leading to low yield thus contributing to food insecurity (Iseki et al 2021; Bisikwa 2011) due to grain scarcity. It is an increasingly important plant protein-rich candidate crop that is now been promoted for cultivation by farmers in many disadvantage and under-privilege communities of South Africa to deal with food insecurity challenges (Kamara et al 2018; Alemu et al 2016; Wheeler and Von Braun 2013).

Arbuscular mycorrhizae fungi (AMF) are soil microorganisms that provide a symbiotic relationship with plant roots (Aguila et al 2022). This symbiotic relationship supports their host plants with increasing access to nutrient and water uptake from soil while protecting them from various biotic and abiotic stressors (Powell and Rilling 2018). The importance of this relationship has been widely acknowledged in both ecological and agricultural systems due to their benefits towards enhancing both growth and yields of crops (Oyewole et al 2017; Kim et al 2017; Olawuyi et al 2012). Additionally, the application of *Arbuscular mycorrhizae fungi* represent a latent potential opportunity to enhance food security globally (Rodriguez and Sanders 2015). AMF is marketed as Mycoroot^{TM} in South Africa (Mukhongo et al 2016). Mycoroot^{TM} is a commercialised product in granular form composed of native isolates of AMF use as bio-inoculants for fertility management strategy and inexpensive way to improve crop yields (Sharma et al 2013; Maboko et al 2013). Enhancing mutualistic relationships with advantageous soil microbes, such *Arbuscular mycorrhizae fungi*, may be a key to effective cowpea production.

Compost is regarded as a very valuable material utilised as an organic fertiliser and soil conditioner (Chia et al 2020). It influences soil substrate size distribution thereby allowing porosity for the aeration process and increases surface area for water infiltration (Adugna et al 2016). Previous research works have shown that compost can help improve the soil's ability to retain water thus improving the productivity of cultivated crops such as chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*) (Chukwudi et al 2022; Wu et al 2020; Ditta et al 2018; Ahmadpour and Hossain 2017). Biochar on the other hand is a carbon rich material produced from pyrolyzing biomass under anaerobic conditions at temperatures between 300-700°C (Liang et al 2021). Biochar utilization as an organic amendment has been reported to promote increase soil organic matter content whilst mitigating the negative impacts of moisture shortages on plant development and crop yield (Ullah et al 2021; Gavili and Haghighi 2019). The application of biochar improves soil properties by increasing absorption of water and nutrients due to its porous nature thereby aiding crop productivity (Tan et al 2015; Sohi et al 2010). Numerous reports have demonstrated that application of a combination of biochar and compost promote synergistic effects that enhance productivity in agricultural crops (Tammeorg et al 2014; Lui et al 2012; Downie 2011). Integrating biochar and compost as soil organic amendments consist of multiple benefits in agroecosystems such as enhanced Nitrogen (N) fixation on legumes and improvements on growth and yields in cereal-legumes intercropping systems including cowpeas as reported Cobb et al (2018) and Liu et al (2017).

Despite some degrees of the drought tolerance ability of this important crop, cowpea have not been adequately research and promoted in South Africa on the association of *Arbuscular mycorrhizae fungi* with variable soil amendment rates such as biochar and compost in mitigating moistures stress (Safaei Asadabadi et al 2021; Gerrano et al 2019). AMF inoculation along with compatible combination of biochar and compost soil amendments rates offers a cheaply efficient tool to alleviate moisture stress and promote the health status of cowpeas rather than the use of expensive inorganic agrochemicals, considering the fact that small-scale farmers in South Africa mostly cultivate this crop. Henceforth, the objective of the study is to evaluate the sole and combined effect of Mycoroot™ inoculation and variable biochar-compost mixtures on cowpea yield and yield attributes with and without moisture stress. Furthermore, the sensitivity of cowpea yield due to moisture stress has not been adequately established

(Ahmed and Suliman 2010). Cowpea production is gradually increasing in South Africa, therefore it is important to gather well-researched information on the sensitivity of moisture stress in cowpea yield that will be accessible to small scale farmers, as moisture stress tends to present unfavourable growing conditions that alters growing seasons which subsequently reduces yield productivity. Therefore, it is hypothesised that the combined us of Mycoroot™ inoculation with variable biochar-compost mixtures will have no effect on cowpea yield and yield attributes under moisture stress condition.

4.2 Materials and Methods

Detailed description of the methodology including trial layout for the study are as previously provided in sections 3.2.1 to 3.2.4 in chapter 3.

4.3 Data collection

4.3.1 Yield data

At maturity, yield parameters were recorded after harvesting. The number of pods per pot was counted to determine the treatment effect on pods. Thereafter, oven dried at 65°C for 48 hours to determine dry pod weight. Pod length was determined using a ruler to measure length. Thereafter, the pods were threshed by hand to count the total number of seeds, seed cavities and haulm weigh per pot and to further determine the treatment effect on seed yield. Seed and haulm weight was determined by using an electronic weighing balance. Shoots were cut at ground level while roots were carefully removed from the pots, washed of soil to count the number of nodules. For fodder and root dry weight determination, plant shoots and roots were oven dried at 65°C for 48 hours to determine their dry weight.

4.3.2 Statistical analysis

All data collected was subjected to analysis of variance (ANOVA) using Statistic 10. The significant difference between mean of treatments was tested using Tukey’s test at 5% level of significance. The statistical model used for data analysis was as presented under section 3.3. Similarly, Pearson correlation analysis was performed to determine the relationship between grain yield and measured yield attributes.

4.4 Results

4.4.1 Treatment effects on yield and yield attributes of selected cowpea

There is variation among the main treatment factors. Moisture levels showed a significant effect ($p<0.05$) in all yield parameters except root dry weight, while soil textural types significantly influenced ($p<0.05$) all measured yield parameters (Table 4.1). Unexpectedly, soil amendments and inoculation had no significant effect ($p>0.05$) on all measured yield parameters (Table 4.1). Furthermore, interaction between moisture and soil textural types had a significant effect on mean dry weight, number of seeds, fodder weight, seed weight, mean haulm weight and mean pod length (Table 4.1). Interaction between soil amendments and soil types had a significant effect ($p<0.05$) on fodder weight and mean pod length (Table 4.1).

4.2.2: Correlation analysis

Correlation analysis revealed that number of pods was significantly and positively correlated with all measured yield attributes of mean pod dry weight, mean pod cavity, number of seeds, number of nodules, fodder weight, root dry weight, seed weight, mean haulm weight and mean pod length (Table 4.3). Likewise, positive and strong correlation was obtained between mean pod dry weight and mean pod cavity, number of seeds and number of nodules, fodder weight and root dry weight. Moreover, a significant and positive correlation was observed form seed weight, mean haulm weight as well as mean pod length.

Table 4. 1: p-values of ANOVA for the measured yield parameters

Factor	No Pods	Pod dry weight (g)	No pod cavities	No Seeds	No nodules	Fodder weight (g)	Root dry weight (g)	Seed weight (g)	Haulm weight (g)	Pod Length (cm)
Inoculation (IN)	0.948	0.062	0.567	0.441	0.871	0.875	0.421	0.601	0.847	0.515
Moisture levels (ML)	0.000***	0.000***	0.023*	0.000***	0.045*	0.000***	0.065	0.000***	0.000***	0.497
Soil Amendments (SA)	0.345	0.504	0.705	0.107	0.531	0.267	0.189	0.318	0.100	0.407
Soil Types (ST)	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.005**	0.000***	0.000***	0.020*
ML*ST	0.558	0.000***	0.192	0.004**	0.957	0.004**	0.351	0.000***	0.010**	0.054*
SA*ST	0.415	0.902	0.380	0.758	0.362	0.055*	0.421	0.600	0.576	0.054*

*= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

Table 4. 2: Effects of inoculation, soil moisture levels, soil amendments and soil textural types on measured yield parameters

Factor	No Pods	Pod dry weight (g)	No of pod Cavities	No Seeds	No Nodules	Fodder weight (g)	Root dry Weight (g)	Seed weight (g)	Haulm weight (g)	Pod Length (cm)
Inoculation (IN)										
Inoculated	4.34a	7.61a	10.53a	31.95a	19.64a	9.4a	7.62a	5.55a	1.29a	14.03a
Non-inoculated	4.33a	6.94a	10.31a	30.75a	19.92a	9.13a	8.05a	5.39a	1.28a	13.82a
!Critical value	0.474	0.707	0.754	3.081	3.425	1.142	1.054	0.616	0.136	0.645
Moisture levels (ML)										
Adequate moisture	4.89a	8.13a	10.86a	35.5a	21.53a	10.19a	8.33a	6.32a	1.55a	14.04a
Moisture stressed	3.78b	6.42b	9.98b	27.2b	18.03b	7.98b	7.34	4.61b	1.01b	13.82a
!Critical value	0.474	0.707	0.754	3.081	3.425	1.142	1.054	0.616	0.126	0.645
Soil Amendments (SA)										
75Bio25Comp	4.63a	7.72a	10.78a	34.5a	18.09a	8.47a	7.67a	5.86a	1.39a	14.34a
50Bio50Comp	4.47a	7.23a	10.44a	31.06a	21.34a	8.55a	7.12a	5.31a	1.30a	13.55a
25Bio75Comp	4.16a	7.17a	10.19a	30.63a	20.66a	9.63a	8.75a	5.61a	1.30a	13.88a
Control	4.09a	6.98a	10.28a	29.22a	19.03a	9.69a	7.78a	5.09a	1.15a	13.94
!Critical value	0.883	1.316	1.404	5.737	6.378	2.127	1.962	1.146	0.253	1.201
Soil Types (ST)										
Sandy loam	4.88a	9.72a	11.56a	41.08a	24.14a	12.37a	8.60a	7.95a	1.48a	14.31a
Loamy sand	3.80b	4.83b	9.28b	21.63b	15.42b	5.80b	7.06b	2.98b	1.08b	13.54a
!Critical value	0.474	0.707	0.754	3.081	3.425	1.142	1.054	0.616	0.136	0.645

*! Implies critical value for comparison (Tukey HSD)

Table 4. 3: Pearson correlation analysis between grain yield and yield attributes

Parameters	No Pods	Pod dry weight	No of pod cavities	No seeds	No nodules	Fodder weight	Root dry weight	Seed weight	Haulm weight	Pod length
No pods	1									
Pod dry weight	0.951***	1								
No pod cavities	0.945***	0.941***	1							
No seeds	0.946***	0.969***	0.941***	1						
No nodules	0.849***	0.866***	0.876***	0.853***	1					
Fodder weight	0.863***	0.909***	0.905***	0.910***	0.861***	1				
Root dry weight	0.894***	0.881***	0.929***	0.887***	0.844***	0.905***	1			
Seed weight	0.921***	0.976***	0.905***	0.967***	0.835***	0.899***	0.859***	1		
Haulm weight	0.968***	0.952***	0.937***	0.952***	0.859***	0.878***	0.891***	0.944***	1	
Pod length	0.939***	0.920***	0.974***	0.920***	0.877***	0.886***	0.930***	0.880***	0.934***	1

*** indicates significance

4.5 Discussion

4.5.1 Number of pods

In the present study, the application of *Arbuscular mycorrhizae fungi* (AMF) inoculation did not significantly affect the number of pods. The number pods were similar in both *Arbuscular mycorrhizae fungi* levels (inoculated and uninoculated). These findings contradict the findings of Rocha et al (2020) and Kazadi et al (2020) who reported an increase in the number of pods following the application of *Arbuscular mycorrhizae fungi* inoculation. The lack of positive response could be as a result of poor adaptation of the fungal and pH that affect the frequency of root colonization by AMF (Kazadi et al 2020). Moisture levels had a positive effect on number of pods. Moisture stress reduced number of pods produced. These results are in agreement with those of Yahaya et al (2019). The reduction in the number of pods under moisture stress may be due to the flower abortion experienced by the plants during the period of moisture stress. The number pods did not respond positively to the application of soil amendments rates. Despite the non-statistical difference, soil amended treatments performed better than the control. Contrarily to the current findings, Agegnehu et al (2015a) reported that organic amendments with biochar and compost increased the number of pods. Soil textural types showed a significant effect on pod yield. Sandy loam soil recorded the highest pod number compared to loamy sand soil. Results from the study are in line with the findings of İç et al (2010), who reported sandy loam soil possess good moisture retention thereby improving yield.

4.5.2 Seed yield and seed weight

Seed yield is the total end product of cowpea production which is important for it economic value (Kardile et al 2018). Seed yield is correlated with seed weight. The application of AMF had no significant effect on seed yield and seed weight. Rocha et al (2019) and Erman et al (2011) reported similar results. This can be ascribed to the fact that improvements in nutrients acquisition was not enough for the plants to use efficiently to develop and fill the seed cavities. However, other researchers reported significant enhancement in grain yield and weight under AMF association (Aguégué et al 2021; Ghorchiani et al 2018). Moisture levels had a significant effect on seed yield and weight. Adequate moisture treatments performed better in terms of producing seed yield and weight as compared to moisture stress. These results confirm those of Abayomi et al (2019) and Farooq et al (2018). Moisture deficit inhibit the process of protein synthesis, which is responsible for seed development and composition, limit embryo development due to lack of photosynthates, resulting in impaired grain yield (Farooq et al 2018). Furthermore, a report from Kedir (2020) documented that moisture stress reduces kernel weight in wheat. Seed yield and weight showed no positive response on the application of soil amendments rates. However, amended soil treatments performed the highest with producing highest seed weight and yield as compared to non-amended soil treatment (control) particularly soil amendment rate 75Bio:25Comp. Contrary to the findings of the study, El-shimi (2022) documented an increase in seed yield and weight due to the addition of biochar and compost as soil ameliorant. The insignificant effect of soil amendments could be ascribed to inadequate concentration of soil amendments. Soil textural types together with the interaction between soil textural types and moisture levels showed a positive effect on seed weight and yield. Sandy loam soil produced under adequate moisture recorded the highest grain yield than loamy sand textural soil type under moisture stress. This could be as a result of loamy sand, consisting of a high sand percentage (Table 3.2) classified as the poorest soil in terms of nutrients availability which manifested in poor performance (Yetunde et al 2022).

4.5.3 Pod characteristics

4.5.3.1 Pod length, cavity, pod dry weight and haulm weight

In the current study, application of AMF inoculation did not impact pod length, cavity, haulm weight and pod weight. However, AMF inoculated treatment recorded the highest mean pod length, cavity, weight wand haulm weight than in non-inoculated treatments. Our results confirm those of Dobo (2022), who reported an insignificant effect on pod length following AMF inoculation in soybean (*Glycine max*). According to Dobo (2022) compared to natural ecosystems, agricultural soils have been found to have lower AMF levels. Moreover, Pellegrino and Bedini (2014) revealed that poor adaptation AMF and soil pH of the soil could be the reason to the efficiency of AMF inoculation. In contrary, Adeyemi et al (2020) reported an increase in pod dry weight of inoculated treatment. Moisture levels significantly influenced pod characteristics mean pod dry weight, pod cavity and haulm weight except pod length. The highest mean pod characteristics (pod dry weight, pod cavity, haulm weight) were achieved under adequate moisture than in moisture stress. Hamidou et al (2013) in which it was documented that moisture stress decreased reported similar results pod and haulm yield. This may be attributed to the highest flower abortion during moisture stress imposition.

Additionally, Moisture stress that occurs during vegetative stage cause a disruption in yield potential such as premature grain and which results in weight grain reduction in which this tends to affect pod cavity as well as haulm weight. Soil amendments did not positively influence pod length, pod cavity, pod weight and haulm weight. These results contradict the findings of El-shimi (2022) who reported that integrating compost and biochar increased pod development. Valuable nutrients from biochar and compost integration takes time to react with the soil and they are realised in slow rates, this could be the reason as to why soil amendments did not positively influence these parameters. Soil textural types influenced pod length significantly. The highest mean pod length was recorded on sandy loam soil treatments compared loamy sand treatments. Pod cavity, pod dry weight and haulm weight followed a similar trend. Based on these observation, loam soil influenced pod length, pod cavity and pod weight due to it beneficial properties of retaining valuable nutrients and water that support plant development as compare to sandy soil. Moreover, interaction between moisture levels and soil textural types was significant on the pod length, pod dry weight and haulm weight. These measured parameters where more pronounced on well-watered and sandy loam soil treatments than in moisture stressed and loamy sand soil treatments. Due to sandy soil being porous, high permeability and poor drainage contribute to poor crop establishment (Hollister et al 2013).

4.5.4 Fodder weight

Application of AMF inoculation did not significantly affect fodder weight in the present study. Fodder weight was maximum in inoculated plants but not significantly differing from non – inoculated plants. These results are in disagreement with Oliveira et al (2017) who reported an increase in fodder weight due to mycorrhizal inoculation. The ineffectiveness of the inoculation may be attributed to the growing condition, soil pH growing season. According to a study done by Pellegrino and Bedini (2014) on chickpea, mycorrhizal inoculation resulted in an increase in fodder weight in spring. Moisture levels had a positive impact on fodder weight. Moisture stressed pots recorded less fodder weight compare to adequate moisture pots. These finding are similar to Kedir (2020). Moisture stress slows down processes such as photosynthesis thereby decreasing fodder accumulation and causes stunted growth (Waraich et al 2011). Moisture stress decreased the number of leaves and plant height hence the reason fodder weight was less under moisture stressed pots. Similarly, soil amendments did not affect fodder weight. These results contradict with those of Cobb et al (2018) who reported a significant increase fodder in biochar and compost amended plants. Similar results were reported by Trupiano et al (2017).

The lack of soil amendments effect on fodder weight may be attributed to many factors including the type and composition of biochar and compost. Soil textural type had a positive effect on fodder weight. The highest fodder weight was recorded from sandy loam soil. This is attributed to the positive advantages of loam soil such as good nutrients retention (Table 3.2). Surprisingly, interaction between soil amendments and soil textural types was significant. The control in sandy loam soil produced the highest fodder dry weight as compared to application of soil amendment rates in loamy sand that might have been ascribe to the benefits of loam soil as mentioned above.

4.5.5 Root dry weight

Application of natural *Arbuscular mycorrhizae fungi* inoculation did not impact root weight. However, numerous reports suggested that AMF inoculation increase root dry weight (Adeyemi et al 2021). Root dry weight increases significantly following AMF inoculation is often associated with beneficial effects derived from the AMF colonisation mycorrhizal association (Pellegrino and Bedini 2014). However, the benefits of mycorrhizal association is inhibited by lower soil pH which might have affected the results of the study. Moisture levels as well as soil amendments did not impact root dry weight. Moisture levels had no significant effect however under moisture stress, less root dry weight was observed as compared to adequate moisture. Jangpromma et al (2012) revealed that moisture deficit reduces root dry weight. Since the current study was conducted using pots, this might have hindered root elongation due to space constraint imposed. Soil amendments did not have an impact on root weight. Despite insignificance, maximum root dry weight was observed on amended soil particularly 50Bio:50Comp compared to a control. These results are in sharp contrast to the earlier findings reported by Awasthi et al (2019) who observed a statistical significant increase in root dry weight from compost-biochar amended soil compare to the control. The contribution of biochar and compost soil amendments have been reported minimal in various studies (Borchard et al 2014). Soil textural types significantly influenced root weight. Root weight was higher on sandy loam soil as compared to loamy sand. This is attributed to the good qualities loam soil has for agricultural crops.

4.5.6 Nodule number

Root colonisation by AMF inoculation did not influence the number of nodules. Interestingly, fewer nodules were formed in inoculated plants as compared to non-inoculated plants. In contrast to our findings, reported that AMF inoculation increased nodule number. Tajini et al (2013) and Huang et al (2014) reported similar reports in common beans and white clover respectively. Following AMF inoculation increased the absorption of nutrients such as P, which may have contributed to more nodule production (Oruru et al 2018). Soil pH affect the frequency of root colonization, which may have been the cause of ineffectiveness of AMF colonisation. Soil amendments did not impact the number of nodules. These results correlate with Yeboah et al (2020) who indicated that the number of nodules was not affected by integrating biochar and compost as soil amendments. Agegnehu et al (2015b) reported that negative response of crops to soil amendment could be attributed to soil properties changes and pH induced micronutrient deficiencies. Moisture levels positively influenced the number of nodules. Moisture stress treatments recorded a sharp reduction in the number of nodules as compared to well-watered treatments. Umamahesh et al (2018) reported similar findings. Moisture stress lowers plant investment in the number nodules and invest more energy to other part of the plant. Soil amendments did not significantly influence root dry weight. There were no significantly variation between treatments. Soil textural types significantly influenced the number of nodules. The number of nodule count was substantially higher in the sandy loam soil treatments than in loamy sand soil treatments, supporting the findings of Thapa et al (2018). Nodulation is highly sensitive to moisture dynamics, which is greatly influence by soil substrates. According to Abidi et al (2015) and Khandelwal and Sindhu (2012) reported that nodulation may be affected by water deficit and temperature.

4.5.7 Pearson correlation analysis

The highly significant and positive correlation between seed yield and he number of pods in the current study is in agreement with previous studies. Santos et al (2014) reported that number of pods showed a positive and significant correlation with cowpea seed yield. Similarly, the highly significant and positive correlation between seed yield with pod length, seed dry weight, root dry weight and fodder weight are consistent with the findings reported by Meena et al (2015) and Kamara et al (2017). Furthermore, root dry weight, number of seeds positively collated with seed weight, supporting the findings of Alidu (2018). The implication of these observations is that these parameters can be useful in selecting traits for the improvement of cowpea yield (Iqbal et al 2010).

4.6 Conclusion

In conclusion, the findings revealed that moisture stress and loamy sand soil significantly reduces cowpea yield while maximum yield was obtained from adequate water and sandy loam soil. The application of Mycoroot^{TM} inoculation showed inconsequential effect on the measured yield parameters. However, despite statistical insignificance, Mycoroot^{TM} inoculation managed to enhanced some yield attributes such as pod characteristics (pod length, cavity, pod dry weight and haulm weight) and fodder weight indicating potential effectiveness of the inoculant. Based on the results obtained, soil amendment at 75:25 Biochar and Compost mix ratio achieved 15.1% higher seed weight than the control while soil amendment with 50:50 biochar and compost mix ratio produced the highest root dry weight. This suggests that cowpea yield performance was improved in amended soil. Based on the findings in this study, the hypothesis that combined Mycoroot™ inoculation with variable biochar-compost mixtures will have no effect on cowpea yield and yield attributes under moisture stress condition is hereby rejected. Integrated use of Mycoroot™ as inoculant containing native *Arbusculr mycorrhizae fungi* with 75:25 biochar and compost mix ratio as soil amendment offers an appropriate agronomic approach that can assist farmers with enhancing cowpea seed yield up to 15.1% in moisture deficient and low fertility soils.

References

Abayomi, Y.A., Afolabi, E.S., and Aderolu, M.A., (2019). Effects of water stress at different stages on growth, grain yield and seed quality of cowpea genotypes. *NISEB Journal* 1:1:1.

Abidi, N., Liyanage, S., Auld, D., Norman, L., Grover, K., Augadi, S., Singla, S., and Trostle, C., (2015). Challenges and opportunities for increasing guar production in the United States to support unconventional oil and gas production. Chapter 12*: Challenges and Opportunities for Increasing Guar Production in the United States to Support Unconventional Oil and Gas Production* 207-225.

Adeyemi, N.O., Atayese, M.O., Olubode, A.A., and Akan, M.E., (2020). Effect of commercial arbuscular mycorrhizal fungi inoculant on growth and yield of soybean under controlled and natural field conditions. *Journal of Plant Nutrition 43*:487-499.

Adeyemi, N.O., Atayese, M.O., Sakariyawo, O.S., Azeez, J.O., Olubode, A.A., Ridwan, M., Adebiyi, A., Oni, O., and Ibrahim, I., (2021). Influence of different arbuscular mycorrhizal fungi isolates in enhancing growth, phosphorus uptake and grain yield of soybean in a phosphorus deficient soil under field conditions. *Communications in Soil Science and Plant Analysis 52*:1171-1183.

Adugna, G., (2016). A review on impact of compost on soil properties, water use and crop productivity. *Academic Research Journal of Agricultural Science and Research* 4:93-104.

Agegnehu, G., Bass, A.M., Nelson, P.N., Muirhead, B., Wright, G., and Bird, M.I., (2015a). Biochar and biochar-compost as soil amendments: effect on peanut yield soil properties and greenhouse gas emissions in tropical North Queensland, Australia. *Agriculture, Ecosystem and Environment* 213:72-85.

Agegnehu, G., Bird, M., Nelson, P., and Bass, A., (2015b). The maeliorating effects of biochar and compost in soil quality and plant growth on a Ferralsol. *Soil Research* 53:1-12.

Aguégué, M.R., Ahoyo Adjovi, N.R., Agbodjato, N.A., Noumavo, P.A., Assogba, S., Salami, H., Salako, V.K., Ramón, R., Baba-Moussa, F., Adjanohoun, A., and Glele Kakaï, R., (2021). Efficacy of native strains of arbuscular mycorrhizal fungi on maize productivity on ferralitic soil in Benin. *Agricultural Research* 11:627-641.

Aguila, S.R.D., La Sota-Ricaldi, D., Maria, A., Corazon-Guivin, M.A., and López-García, Á., (2022). Phylogenetic diversity of arbuscular mycorrhizal fungal communities' increases with crop age in coffea arabica plantations. *Journal of Soil Science and Plant Nutrition 22*:32913303.

Ahmadpour, R., and Hossain Zade, S.R., (2017). Evaluating the effects of water stress and urban waste compost on morpho-physiological indices and yield components of lentil (*Lens culinaris Medik*). *Journal of Iranian Plant Ecophysiological Research* 12:42-56.

Ahmed, F.E., and Suliman, A.S.H., (2010). Effect of water stress applied at different stages of growth on seed yield and water-use efficiency of Cowpea. *Agriculture and Biology Journal of North America* 1:534-540.

Alemu, M., Asfaw, Z., Woldu, Z., Fenta, B.A., and Medvecky, B., (2016). Cowpeas (*Vigna unguiculata (L) Walp.)* (Fabaceae) landrance diversity in northern Ethopia. *International Journal of Biodiversity and Conversation* 11:297-309.

Alidu, M.S., (2018). Evaluation of cowpea genotypes for drought tolerance using the pot screening approach. *Asian Research Journal of Agriculture* 10:1-11.

Awasthi, M.K., Wang, Q., Chen, H., Liu, T., Awasthi, S.K., Duan, Y., Varjani, S., Pandey, A., and Zhang, Z., (2019). Role of compost biochar amendment on the (im) mobilization of cadmium and zinc for Chinese cabbage (*Brassica rapa L.*) from contaminated soil. *Journal of Soils and Sediments* 19:3883-3897.

Barros, J.R.A., Angelotti, F., Santos, J.D.O., Silva, R., Dantas, B.F. and De Melo, N.F., (2020). Optimal temperature for germination and seedling development of cowpea seeds. In Embrapa Semiárido-Artigo em anais de congresso (ALICE). *Revista Colombiana de Ciencias Hortícolas* 14:1-19.

Bisikwa, J., (2011). McKnight foundation collaborative crops research project No. 09-480 improving food security through participatory development of high yielding and pest resistant cowpea varieties in Uganda Annual Progress Report-Narrative. Breeding drought-tolerant cowpea: constraints, accomplishments, and prospects. *Euphytica* 167:353-370.

Chia, W.Y., Chew, K.W., Le, C.F., Lam, S.S., Chee, C.S.C., Ooi, M.S.L., and Show, P.L., 2020. Sustainable utilization of bio-waste compost for renewable energy and soil amendments. *Environmental Pollution* 267:115662.

Chukwudi, U.P., Mavengahama, S., Kutu, F.R., and Motsei, L.E., (2022). Heat stress, varietal difference, and soil amendment influence on maize grain mineral concentrations. *Agriculture* 12:1633.

Cobb, A.B., Wilson, G.W., Goad, C.L., and Grusak, M.A., (2018). Influence of alternative soil amendments on mycorrhizal fungi and cowpea production. *Heliyon* 4:00704.

Ditta, A., Muhammad, J., Imtiaz, M., Mehmood, S., Qian, Z., and Tu, S., (2018). Application of rock phosphate enriched composts increases nodulation, growth and yield of chickpea. *International Journal of Recycling of Organic Waste in Agriculture* 7:33-40.

Dobo, B., (2022). Effect of arbuscular mycorrhizal fungi (AMF) and rhizobium inoculation on growth and yield of *Glycine max* L. Varieties. *International Journal of Agronomy* 2022.

Downie, A., (2011). Biochar production and use: environmental risks and rewards. *Applied Soil Ecology* 15:3-6.

El-Shimi, N.M., (2022). Effect of Biochar, Compost and bio-fertilizer on pea yield then, Study its residual effect on the subsequent pepper crop. *Medicon Agriculture and Environmental Sciences* 2:13-31.

Erman, M., Demir, S., Ocak, E., Tüfenkçi, Ş., Oĝuz, F., and Akköprü A., (2011). Effects of Rhizobium, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum L.)* under irrigated and rainfed conditions 1-Yield, yield components, nodulation and AMFcolonization. *Field Crops Research* 122:14-24.

Farooq, M., Hussain, M., Usman, M., Farooq, S., Alghamdi, S.S., and Siddique, K.H., (2018). Impact of abiotic stresses on grain composition and quality in food legumes. *Journal of Agricultural and Food Chemistry* 66:8887-8897.

Gavili, E., Moosavi, A.A., and Haghighi, A.A.K., (2019). Does biochar mitigate the adverse effects of drought on the agronomic traits and yield components of soybean? *Industrial Crops and Products* 128:445-454.

Gerrano, A.S., Jansen van Rensburg, W.S., and Kutu, F.R., (2019). Agronomic evaluation and identification of potential cowpea (*Vigna unguiculata L. Walp*) genotypes in South Africa. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science* 69:295-303.

Ghorchiani, M., Etesami, H., and Alikhani, H.A., (2018). Improvement of growth and yield of maize under water stress by co-inoculating an arbuscular mycorrhizal fungus and a plant growth promoting rhizobacterium together with phosphate fertilizers. *Agriculture. Ecosystems and Environment* 258:59-70.

Hamidou, F., Halilou, O., and Vadez, V., (2013). Assessment of groundnut under combined heat and drought stress. *Journal of Agronomy and Crop Science* 199:1-11.

Hollister, C.C., Bisogni, J.J., and Lehmann, J., (2013). Ammonium, nitrate, and phosphate sorption to and solute leaching from biochars prepared from corn stover (*Zea mays L.*) and oak wood (*Quercus spp.*). *Journal of Environmental Quality* 42:137-144.

Horn, L.N., Nghituwamata, S.N., and Isabella, U., (2022). Cowpea Production Challenges and Contribution to Livelihood in Sub-Saharan Region. *Agricultural Sciences* 13:25-32.

İç, S., Gülser, C., Candemir, F., and Demir, Z., (2010). Effects of plant growth on some physical properties of different textured soils. In Proceedings of the International Soil Science Congress on Management of Natural Resources to Sustain Soil Health and Quality. Kizilkaya, R., Gülser, C., Dengiz, O., 1072-1077.

Iqbal, Z., Arshad, M., Ashraf, M., Naeem, R., Malik, M.F., and Waheed. A., (2010). Genetic divergence and correlation studies of soybean (*Glycine max (L.) Merrill*.) genotypes. *Pakistan Journal of Botany* 42:971-976.

Iseki, K., Ikazaki, K., and Batieno, J.B., (2021). Cowpea yield variation in three dominant soil types in the Sudan Savanna of West Africa. *Field Crops Research* 261:108012.

Jangpromma, N., Thammasirirak, S., Jaisil, P., and Songsri, P., (2012). Effects of drought and recovery from drought stress on above ground and root growth, and water use efficiency in sugarcane (*'Saccharum officinarum'L.*). *Australian Journal of Crop Science 6*:1298-1304.

Kamara, A.Y., Ewansiha, S., Ajeigbe, H., Omoigui, L., Tofa, A.I., and Karim, K.Y., (2017). Agronomic evaluation of cowpea cultivars developed for the West African Savannas. *Legume Research-An International Journal* 40:669-676.

Kamara, A.Y., Omoigui, L.O., Kamai, N., Ewansiha, S.U., and Ajeigbe, H.A., (2018). Improving cultivation of cowpea in West Africa. *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)* 2:235-252.

Kardile, P.B., Dahatonde, K.N., Burondkar, M.M., and Bhave, S.G., (2018). Effect of moisture stress conditions on yield and yield attributing characters of four cowpeas (*Vigna unguiculata L. Walp*) Genotypes. *International Journal of Current Microbiology and Applied Science* 4:2631-2636.

Kazadi, A.T., Shutcha, M.N., Baert, G., Haesaert, G. and Mundende, R.P.M., (2020). Effect of Soil Properties on Arbuscular Mycorrhizae Fungi (AMF) Activity and Assessment of Some Methods of Common bean (*Phaseolus vulgaris L.*) Inoculation in Lubumbashi Region (DR. Congo).

Kedir, U., (2020). The effects of nitrogen and moisture stress on yield and quality of wheat: A review. *International Journal of Research Studies in Bioscience* 8:13-20.

Khandelwal, A., and Sindhu, S.S., (2012). Expression of 1-aminocyclopropane-1-carboxylate deaminase in rhizobia promotes nodulation and plant growth of clusterbean (*Cyamopsis tetragonoloba L.*). *Research Journal of Microbiology* 7:158.

Kim, S.J., Eo, J.K., Lee, E.H., Park, H., and Eom, A.H., (2017). Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology* 45:20-24.

Liang, L., Xi, F., Tan, W., Meng, X., Hu, B., and Wang, X., (2021). Review of organic and inorganic pollutants removal by biochar and biochar-based composites. *Biochar* 3:255-281.

Liu, L., Wang, Y., Yan, X., Li, J., Jiao, N., and Hu, S., (2017). Biochar amendments increase the yield advantage of legume-based intercropping systems over monoculture. *Agriculture, Ecosystems and Environment* 237:16-23.

Lui, J.H., Schulz, S., Brandl, H,M., Miehtke, B., Huwe, B., and Glaser, B., (2012). Short-term effect of biochar and compost on soil fertility and water status of a Dystric cambisol in NE Germany under field conditions. *Journal of Plant Nutrition and Soil Science* 10:698-707.

Maboko, M.M., Bertling, I., and Du Plooy, C.P., (2013). Arbuscular mycorrhiza has limited effects on yield and quality of tomatoes grown under soilless cultivation. *Acta Agriculturae Scandinavica, Section B–Soil and Plant Science* 63:261-270.

Meena, H.K., Krishna, K.R., and Singh, B., (2015). Character associations between seed yield and its components traits in cowpea [*Vigna unguiculata (L.) Walp*.]. *Indian Journal of Agricultural Research* 49:567-570.

Mekonnen, T.W., Gerrano, A.S., Mbuma, N.W., and Labuschagne, M.T., (2022). Breeding of vegetable cowpea for nutrition and climate resilience in Sub-Saharan Africa: progress, opportunities, and challenges. *Plants* 11:1583.

Mfeka, N., Mulidzi, R.A., and Lewu, F.B., (2019). Growth and yield parameters of three cowpeas (*Vigna unguiculata L. Walp*) lines as affected by planting date and zinc application rate. *South African Journal of Science* 115:27-34.

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* 10:565.

Mukhongo, R.W., Tumuhairwe, J.B., Ebanyat, P., AbdelGadir, A.H., Thuita, M., and Masso, C., (2016). Production and use of arbuscular mycorrhizal fungi inoculum in sub-Saharan

Africa: challenges and ways of improving. *International Journal of Soil Science* 11:108-122.

Olawuyi, O.J., Ezekiel-Adewoyin, D.T., Odebode, A.C., Aina, D.A., and Esenbamen, G.E., (2012). Effect ofarbuscular mycorrhizal (*Glomus clarum*) and organomineral fertilizer on growth and yield performance of okra (*Abelmoschus esculentus*). *African Journal of Plant Science* 6:84-88.

Oliveira, R.S., Carvalho, P., Marques, G., Ferreira, L., Nunes, M., Rocha, I., Ma, Y., Carvalho, M.F., Vosátka, M. and Freitas, H., (2017). Increased protein content of chickpea (*Cicer arietinum L.*) inoculated with arbuscular mycorrhizal fungi and nitrogen-fixing bacteria under water deficit conditions. *Journal of the Science of Food and Agriculture* 13:4379-4385.

Oruru, M.B., Njeru, E.M., Pasquet, R., and Runo, S., (2018). Response of a wild-type and modern cowpea cultivars to arbuscular mycorrhizal inoculation in sterilized and non-sterilized soil. *Journal of Plant Nutrition* 41:90-101.

Oyewole, B.O., Olawuyi, O.J., Odebode, A.C., and Abiala, M.A., (2017). Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. *Biotechnology Reports* 14:8-15.

Pellegrino, E. and Bedini, S., (2014). Enhancing ecosystem services in sustainable agriculture: biofertilization and biofortification of chickpea (*Cicer arietinum L.)* by arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 68:429-439.

Powell, J.R., and Rillig, M.C., (2018). Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytologist*, *220*:1059-1075.

Rocha, I., Ma, Y., Vosátka, M., Freitas, H., and Oliveira, R.S., (2019). Growth and nutrition of cowpea (*Vigna unguiculata*) under water deficit as influenced by microbial inoculation via seed coating. *Journal of Agronomy and Crop Science* 205:447-459.

Rocha, I., Souza-Alonso, P., Pereira, G., Ma, Y., Vosátka, M., Freitas, H. and Oliveira, R.S., (2020). Using microbial seed coating for improving cowpea productivity under a low-input agricultural system. *Journal of the Science of Food and Agriculture* 3:1092-1098.

Rodriguez, A. and Sanders, I.R., (2015). The role of community and population ecology in applying mycorrhizal fungi for improved food security. *The ISME Journal 9*:1053-1061.

Safaei Asadabadi, R., Hage-Ahmed, K., and Steinkellner, S., (2021). Biochar, compost and arbuscular mycorrhizal fungi: a tripartite approach to combat Sclerotinia sclerotiorum in soybean. *Journal of Plant Diseases and Protection* 128:1433-1445.

Santos, A.D., Ceccon, G., Davide, L.M.C., Correa, A.M., and Alves, V.B., (2014). Correlations and path analysis of yield components in cowpea. *Crop Breeding and Applied Biotechnology* 14:82-87.

Sharma, S.B., Sayyed, R.Z., Trivedi M.H., and Gobi, T.A., (2013). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2:10.1186/2193-1801-2-587

Simunji, S., Munyinda, K.L., Lungu, O.I., Mweetwa, A.M., and Phiri, E., (2019). Evaluation of cowpea (*Vigna unguiculata L. walp*) genotypes for biological nitrogen fixation in maizecowpea crop rotation. *Sustainable Agriculture Research* 8:82-93.

Sohi, S.P., Krull, E., Lopez-Capel, E., and Bol, R., (2010). A review of biochar and its use and function in soil. *Advances in Agronomy* 105:47-82.

Tajini, F., Trabelsi, M., and Drevon, J.J., (2012). Combined inoculation with Glomus intraradices and Rhizobium tropici CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris L.*). *Saudi Journal of Biological Sciences* 2:157-163.

Tammeorg, P., Simojoki, A., Makela, P., Stoddard, F.L., Alakukku, L., and Helenius, J., (2014). Short-term effect of biochar on soil properties and wheat yield formation with meat bone meal and inorganic fertiliser on a boreal loamy sand. *Agriculture, Ecosystem and Environment* 1:2-7.

Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y. and Yang, Z., (2015). Application of biochar for the removal of pollutants from aqueous solutions. *Chemosphere* 125:70-85.

Thapa, S., Adams, C.B., and Trostle, C., (2018). Root nodulation in guar: Effects of soils, Rhizobium inoculants, and guar varieties in a controlled environment. *Industrial Crops and Products* 120:198-202.

Trupiano, D., Cocozza, C., Baronti, S., Amendola, C., Vaccari, F.P., Lustrato, G., Di Lonardo, S., Fantasma, F., Tognetti, R., and Scippa, G.S., (2017). The effects of biochar and its combination with compost on lettuce (*Lactuca sativa L.*) growth, soil properties, and soil microbial activity and abundance. *International Journal of Agronomy* 2017.

Ullah, N., Ditta, A., Imtiaz, M., Li, X., Jan, A.U., Mehmood, S., Rizwan, M.S., and Rizwan, M., (2021). Appraisal for organic amendments and plant growth-promoting rhizobacteria to enhance crop productivity under drought stress: A review. *Journal of Agronomy and Crop Science* 207:783-802.

Umamahesh, Y.Y.P.S.V., Reddy, D.M., and Sumathi, V., (2018). Influence of moisture stress on mung bean genotypes for morpho-physiological traits. *Andhra Pradesh Journal of Agricultural Science* 2:78-94.

Waraich, E.A., Ahmad, R., and Ashraf, M.Y., (2011). Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Science* 5:764-777.

Wheeler, T., and Von Braun. J., (2013). Climate change impacts on global food security. *Science* 341:508-513.

Wu, M., Guo, X., Wu, J., and Chen, K., (2020). Effect of compost amendment and bioaugmentation on PAH degradation and microbial community shifting in petroleumcontaminated soil. *Chemosphere* 256:126998.

Yahaya, D., Denwar, N., and Blair, M.W., (2019). Effects of moisture deficit on the yield of cowpea genotypes in the Guinea Savannah of Northern Ghana. *Agricultural Sciences* 4:577595.

Yeboah, E., Asamoah, G., Ofori, P., Amoah, B., and Agyeman, K.O.A., (2020). Method of biochar application affects growth, yield and nutrient uptake of cowpea. *Open Agriculture* 5:352-360.

Yetunde, B.O., Gani, O.K., Emmanuel, J., and Christiana, S., (2022). Phosphate leachate losses in sandy soil amended with different fertilizers and effects on cowpea yield. *Journal of Soil Science and Environmental Management* 12:1-10.

CHAPTER 5

Integrated MYCOROOT™ inoculation and biochar-compost mixture application under variable soil moisture conditions enhance proteins, secondary metabolites and mineral composition in cowpea grains.

Abstract

Cowpea (*Vigna unguiculata L.*) is a protein-rich grain that is grown in various parts of SubSaharan Africa (SSA). Its benefits include among others, an inexpensive plant-protein source and a vital constituents of essential minerals for human nutrition and health. Mycoroot^{TM} is a South African locally produced biofertiliser that is gaining interest among scientists as an unconventional approach to enhance legume crops performance including grain qualities. A greenhouse experiment was undertaken at the University of Mpumalanga to examine the effect of integrated Mycoroot^{TM} inoculation and biochar-compost mixture application ratios as an agronomic package to enhance protein, secondary metabolites and mineral composition in cowpea grains under variable moisture conditions. The trial comprised of 4 soil amendments derived from different mix ratios of biochar (BC) and compost (C), 2 AMF levels (inoculated and uninoculated) and 2 soil moisture regimes (adequate soil moisture and moisture stressed) as main treatment factors; and evaluated on 2 distinct soil textural types (sandy loam and loamy sand). The soil amendments comprised of 50%biochar50%compost (50:50 BC/C), 75%biochar25%compost (75:25 BC/C), 25%biochar75%compost (25:75 BC/C) and a control with no amendment. The treatment factors were combined and laid out in a 2x2x4x2 factorial design fitted into RCBD with each replicated 4 times. Results of the study revealed that Mycoroot^{TM} inoculation resulted in 0.26 G/dm reduction of grain flavonoid content under both moisture levels. Higher flavonoid concentration of 0.38 G/dm was recorded from un-inoculated treatments compare to 0.26 G/dm from inoculated treatments. Similarly, Mycoroot^{TM} inoculation increases anthocyanin and protein contents in cowpea grain under moisture stressed condition. The different soil amendments exerted significant ($p<0.05$) effect on anthocyanin and flavonoids but had inconsequential effect on protein and TSS content. Although none of Mycoroot^{TM} inoculation or soil amendments exerted any significantly effect on the mineral composition of cowpea grain except for Zn content that was increased by the 75% biochar and 25% compost mix ratio. The variation in soil moisture level had positive effect on cowpea grain P content with higher concentration recorded in moisture stressed cowpea plants.

Keywords: Cowpea grains**,** moisture stress**,** *arbuscular mycorrhizal fungi*, biochar, compost, soil textural types, flavonoids, TSS, Anthocyanin, nutrients

5.1 Introduction

Cowpeas (*Vigna unguiculata L.*) are leguminous crops cultivated mostly by smallholder farmers in semi-arid and arid areas (Singh and Das 2022; Nkomo et al 2021). They are known to be second-most valuable food source after cereals due to their inexpensiveness and the high amount of proteins contained in their grains and the different plant parts (Maphosa and Jideani 2017; Kumar et al 2016; Pereira et al 2014). The crop is among the well-known local African grain and vegetable crops that are high in nutrients, vitamins and minerals, possessing a great potential to maximising food and nutritional security (Owade et al 2020; Okonya and Maass 2014). According to Diouf (2011), cowpea grains consist of a significant amount of micronutrients such as iron and calcium while the crude protein content ranges from 23 and 32% supporting millions of people in developing countries and underprivileged communities (Hall 2012). Several research works have revealed that cowpeas are a good source of vital minerals such as Calcium (Ca), Copper (Cu), Iron (Fe), Phosphorus (P) and Zinc (Zn) that are important for human nutrition and health (Asiwe 2022; Alamu et al 2016). Secondary metabolites are involved in various physiological functions of plants that exhibit defensive effects in attempt to respond against adverse environmental stresses such as moisture stress (Shojaie et al 2016). Cowpeas and common bean (*Phaseolus vulgaris*) are some of the leguminous crops that contain high levels of secondary metabolites such anthocyanin and flavonoids (Shishehbor and Hemmati 2022; Harmankaya et al 2016). Secondary metabolites are utilised to enhance flavours in various human diets and assisting plants with survival under harsh environmental conditions by promoting glutamic acid-mediated proline biosynthesis pathway essential for osmotic regulation under drought stress (Qu et al 2019; Tiwari and Rana 2015; Ramakrishna and Ravishankar 2011).

Crop is drought tolerant, making it to be an important crop in drought prone areas (Sanjeev et al 2018). However, one of the significant abiotic stresses that impair cowpea plants' growth and yield is moisture stress (Ritte et al 2022; Etienne et al 2018; Gagné-Bourque et al 2016). Moisture stress reduces crop productivity by affecting plant organs (xylem) responsible for water and nutrient acquisition and acquisition thus contributing to reduced food availability, malnutrition, famine and exacerbate the risk of food insecurity especially in rural poor communities (Masih et al 2014). In addition, plants develop defence mechanisms that provide protection and serve as coping strategies against environmental stresses such as moisture stress (Rao et al 2013). Secondary metabolites assist crops under moisture deficits conditions by inducing in-vivo drought signalling whereby drought stress is perceived in the root system network as a stress signal through cell to cell signalling networks, which then travels to the leaves via xylem and then triggers stomatal closure through the vascular to cell guards signalling, preventing water loss (Yadav 2021). There is a research gap currently on the effect of moisture stress on the availability of secondary metabolites and mineral content in cowpea production.

In South Africa, Mycoroot^{TM} products containing local soil specie of *Arbuscular mycorrhizae fungi* (AMF) are commercially packaged and marketed agricultural inputs that have potential to colonise with roots of host plants in a symbiotic relationship and are directly linked to a number of advantages (Qiao et al 2015; Mukhongo et al 2016). These microorganisms create large hyphal networks that facilitate root absorption under adverse environmental conditions (Sharma et al 2021). This symbiosis relationship is essential for plant growth, yield, and in nutrient limiting conditions providing greater tolerance to numerous abiotic stressors such as drought (Ercoli et al 2017; Fileccia et al 2017; Soka and Ritchie 2014). Abdel-Fattah and Asrar (2012) revealed that the presence of AMF in the soil helps to prolong the availability of nutrients in soil such as phosphorus (P) that plays a vital role in plant growth and development. AMF have been reported to possess the ability to boost the host plants' capacity to withstand water stress by simplifying water and nutrient acquisition in plant roots (Ingraffia et al 2019; Delavaux et al 2017). Furthermore, improvements in grain nutrient P, K, Zn contents due to AMF application have been described by Mehmood et al (2022) and Watts-William and Gilbert (2021). Therefore, a key to efficient cowpea grain production may be prompted by the use of mutualistic association benefits such as *Arbuscular mycorrhizae fungi* such as Mycoroot^{TM}.

Biochar is an organic waste material utilised in agricultural systems to improve soil fertility, conserve water and enhance crop yields (Lusiba et al 2021; Ding et al 2016; Petter and Madari 2012). It is a carbon-rich material derived from decomposition of lignocellulosic biomass at relatively low temperature, with little or no oxygen present (Novotny et al 2015). According to Spokas et al (2012), the use of biochar contributes to food security and mitigation of environmental related issues. Many studies have reported that biochar is among the cost-effective organic soil amendments utilised for increasing agricultural productivity. It is a nutrient carrier that retains moisture to the soil, increase soil pH and cation-exchange capacity, alleviate nutrient and drought stresses which collectively improves crop yields required to feed population and subsequently restore degraded lands (Joseph et al 2021; Yadav et al 2019; Hagemann et al 2018). Compost on the other hand is a by-product rapid microbial decomposition of organic materials under controlled aerobic condition (Adugna 2016).

Compost performs several functions to better the quality and state of the soil such as increasing available plant water, water holding capacity and soil fertility (Wu et al 2020). Integrated use of biochar and compost to act as soil conditioners improves soil physical properties thereby increasing plant yield such as Faba beans (*Vicia faba*) and Soybean (*Glycine max*) (Haddad et al 2022; Lui et al 2012).

Combining AMF inoculation with integrated use of biochar and compost presents a promising tool that can lead to positive improvement in plant and soil conditions to achieve higher grain yields. Furthermore, using these microorganisms as inoculants may contributes to a more viable production system that is less dependent on inorganic fertilizers. There are numerous works regarding individual application of AMF inoculation and biochar-compost amendments rates to agricultural crops, however the interactive effects of integrating AMF inoculation and varied biochar and compost mixtures as an agronomic package under moisture stress in cowpea production has not been well established. Therefore, it is hypothesised that the combination of Mycoroot™ inoculation with variable biochar-compost mixt ratios will significantly improve cowpea mineral, protein and secondary metabolites content under two moisture regimes

5.2 Materials and methods

5.2.1 Description of the greenhouse trial

Detailed description of the methodology greenhouse trial including trial layout were as previously provided in sections 3.2.1 to 3.2.4 in chapter 3.

5.2.2 Seed preparation and milling

Seeds obtained from the greenhouse trial yield were grounded into fine powder using a grinding blander and sieved using a 0.1 mm sieve.

5.2.3 Protein content determination in the milled seed samples

The total protein was determined using the Kjeldahl method that involved digestion followed by distillation and titration (Omenna et al 2016). Digestion of 0.5 g weighed dried sample in a Kjeldhal tube mixed with 1 g catalyst mixture of K_2SO_4 and $CuSO_4$ mixture (Kjeldhal tablet), and 15 ml concentrated H_2SO_4 carefully added along the wall of the Kjeldhal tube. The tubes containing samples and digestion mixtures were placed in the digestion apparatus for the process of digestion set at a temperature of 350°C and about 2 hours until the solution became clear. The tubes were removed and allowed for cooling to occur at room temperature and then 50 ml of distilled water was added. Digestion was done on a blank sample as well.

Distillation process: Approximately 10 ml of 1% boric acid (10g/l) plus 2 drops of bromocresol green indicator (100 mg in 100 ml ethanol) was added onto a 250 ml conical flask. Each Kjeldhal tube containing the digested sample and the conical flask were both attached onto the preheated distillation apparatus. The digest was distilled until the volume of the distillate in the receiving flask increased up to 40 ml. The flask containing boric acid and distillate was removed and prepared for the titration process.

Titration: The NH_3 trapped into the boric acid in the distillation flask was titrated with 0.01 of hydrochloric acid (HCl).

Calculation:

$$\mathrm{N\%} = \frac{(V1 - V2)xNx14.01}{\mathrm{Wx1000}} \, x\ 100\%$$

V1= volume of the HCl used for the sample (ml)

V2= volume of the HCl used for the blank (ml)

W= weight of the dry sample (g)

N= normality of HCl solution

14.01= atomic weight of Nitrogen (g)

5.2.4 Determination of Anthocyanin

Cowpea seeds were blended to fine powder using a blender, sieved at 0.85 mm. One gram of blended sample was mixed with 9 ml of acidified acetone solvent following the ratio 70:29.5:0.5 of Acetone:water:acetic acid. The mixture was homogenised and centrifuged at 4000 rpm for 20 minutes at 4°C. The supernatant was separated, and the extracted residue underwent the same process for the second time under the same conditions. The extracts were diluted using a mixture of 1:5 ratio of 0.025 M (pH 1.0) potassium chloride and 0.4 M (pH 4.5) sodium acetate solution as buffer. Measurement of absorbance at absorption spectrum of 710 nm on all samples using spectrophotometer with values observed expressed in Gdm^{-1} (AquinoBolanos et al 2016).

5.2.5 Determination of flavonoids

A sample of 1g grounded seed powder was weighed and mixed with 9ml of acidified methanol prepared to the ratio 79:20:1 ($MeOH:H_2O:HCl$). The mixture was incubated for 72 hours in darkness for auto-extraction, after which the mixture was centrifuge at 4000 rpm or 20 minutes 4^0C. The absorbance of the clear supernatant liquid was measured spectrometrically using a double beam spectrophotometer at 300 nm, and values observed expressed in Abs Gdm^{-1} as described by Makoi et al (2010).

5.2.6 Determination of total soluble sugars (TSS)

Using a grinding blender, cowpeas seeds from the greenhouse trial were grounded into fine powder, sieved using a 0.1 mm sieve. About 1g of powder sample was weighed and then transferred into 50 ml microfuge tubes. 40 ml of 80% ethanol was added, homogenized for 1 minute and the tubes were placed in a water bath at 80^0 C for 20 minutes, thereafter the cooling down process was allowed at room temperature. After this treatment, the tubes were subjected to centrifuge at 6000 rpm for 10 minutes (Al-Amri 2023). Using a pipette, a drop of the supernatant was carefully put on the surface of the refractometer's prizm. The total soluble sugar values were observed and recorded.

5.2.7 Laboratory determinations on milled cowpea grain samples

5.2.7.1 Determination of the mineral composition of cowpea grains

All mineral analyses were undertaken following methodology described in AgriLaboratory Association of Southern Africa (AgriLASA) Handbook of Feeds and Plant Analysis, method 6.3.1 and 6.5.1. Approximately 0.5 g sample of seed powder was weighed out into 25 cm^3 calibrated tube with 4 ml of 55% nitric acid and 70% perchloric acid added and allowed to stay overnight**.** Thereafter, the tubes were placed on a digestion block with power on to digest samples for 2 hours at 100°C and then for 6 hours at 180°C on digestion block. Digestion was completed when the digest became colourless. The cooling process was allowed overnight, and calibrated thereafter made up to 25 cm^3 mark with de-ionized water. The concentration of Ca, Zn, Cu and Fe in the digests were measured using atomic absorption spectrophotometer while

K concentration was measured using fame emission spectrophotometer conditions as indicated in Table 5.1 below.

Table 5. 1: Spectrophotometer condition used for K, Ca, Zn, Cu and Fe determination

Element	Wavelength (nm)	Slit width (nm)	Working range (ppm)	Flame type	Type of spectroscopy
Calcium	422.7	0.5	1 - 4	Air-acetylene	Atomic Absorption
Potassium	766.5	0.1	0.03 - 2.0	Air – acetylene	Flame emission
Zinc	213.9	0.5	0.4 – 1.5	Air – acetylene	Atomic Absorption
Copper	324.7	0.5	1 – 5	Air – acetylene	Atomic Absorption
Iron	248.3	0.2	2 – 9	Air – acetylene	Atomic Absorption

Source: AgriLaboratory Association of Southern Africa (AgriLASA) Handbook of Feeds and Plant Analysis

Five standard solutions for each element, including the blank were prepared for the reading of the concentration of all elements in each digest sample. The sample extraction factor of 50 was included in the standards (25 cm^3/0.5 g (v/m ratio) = 50). To prevent ionization interferences in Ca and K determinations, an ionization buffer (2000 ppm Strontium chloride) was added to each standard and sample solutions (Maseko et al 2022). Strontium chloride was the releasing agent for the flame spectroscopy. Standard solutions were aspirated into the flame starting with the lowest concentration and the corresponding absorbance read. A calibration graph was drawn relating absorbance to the concentration in ppm of nutrient present. Proceeded with sample solutions. The concentration of the sample was directly read from the calibration graph in ppm. Results of Zn, Cu and Fe were reported in mg/kg whilst K and Ca were reported in percent (%).

5.2.7.2 P determination

Standard solutions containing 0, 10, 20, 30, 40 and 60 ppm P were prepared. The sample dilution factor of 50 was included in the standards. Using an automated P analyser, the sample containing reagents comprising of vanado-molydate and stannous chloride solution run through the system for 30 minutes with a set baseline. The determination of P was based on colorimetric method in which a blue colour was formed by the reaction of ortho-phosphate and the molybdate ion. The phospho-molybdenum complex read at 660 nm and the measured P and results reported in percent.

5.3 Statistical analysis

All data from laboratory analyses namely mineral composition, secondary metabolites, proteins and TSS were subjected to analysis of variance (AVONA) using Statistic 10. The significant difference between mean of treatments tested using Tukey's test at 5% level of significance. The statistical model that described ANOVA use for the data analysis is as presented in 3.3. Pearson correlation analysis was performed to determine the relationship between measured mineral composition and grain contents.

5.4 Results

5.4.1 Treatment effect on grain proteins, secondary metabolites and Total soluble sugars (TSS) of selected cowpea.

5.4.1.1 Protein

Mycoroot^{TM} inoculation, soil amendments, moisture levels and soil textural types had no significant effect ($p>0.05$) on protein content (Table 5.2). Interestingly, interaction between Mycoroot^{TM} inoculation and moisture levels had a significant effect ($p<0.05$) on protein content (Table 5.2). Similarly, the interaction between soil moisture levels and soil textural types as well as soil moisture levels and soil amendments interaction exerted significant ($p<0.05$) effect on protein content (Table 5.2).

5.4.1.2 Anthocyanin

Variation in soil moisture levels and soil amendments exerted significant ($p<0.05$) effect on anthocyanin content (Table 5.2). However, Mycoroot^{TM} inoculation and soil textural types had no significant ($p>0.05$) on anthocyanin content (Table 5.2). Moreover, the interaction between inoculation and moisture levels as well as soil textural types and soil amendments exerted significant ($p<0.05$) effect on anthocyanin content (Table 5.2).

5.4.1.3 Flavonoids

Application of Mycoroot^{TM} inoculation and soil amendments showed a significant effect ($p<0.05$) on flavonoids content (Table 5.2). While, moisture levels and soil textural types showed no significant effect ($p>0.05$) on flavonoid content. Moreover, interaction between inoculation and soil amendments had a positive effect ($p<0.05$) on flavonoids content (Table 5.2). Also, interactions between moisture levels and soil amendments and interaction between soil textural types and soil amendments had a significant effect ($p<0.05$) on flavonoid content (Table 5.2).

5.4.1.4 Total soluble sugar (TSS)

Application of Mycoroot^{TM} inoculation, soil amendments, moisture levels and soil textural types had no significant ($p>0.05$) on TSS content (Table 5.2). However, interaction between moisture levels and soil amendments had a significant effect ($p<0.05$) on TSS content (Table 5.2).

Table 5. 2: p-values of ANOVA of measured parameters Anthocyanin, Flavonoids, TSS and Proteins

Source of variation	Proteins (%)	Anthocyanin (mg/g DM)	Flavonoids (G/dm)	TSS (^{O}Brix)
Inoculation (IN)	0.377	0.595	0.000***	0.313
Moisture Levels (ML)	0.128	0.025*	0.143	0.073
Soil Amendments (SA)	0.392	0.000***	0.000***	0.833
Soil Types (ST)	0.101	0.859	0.363	0.459
IN*ML	0.055*	0.033*	0.3343	0.997
IN*SA	0.227	0.067	0.010**	0.718
ML*ST	0.039*	0.137	0.125	0.211
ML*SA	0.049*	0.504	0.005**	0.044*
ST*SA	0.325	0.007**	0.001**	0.257

*= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

Table 5. 3: Mean separation of measured parameters Anthocyanin, Flavonoids, TSS and Proteins

Source of variation	Proteins (%)	Anthocyanin (mg/g DM)	Flavonoids (g/dm)	TSS (^{O}Brix)
Inoculation (IN)				
Inoculated	29.15a	0.31a	0.26b	20.17a
No inoculation	28.16a	0.31a	0.38a	20.00a
*!Critical Value	2.219	0.037	0.049	0.328
Soil Moisture Levels (ML)				
Moisture stressed	27.71a	0.33b	0.30a	19.92a
Adequate moisture	29.60a	0.29a	0.34a	20.25a
*!Critical Value	2.460	0.037	0.054	0.362
Soil Amendments (SA)				
25Bio75Comp	28.28a	0.32a	0.28b	19.99a
50Bio50Comp	30.66a	0.25b	0.44a	20.13a
75Bio25Comp	27.51a	0.31ab	0.28b	20.13a
Control	28.17a	0.36a	0.29b	20.19a
*!Critical Value	5.592	0.041	0.105	0.708
Soil textural types (ST)				
Sandy loam	29.78a	0.31a	0.33a	20.01a
Loamy sandy	27.53a	0.31a	0.31a	20.16a
*!Critical Value	2.695	0.040	0.058	0.390

*! Implies critical value for comparison (Tukey’s HSD)

5.4.2 Treatment effects on mineral composition of selected cowpea

The results of the treatment effect on P, K, Ca, Zn, Cu and Fe contents of cowpea grain are contained in Table 5.4. None of the P, K, Ca, Zn, Cu and Fe contents of cowpea grains significantly affected by the application of Mycoroot^{TM} inoculation, soil textural types and soil amendments treatments. However, moisture levels and its interaction with Mycoroot^{TM} inoculation had a positive effect ($p<0.05$) on P content (Table 5.4). Interaction between soil textural types and soil amendments significantly affected ($p<0.05$) Cu content in cowpea grain (Table 5.4). Significant and positive effect obtained between mineral compositions (Table

5.5). The correlation analysis performed revealed that mineral P content was significantly and positively correlated with all five mineral contents namely K, Ca, Zn, Cu and Fe (Table 5.6). Similarly, a strong and positive correlation was observed between K and Ca, Zn and Cu mineral contents. Moreover, there was a significant and positive correlation was obtained between Cu and Fe grain contents (Table 5.6).

Table 5. 4: p-values of ANOVA for the measured parameters

Factor	(%)			(mg/kg)		
	P	K	Ca	Zn	Cu	Fe
Soil Types (ST)	0.065	0.396	0.951	0.793	0.145	0.942
Soil Amendments (SA)	0.539	0.806	0.357	0.624	0.475	0.970
Moisture Levels (ML)	0.043*	0.121	0.951	0.158	0.126	0.354
Inoculation (IN)	0.236	0.218	0.430	0.234	0.394	0.249
ST*SA	0.079	0.376	0.871	0.097	0.036*	0.335
ML*IN	0.049*	0.315	0.951	0.073	0.145	0.157

P=Phosphorus; K=Potassium; Ca= Calcium; Zn= Zinc; Cu= Copper; Fe= Iron; *= indicates significant effect of treatments at 5% **=indicates significant effect of treatments at 1% and ***= indicates significant effect of treatment at 0.1%

Table 5. 5: Effects of inoculation, soil moisture levels, soil amendments and soil textural types on cowpea nutrients

Factors	(%)			mg/kg		
	P	K	Ca	Zn	Cu	Fe
Soil Types (ST)						
Loamy sandy	0.30a	1.23a	0.10a	42.60a	4.54a	73.06a
Sandy loam	0.27a	1.19a	0.10a	43.28a	4.31a	72.88a
!Critical value	0.0333	0.0970	0.0216	5.4907	0.3221	5.4862

Soil Amendments (SA)						
Control	0.31a	1.24a	0.10a	43.34a	4.61a	73.63a
25Bio75Comp	0.28a	1.21a	0.09a	42.45a	4.40a	72.13a
50Bio50Comp	0.28a	1.18a	0.09a	40.6a	4.40a	72.63a
75Bio25Comp	0.27a	1.22a	0.11a	45.35a	4.28a	73.50a
!Critical value	0.0639	0.1863	0.0141	10.545	0.6186	10.536
Moisture Levels (ML)						
Moisture stressed	0.30a	1.25a	0.10a	44.84a	4.30a	74.19a
Adequate moisture	0.27b	1.18a	0.10a	41,03a	4.51a	71.75a
!Critical value	0.0333	0.097	0.0216	5.4907	0.3221	5.4862
Inoculation (IN)						
Non-inoculated	0.30a	1.24a	0.9a	44.52a	4.36a	74.50a
Inoculated	0.28a	1.18a	0.10a	41.35a	4.49a	71.44a
!Critical value	0.0333	0.097	0.0216	5.4907	0.3221	5.4862

*! Implies critical value for comparison (Tukey's HSD); P = Phosphorus; K = Potassium; Ca = Calcium; Cu = Copper; Fe = Iron

Table 5. 6: Pearson correlation coefficients among mineral composition of cowpea grain

	% P	% K	% Ca	Zn mg/kg	Cu mg/kg	Fe mg/kg
P	1					
K	0.995***	1				
Ca	0.940***	0.959***	1			
Zn	0.990***	0.991***	0.961***	1		
Cu	0.991***	0.995***	0.957***	0.989***	1	
Fe	0.992***	0.999***	0.963***	0.992***	0.996***	1

*** indicates significance

5.5 Discussion

5.5.1 Treatment factors and their interaction effects on protein and secondary metabolites.

In the present study, the application of Mycoroot^{TM} inoculation did not significantly affect seed protein content. However, interaction between AMF inoculation and moisture levels showed significant effect on protein content. Under moisture stress, AMF inoculated treatments recorded the highest amount of protein content than in non-inoculated treatments. These results corroborate the findings by Oliveira et al (2017) and Habibzadeh et al (2013). The positive response from application of AMF is associated with an increased root length density and improve nutrition under moisture stress (El-Sawah et al 2021). Moisture levels did not exert a significant response on the concentration of protein in seeds. However, based on the comparison of the two moisture levels, the moisture stressed treatments resulted in reduced percentage seed protein content which in agreement with earlier findings by Ghanbari et al (2013). It appears that the decrease in grain protein content may have been caused by the drastic reduction in photosynthetic process due to moisture stress, which interrupted the materials that are responsible for protein synthesis (Maleki et al 2013). Researchers believed that moisture stress reduces the rate of Nitrogen partitioning and fixation which lowers the rate of protein formation in seeds. Soil amendments did not significantly influence protein content in cowpea seeds. Contrary to these findings, Ramzani et al (2017) revealed that sole application of biochar increased protein content in quinoa seeds. It has been reported that the increase in seed protein content is influenced by the improvement in soil health and nutrition for the plants (Shahbaz et al 2018). Soil textural types had no significant effect on protein content. However, high protein contents were recorded in cowpea seeds obtained from loamy sand than sandy loam soil. Loamy sand soil possesses good drainage capacities and excellent moisture retention, which is beneficial to crops (İç et al 2010).

Anthocyanin are a class of polyphenolic compounds widely distributed in plants in which they play a significant role in plant propagation and plant defence mechanisms against various abiotic and biotic stresses (Alappat and Alappat 2020; Liu et al 2018). Anthocyanin content was not significantly affected by the presence of AMF inoculation. These results are in disagreement with the findings of Zhang et al (2019) who reported that inoculation with AMF improves anthocyanin content and mainly acts as barriers and protectors against drought stress. The lack of *Arbscular Mycorrhizae fungus* inoculation effect could be attributed to the length and severity of drought stress which affects the colonisation of the fungus (Jongen et al 2022).

Moisture levels had a positive effect on anthocyanin content. Unexpectedly, highest anthocyanin contents were observed from moisture stressed treatments seeds than in adequate moisture treatments. This suggest that moisture stress favoured the accumulation of anthocyanin in seeds, perhaps to store molecules with a higher number of sugars as energy reservoirs during moisture stress conditions (Hinojosa-Gómez et al 2020). Similarly, these findings were also in agreement with Kamali and Mehraban (2020) and Haneef et al (2014) who documented improvements in anthocyanin contents under drought stress.

Soil amendments showed statistical difference on the contents of anthocyanin. Non-amended soil treatments (control) recorded high concentration of anthocyanin compared to amendment soil. These results do not correlate with the findings of Lui et al (2021) who documented that the application of compost and biochar results in an increase in the total anthocyanin contents. However, there were slight differences between individual amendment treatment combinations, making it difficult to compare data in literature. Treatment with more compost (25Bio:75Comp) recorded high amounts of anthocyanin and treatment with 50% of biochar and 50% compost recorded the least while the control remained the highest. Soil textural types had no positive influence on anthocyanin content. These results disagree with the findings of Cheng et al (2014) whereby rapid concentration of anthocyanin was observed in soil rich with sand similar, which is similar to sandy loam.

Flavonoids are defined as phenolic chemicals concentrated in seed coats that make up majority of colouring in grains (Anjos Barros et al 2020). AMF inoculation showed significant difference in the concentration of flavonoids, supporting the findings of Lu et al (2015). Interestingly, the highest contents of flavonoid were observed on non-inoculated seeds than in inoculated seeds, suggesting that the presence of AMF lowered flavonoid contents. Contrarily to these findings, Jerbi et al (2022) documented a significant increase in gran flavonoids following AMF inoculation in Barley (*Hordeum vulgare ssp. nudum L.*). Additionally, flavonoid concentration in plants inoculated with AMF regulate and maintain the developmental stage of Arbucular mycorrhorrhizal fungus symbiosis (Lu et al 2015). There was no significant effect influenced by moisture levels on flavonoid concentration. However, flavonoid concentrations found to be less in moisture stressed treatments compared to flavonoid concentration in adequate moisture treatments.

Moisture stress significantly reduced the amount flavonoids concentration, which confirmed the results of Gholinezhad and Darvishzadeh (2021). Contrary to this, numerous reports have documented that flavonoids concentrations tend to increase when crops suffer from environmental stresses such as drought (Kumar and Sharma 2018; Hodaeia et al 2018). However, secondary metabolites accumulations such as flavonoids are strongly depended on growing conditions which might have been a factor in influencing our end results (Pradhan et al 2017). Soil amendments showed a significant effect on the contents of flavonoids, supporting the findings of Kasmaei et al (2019). Amended soil treatment 50Bio:50Comp recorded a significant increase in flavonoids concentration with 0.44 G/dm compared to the control 0.29 G/dm. It has been reported that the integration of organic amendments (biochar and compost) favours accumulation of flavonoids which is a great indication of nutrient status (Bozzolo et al 2017; Salama et al 2015). Soil textural types showed no relevant variation on flavonoid concentration. However, previous research work from Lin et al (2010) described a positive increase in flavonoid concentration was observed on loam (loamy sand) soil compared to sandy (sandy loam) soil.

5.5.2 Total Soluble Sugar

Plants adapt to environmental stresses through various forms of adaptive strategies in response to these stresses such as altering their metabolic adjustments that results in the production of organic solutes such as sugars (Choudhary et al 2023). Total soluble sugars are components that perform a crucial function in the maintenance of water availability and osmotic adjustments in various crops exposed to adverse water limiting conditions (Dien et al 2019). In the current study, the application of AMF did not impact the total soluble sugars. These results contradict of Sheteiwy et al (2022) and Garg et al (2018), where they were reported that the introduction of AM fungi in the soil increased the concentration of total soluble sugars in chickpea seeds and peanuts, respectively. This is attributed by the ability of AMF to induce metabolic pathways which becomes beneficial to crops (Gao et al 2020; Begum et al 2019). Moisture levels and soil amendments were not significant on the total soluble sugars present in the seeds. Comparison showed that moisture stress recorded the lowest TSS values than in adequate moisture.

Soil moisture stress reduce the concentration of sugar in seed (Nacer 2012). According to reports, moisture stress has an impact on the translocation of total sugars due to impaired photosynthetic processes during the development stages (Wijewardana et al 2019; Nacer 2012). Unexpectedly, soil amendment rates treatments performed the least in influencing the concentration of total soluble sugars than the control, which recorded more. In contrast, Das et al (2022) detailed that seed soluble sugars increases in response to application of organic soil amendments. In addition, interaction between soil amendments and moisture levels exacted a significant impact. Treatment 50Bio:50Comp recorded the highest TSS values under moisture stress when compared to other soil amendment combinations including the control. Organic fertilisers under moisture limited conditions enhance relative water content in crop there by increasing the concentration of total soluble sugars (Roy et al 2022; Salehi et al 2016).

5.5.3 Treatments and their interaction effects on mineral composition of cowpea grain

Plant minerals are nutrients essential for the maximum development and functioning of humans and animals as they are required in cell and tissue development (Watts-Williams and Gilbert 2021 et al 2017). In the present study, the application of Mycoroot^{TM} inoculation had no significant effect on P, K, Zn, Fe, Cu, grain contents. In contrast, similar trends from previous research work reported significant improvements in grain nutrient P, K, Zn content following AMF inoculation application respectively (Mehmood et al 2022; Watts-William and Gilbert 2021). The AMF's mechanism for balancing and optimizing plant nutrients and Zn uptake was responsible for this improvement. However, according to Watts-Williams and Cavagnaro (2018) and Ercoli et al (2017) reported that seeds from mycorrhizal inoculated plants may have more or less Zn, Fe, or P than the non-mycorrhizal control. Inoculants are comprised of living organisms, their introduction in an environment occasionally causes unexpected effect on the soil, which affect nutrient uptake by plants (Koch et al 2011). According to Asiwe (2022) nutrient content in cowpea grains is reliant on the availability of soil nutrients for uptake suggesting that if soil is low in nutrient contents, that could result in low nutrient uptake by the growing plant thus limiting the nutrient concentration in the grain. Moisture levels showed no significant effect on K, Ca, Zn, Cu and Fe grain contents. Interestingly, moisture stressed treatment had significantly higher P contents. Grain nutrient is strongly impacted by drought, for instant drought conditions improved the concentration of P irrespective of the stress pattern whether gradual or sudden (Farooq et al 2018). The application of variable mix ratios of biochar-compost as soil amendments had no significant effect on all measured grain nutrients. Despite statistical deference of soil amendment ratios on Fe, Cu, Ca, K and P mineral composition, Zn content was found greater on soil amendment ratio 75Bio:25Comp than other soil amendment rates. These results concur with the findings of Lusiba et al (2021) who escribed the increase of Zn in chickpeas (*Cicer arietinum*) due to the improvement of pH by biochar.

5.6 Conclusion

In conclusion, results from the study revealed that Mycoroot^{TM} had no statistical effect on protein, anthocyanin and flavonoids. However, significant effects of Mycoroot^{TM} inoculation and its interaction with moisture levels were noticed. Under moisture stress, the application of Mycoroot^{TM} inoculation significantly increased protein and anthocyanin contents suggesting that Mycoroot^{TM} inoculation can alleviate negative effects of moisture stress, which, in turn, led to a better grain quality in terms of increased protein for solving malnutrition while increased anthocyanin contents can assist with better adaptation to harsh environments. Moreover, based on the results obtained, moisture stress increased P grain content compared to adequate moisture, which can be ascribed to the benefits of Mycoroot^{TM} inoculation as supported by literature. Soil amendment rate 50Bio:50Comp gave the highest flavonoids concentration suggesting that cowpea grain secondary metabolite was improved in amended soil. Zn was improved by soil amendment rate 75Bio25Comp. In this study, the hypothesis that sole and combined Mycoroot™ inoculation and variable biochar-compost mixtures will have a significant effect on cowpea mineral, protein and secondary metabolites content under two moisture regimes is hereby accepted. Integrated use of Mycoroot™ as inoculant containing native *Arbuscular mycorrhizae fungi* with 50Bio:50Comp mixture as soil amendment presents an appropriate agronomic approach that can assist farmers with improving cowpea protein, secondary metabolites and mineral contents in moisture deficient and low fertility soils.

References

Abdel-Fattah, G.M., and Asrar, A., (2012). Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum L.*) plants grown in saline soil. *Acta Physiologiae Plant* 34:267-277.

Adugna, G., (2016). A review on impact of compost on soil properties, water use and crop productivity. *Academic Research Journal of Agricultural Science and Research* 4:93-104.

African continent: a geospatial and long-term perspective. *Hydrology and Earth System Sciences* 18:3635-3649.

Al-Amri, S., (2023). Effect of presoaking various growth regulators on the yield and biochemical characteristics of cowpea plants (*Vigna sinensis L.*). *Journal of King Saud University-Science* 35:102385.

Alamu, E.O., Maziya-Dixon, B., Popoola, I., Gondwe, T., and Chikoye, D., (2016). Determination of quality traits, and the nutrient and mineral contents of Cowpea varieties in South Africa. *Journal of Food, Nutrition and Research* 4:664-670.

Alappat, B., and Alappat, J., (2020). Anthocyanin pigments: Beyond aesthetics. *Molecules* 25:5500.

Anjos Barros., N.V.D., Abreu, B.B.D., Sampaio da Silva, D.T., Soares, A.K.D.O., Rocha, M.D.M., and Reis Moreira-Araújo, R.S.D., (2020). Identification and Quantification of Phenolic Compounds in Grains of Biofortified Cowpea Cultivars, Before and After Cooking. *Current Nutrition and Food Science* 16:105-113.

Asiwe, J.N.A., (2022). Determination of quality traits, and the nutrient and mineral contents of Cowpea varieties in South Africa. *African Journal of Food, Agriculture, Nutrition and Development* 22:20331-20348.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N., and Zhang, L., (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in Plant Science* 10:1068.

Bozzolo, A., Pizzeghello, D., Cardinali, A., Francioso, O., and Nardi, A., (2017). Effects of moderate and high rates of biochar and compost on grapevine growth in a greenhouse experiment. *AIMS Agriculture and Food* 2:113-128.

Cheng, G., He, Y.N., Yue, T.X., Wang, J., and Zhang, Z.W., (2014). Effects of climatic conditions and soil properties on Cabernet Sauvignon berry growth and anthocyanin profiles. *Molecules* 19:13683-13703.

Choudhary, S., Wani, K.I., Naeem, M., Khan, M.M.A., and Aftab, T., (2023). Cellular responses, osmotic adjustments, and role of osmolytes in providing salt stress resilience in higher plants: polyamines and nitric oxide crosstalk. *Journal of Plant Growth Regulation* 42:539-553.

Das, S.K., Ghosh, G.K., and Avasthe, R., (2022). Biochar and organic manures on produce quality, energy budgeting, and soil health in maize-black gram system. *Arabian Journal of Geosciences* 15:1-15.

Delavaux, C.S., Smith-Ramesh, L.M., and Kuebbing, S.E., (2017). Beyond nutrients: a metaanalysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* 98:2111-2119.

Dien, D.C., Mochizuki, T., and Yamakawa, T., (2019). Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in Rice (*Oryza sativa L.*) varieties. *Plant Production Science* 22:530-545.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., and Zheng, B., (2016). Biochar to improve soil fertility. A review. *Agronomy Sustainable Development* 36:36.

Diouf, D., (2011). Recent advances in cowpea (*Vigna unguiculata L. Walp*) "omics" research for genetic improvement. *African Journal of Biotechnology* 10:2803-2810.

El-Sawah, A.M., El-Keblawy, A., Ali, D.F.I., Ibrahim, H.M., El-Sheikh, M.A., Sharma, A., Alhaj Hamoud, Y., Shaghaleh, H., Brestic, M., Skalicky, M., and Xiong, Y.C., (2021). Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria enhance soil key enzymes, plant growth, seed yield, and qualitative attributes of guar. *Agriculture* 11:194.

Ercoli, L., Schüßler, A., Arduini, I., and Pellegrino, E., (2017). Strong increase of durum wheat iron and zinc content by field-inoculation with arbuscular mycorrhizal fungi at different soil nitrogen availabilities. *Plant and Soil* 419:153-167.

Etienne, P., Diquelou, S., Prudent, M., Salon, C., Maillard, A., and Ourry, A., (2018). Macro and micronutrient storage in plants and their remobilization when facing scarcity: the case of drought. *Agriculture* 8:14.

Farooq, M., Hussain, M., Usman, M., Farooq, S., Alghamdi, S.S., and Siddique, K.H., (2018). Impact of abiotic stresses on grain composition and quality in food legumes. *Journal of Agricultural and Food Chemistry* 66:8887-8897.

Fileccia, V., Ruisi, P., Ingraffia, R., Giambalvo, D., Frenda, A.S., and Martinelli, F., (2017). Arbuscular mycorrhizal symbiosis mitigates the negative effects of salinity on durum wheat. *Plos One* 12:0184158.

Gagné-Bourque, F., Bertrand, A., Claessens, A., Aliferis, K. A., and Jabaji, S., (2016). Alleviation of drought stress and metabolic changes in timothy (*Phleum pratense L.*) colonized with Bacillus subtilis B26. *Frontiers in Plant Science* 7:584.

Gao, C., El-Sawah, A.M., Ali, D.F.I., Alhaj Hamoud, Y., Shaghaleh, H., and Sheteiwy, M.S., (2020). The integration of bio and organic fertilizers improve plant growth, grain yield, quality and metabolism of hybrid maize (*Zea mays L.). Agronomy* 10:319.

Garg, N., and Bharti, A., (2018). Salicylic acid improves arbuscular mycorrhizal symbiosis, and chickpea growth and yield by modulating carbohydrate metabolism under salt stress. *Mycorrhiza 28*:727-746.

Ghanbari, A.A., Mousavi, S.H., Gorji, A.M., and Idupulapati, R.A.O., (2013). Effects of water stress on leaves and seeds of bean (*Phaseolus vulgaris L.*). *Turkish Journal of Field Crops* 18:73-77.

Gholinezhad, E., and Darvishzadeh, R., (2021). Influence of arbuscular mycorrhiza fungi and drought stress on fatty acids profile of sesame (*Sesamum indicum L.*). *Field Crops Research* 262:108035.

Habibzadeh, Y., Pirzad, A., Zardashti, M.R., Jalilian, J., and Eini, O., (2013). Effects of arbuscular mycorrhizal fungi on seed and protein yield under water-deficit stress in mung bean. *Agronomy Journal* 105:79-84.

Haddad, S.A., Mowrer, J., and Thapa, B., (2022). Biochar and compost from cotton residues inconsistently affect water use efficiency, nodulation, and growth of legumes under arid conditions. *Journal of Environmental Management* 307:114558.

Hagemann, N., Subdiaga, E., Orsetti, S., de la Rosa, J.M., Knicker, H., Schmidt, H.P., Kappler, A., and Behrens, S., (2018). Effect of biochar amendment on compost organic matter composition following aerobic composting of manure. *Science of the Total Environment* 613:20-29.

Haneef, I., Faizan, S., Perveen, R., and Kausar, S., (2014). Impact of bio-fertilizers and different levels of cadmium on the growth, biochemical contents and lipid peroxidation of Plantago ovata Forsk. *Saudi Journal of Biological Sciences* 21:305-310.

Harmankaya, M., Ceyhan, E., Çelik, A.S., Sert, H., Kahraman, A., and Özcan, M.M., (2016). Some chemical properties, mineral content and amino acid composition of cowpeas (*Vigna sinensis (L.) Savi*). *Quality Assurance and Safety of Crops and Foods* 8:111-116.

Hinojosa-Gómez, J., San Martín-Hernández, C., Heredia, J.B., León-Félix, J., Osuna-Enciso, T., and Muy-Rangel, M.D., (2020). Anthocyanin induction by drought stress in the calyx of roselle cultivars. *Molecules* 25:1555.

Hodaeia, M., Rahimmaleka, M., Arzania, A., and Talebi, M., (2018). The effect of water stress on phytochemical accumulation, bioactive compounds and expression of key genes involved in flavonoid biosynthesis in *Chrysanthemum morifolium L. Industrial Crops and Products* 120:295-304.

İç, S., Gülser, C., Candemir, F., and Demir, Z., (2010). Effects of plant growth on some physical properties of different textured soils. In Proceedings of the International Soil Science Congress on Management of Natural Resources to Sustain Soil Health and Quality. Kizilkaya, R., Gülser, C., Dengiz, O., 1072-1077.

Ingraffia, R., Amato, G., Frenda, A.S., and Giambalvo, D., (2019). Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. *Plos One* 14:0213672.

Jerbi, M., Labidi, S., Laruelle, F., Tisserant, B., Jeddi, F.B., and Sahraoui, A.L.H., (2022). Mycorrhizal biofertilization improves grain yield and quality of hulless Barley (*Hordeum vulgare ssp. nudum L.*) under water stress conditions. *Journal of Cereal Science* 104:103436.

Jongen, M., Albadran, B., Beyschlag, W., and Unger, S., (2022). Can arbuscular mycorrhizal fungi mitigate drought stress in annual pasture legumes?. *Plant and Soil* 472:295-310.

Joseph, S., Cowie, A.L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M.L., Graber, E.R., Ippolito, J.A., Kuzyakov, Y., and Luo, Y., (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *Gcb Bioenergy* 13:1731-1764.

Kamali, S., and Mehraban, A., (2020). Nitroxin and arbuscular mycorrhizal fungi alleviate negative effects of drought stress on Sorghum bicolor yield through improving physiological and biochemical characteristics. *Plant Signaling and Behavior* 15:1813998.

Kasmaei, L.S., Yasrebi, J., Zarei, M., Ronaghi, A., Chasemi, R., Saharkhiz, M.J., Ahmadabadi, Z., and Schnug, E., (2019). Impacts of PGPR, Compost and Biochar of Azolla on Dry Matter Yield, Nutrient Uptake, Physiological Parameters and Essential Oil of Rosmarinus Officinalis L. J. Kult 71:3-13.

Koch, A.M., Antunes, P.M., Barto, E.K., Cipollini, D., Mummey, D.L., and klironomos, J.N., (2011). The effects of arbuscular mycorrhizal (AM) fungal and garlic mustard introductions on native AM fungal diversity. *Biological Invasions* 13:1627-1639.

Kumar, I., and Sharma, R.K., (2018). Production of secondary metabolites in plants under abiotic stress: an overview. *Significances of Bioengineering and Biosciences* 2:196-200.

Kumar, R., Rathore, D.K., Singh, M., Kumar, P., and Khippal, A., (2016) Effect of phosphorus and zinc nutrition on growth and yield of fodder cowpea. *Legume Research-An International Journal* 39:262-267.

Lin, J.T., Liu, S.C., Tsay, G.J., and Yang, D.J., (2010). Composition of flavonoids and phenolic acids in Glycin tomentella Hayata cultivated in various soils. *Food Chemistry* 121:659-665.

Liu, Y., Tikunov, Y., Schouten, R.E., Marcelis, L.F., Visser, R.G., and Bovy, A., (2018). Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: A review. *Frontiers in Chemistry* 6:52.

Lu, F.C., Lee, C.Y., and Wang, C.L., (2015). The influence of arbuscular mycorrhizal fungi inoculation on yam (*Dioscorea spp*.) tuber weights and secondary metabolite content. *Peer Review Journal* 3:1266.

Lui, J.H., Schulz, S., Brandl, H,M., Miehtke, B., Huwe, B., and Glaser, B., (2012). Short-term effect of biochar and compost on soil fertility and water status of a Dystric cambisol in NE Germany under field conditions. *Journal of Plant Nutrition and Soil Science* 10:698-707.

Lusiba, S.G., Odhiambo, J.J.O, Adeleke, R., and Maseko S.T., (2021). The potential of biochar to enhance concentration and utilization of selected macro and macro nutrients for chickpea (*Cicer arientinum*) grown in three contrasting soils. *Rhizosphere* 17:100289.

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

Maleki, A., Naderi, A., Naseri, R., Fathi, A., Bahamin, S., and Maleki, R., (2013). Physiological performance of soybean cultivars under drought stress. *Bulletin of Environment, Pharmacology and Life Sciences* 2:38-44.

Maphosa, Y., and Jideani, V.A. (2017). The role of legumes in human nutrition. *Functional Food-improve Health Through Adequate Food* 1:13

Maseko, K.H., Regnier, T., Anyasi, T.A., Du Plessis, B., Da Silva, L.S., Kutu, F.R., and Wokadala, O.C., (2022). Discrimination of Musa banana genomic and sub-genomic groups based on multi-elemental fingerprints and chemometrics. *Journal of Food Composition and Analysis* 106:104334.

Masih, I., Maskey, S., Mussá, F.E.F., and Trambauer, P., (2014). A review of droughts on the

Mee, C.Y., Balasundram, S.K., and Hanif, A.H.M., (2017). Detecting and monitoring plant nutrient stress using remote sensing approaches: A review. *Asian Journal of Plant Sciences* 16:1-8.

Mehmood, H., Ali, M.A., Hussain, S., Baig, K.S., Sultan, H., Hasan Naqvi, S.A., Shahid, M.N., Ali, S., Alhomaidi, E.A., and Datta, R., (2022). Sole and combined effect of foliar zinc and arbuscular mycorrhizae inoculation on basmati rice growth, productivity and grains nutrient. *Plos One* 17:0266248.

Mukhongo, R.W., Tumuhairwe, J.B., Ebanyat, P., AbdelGadir, A.H., Thuita, M., and Masso, C., (2016). Production and use of arbuscular mycorrhizal fungi inoculum in sub-Saharan Africa: challenges and ways of improving. *International Journal of Soil Science* 11:108-122.

Nacer, B., (2012). Soybean seed phenol, lignin, and isoflavones and sugars composition altered by foliar boron application in soybean under water stress. *Food and Nutrition Sciences* 2012.

Nkomo, G.V., Sedibe, M.M., and Mofokeng, M.A., (2021). Production constraints and improvement strategies of cowpea (*Vigna unguiculata L. Walp.)* genotypes for drought tolerance. *International Journal of Agronomy* 2021:1-9.

Novotny, E.H., Maia, C.M.B.F., Carvalho, M.T.M., Madari, B.E., (2015). Biochar: pyrogenic carbon for agricultural use–a critical review. *Revista Brasileira Ciência Do Solo* 39:321-344.

Okonya, J.S., and Maass, B.L., (2014). Protein and iron composition of cowpea leaves: An evaluation of six cowpea varieties grown in Eastern Africa. *African Journal of Food, Agriculture, Nutrition and Development* 14:9329-9340.

Oliveira, R.S., Carvalho, P., Marques, G., Ferreira, L., Nunes, M., Rocha, I., Ma, Y., Carvalho, M.F., Vosátka, M. and Freitas, H., (2017). Increased protein content of chickpea (*Cicer arietinum L.*) inoculated with arbuscular mycorrhizal fungi and nitrogen-fixing bacteria under water deficit conditions. *Journal of the Science of Food and Agriculture* 13:4379-4385.

Omenna, E.C., Olanipekun, O.T., and Kolade, R.O., (2016). Effect of boiling, pressure cooking and germination on the nutritional and antinutrients content of cowpea (*Vigna unguiculata*). *ISABB Journal of Food and Agricultural Sciences* 6:1-8.

Owade, J.O., Abong, G., Okoth, M., Mwang'ombe, A.W., (2020). A review of the contribution of cowpeas leaves to food and nutrition security in East Africa. *Food Science and Nutrition* 8:36-47.

Pereira, E.J., Carvalho, L.M., Dellamora-Ortiz, G.M., Cardoso, F.S., Carvalho, J.L., Viana, D.S., Freitas, S.C., and Rocha, M.M., (2014). Effects of cooking methods on the iron and zinc contents in cowpea (*Vigna unguiculata*) to combat nutritional deficiencies in Brazil. *Food and Nutrition Research* 58:20694.

Petter, F.A., and Madari, B.E., (2012). Biochar: agronomic and environmental potential in Brazilian savannah soils. *Revista Brasileira de Engenharia Agricola Ambiental* 16:761-768.

Pradhan, J., Sahoo, S.K., Lalotra, S., and Sarma, R.S., (2017). Positive impact of abiotic stress on medicinal and aromatic plants. *International Journal of Plant Sciences (Muzaffarnagar)* 12:309-313.

Qiao, X., Bei, S., Li, C., Dong, Y., Li, H., Christie, P., Zhang, F., and Zhang, J., (2015). Enhancement of faba bean competitive ability by arbuscular mycorrhizal fungi is highly correlated with dynamic nutrient acquisition by competing wheat. *Scientific Reports* 5:1-10.

Qu, X., Wang, H., Chen, M., Liao, J., Yuan, J., Niu, G., (2019). Drought stress-induced physiological and metabolic changes in leaves of two oil tea cultivars. *Journal of the American Society for Science* 144:439-447.

Ramakrishna, A., and Ravishankar, G.A., (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behaviour* 6:1720-1731.

Ramzani, P.M.A., Shan, L., Anjum, S., Ronggui, H., Iqbal, M., Virk, Z.A. and Kausar, S., (2017). Improved quinoa growth, physiological response, and seed nutritional quality in three soils having different stresses by the application of acidified biochar and compost. *Plant physiology and biochemistry* 116:127-138.

Rao, A., Ahmad, S.D., Sabir, S.M., Awan, S.I., Shah, A.H., Abbas, S.R., Shafique, S., Khan, F. and Chaudhary, A., (2013). Potential antioxidant activities improve salt tolerance in ten varieties of wheat (*Triticum aestivum L.). American Journal of Plant Sciences* 4:69-76.

Ritte, I.P., Egnin, M., Idehen, O., Mortley, D., Bernard, G.C., Binagwa, P.H., Brown, A.P., and Bonsi, C.K., (2022). Evaluation of Cowpea Morpho-physiological and Yield Responses to Vegetative and Pre-Anthesis Water-Deficit Stress Tolerance under Greenhouse Conditions. *European Journal of Applied Sciences* 10:391-411.

Roy, R., Núñez-Delgado, A., Wang, J., Kader, M.A., Sarker, T., Hasan, A.K., and Dindaroglu, T., (2022). Cattle manure compost and biochar supplementation improve growth of Onobrychis viciifolia in coal-mined spoils under water stress conditions. *Environmental Research* 205:112440.

Salama, Z.A., El Baz, F.K., Gaafar, A.A., and Zaki, M.F., (2015). Antioxidant activities of phenolics, flavonoids and vitamin C in two cultivars of fennel (*Foeniculum vulgare Mill.*) in responses to organic and bio-organic fertilizers. *Journal of the Saudi Society of Agricultural Sciences* 14:91-99.

Salehi, A., Tasdighi, H., and Gholamhoseini, M., (2016). Evaluation of proline, chlorophyll, soluble sugar content and uptake of nutrients in the German chamomile (*Matricaria chamomilla L.*) under drought stress and organic fertilizer treatments. *Asian Pacific Journal of Tropical Biomedicine* 6:886-891.

Sanjeev, K., Deshpande, B.R., Mani, S.A., Desai, T.K., Nagarathna and Hanchinal, R.R., (2018). Review on Characterization of Cowpea Germplasm in Terms of Distinctness, Uniformity, Stability and Novelty for Morphological, Quality and Yield Attributing Parameters. *International Journal of Current Microbiology and Applied Sciences* 7:1124-1139.

Shahbaz, A.K., Lewinska, K., Iqbal, J., Ali, Q., Iqbal, M., Abbas, F., Tauqeer, H.M., and Ramzani, P.M.A., (2018). Improvement in productivity, nutritional quality, and antioxidative defense mechanisms of sunflower (*Helianthus annuus L.*) and maize (*Zea mays L.*) in nickel contaminated soil amended with different biochar and zeolite ratios. *Journal Environmental Management* 218:256-270.

Sharma, K., Gupta, S., Thokchom, S.D., Jangir, P., and Kapoor, R., (2021). Arbuscular mycorrhiza-mediated regulation of polyamines and aquaporins during abiotic stress: deep insights on the recondite players. *Frontiers in Plant Science* 12:1072.

Sheteiwy, M.S., Ahmed, M., Korany, S.M., Alsherif, E.A., Mowafy, A.M., Chen, J., Jośko, I., Selim, S., and AbdElgawad, H., (2022). Arbuscular Mycorrhizal Fungus “Rhizophagus irregularis” impacts on physiological and biochemical responses of ryegrass and chickpea plants under beryllium stress. *Environmental Pollution* 315:120356.

Shishehbor, P., and Hemmati, S.A., (2022). Investigation of secondary metabolites in bean cultivars and their impact on the nutritional performance of Spodoptera littoralis (Lep.: Noctuidae). *Bulletin of Entomological Research* 112:378-388.

Shojaie, B., Mostajeran, A., and Ghanadian, M., (2016). Flavonoid dynamic responses to different drought conditions: Amount, type, and localization of flavonols in roots and shoots of Arabidopsis thaliana L. *Turkish Journal of Biology* 40:612-622.

Singh, R., and Das, A., (2022). Growth, yield and phosphorus use efficiency of vegetable cowpea (*vigna unguiculata*) varieties as influenced by phosphorus levels under rainfed conditions of semi-arid environment. *Journal of Experimental Agriculture International* 44:28-37.

Soka, G., and Ritchie, M., (2014). Arbuscular mycorrhizal symbiosis and ecosystem processes: Prospects for future research in tropical soils. *Open Journal of Ecology* 2014.

Spokas, K.A., Cantrell, K.B., Novak, J.M., Archer, D.W., Ippolito, J.A., Collins, H.P., Boateng, A.A., Lima, I.M., Lamb, M.C., McAloon, A.J., Lentz, R.D., and Nichols, K.A., (2012). Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *Journal of Environmental Quality* 41:973-989.

Tiwari, R., and Rana, C.S., (2015). Plant secondary metabolites. *International Journal of Engineering Research and General Science* 3:2091-2730.

Watts-Williams, S.J., and Cavagnaro, T.R., (2018). Arbuscular mycorrhizal fungi increase grain zinc concentration and modify the expression of root ZIP transporter genes in a modern barley (*Hordeum vulgare*) cultivar. *Plant Science* 274:163-170.

Watts-Williams, S.J., and Gilbert, S.E., (2021). Arbuscular mycorrhizal fungi affect the concentration and distribution of nutrients in the grain differently in barley compared with wheat. *Plants, People, Planet* 3:567-577.

Wijewardana, C., Alsajri, F.A., Irby, J.T., Krutz, L.J., Golden, B.R., Henry, W.B., and Reddy, K.R., (2019). Water deficit effects on soybean root morphology and early-season vigor. *Agronomy* 9:836.

Wu, M., Guo, X., Wu, J., and Chen, K., (2020). Effect of compost amendment and bioaugmentation on PAH degradation and microbial community shifting in petroleumcontaminated soil. *Chemospher*e 256:126998.

Yadav, A.N., (2021). Microbial biotechnology for bio-prospecting of microbial bioactive compounds and secondary metabolites. *Journal of Applied Biology and Biotechnology* 9:1-6.

Yadav, V., Karak, T., Singh, S., Singh, A.K., and Khare, P., (2019). Benefits of biochar over other organic amendments: Responses for plant productivity (*Pelargonium graveolens L.*) and nitrogen and phosphorus losses. *Industrial Crops and Products* 131:96-105.

Zhang, Y., Li, Y., Li, W., Hu, Z., Yu, X., Tu, Y., Zhang, M., Huang, J., and Chen, G., (2019). Metabolic and molecular analysis of non-uniform anthocyanin pigmentation in tomato fruit under high light. *Horticulture Research* 6:1-21.

CHAPTER 6

General Summary, Conclusion and Recommendations

6.1 Introduction

Cowpea is a nutrient-rich leguminous crop cultivated in most tropical and sub-tropical regions (Weng et al 2019). The crop plays significant roles in meeting the diets of humans and animals as sources of dietary proteins, vitamins and minerals (Owade et al 2020; Ibro et al 2014). It is a very resilient crop, recognised for surviving and performing well under challenging agricultural conditions such as moisture stress (Olorunwa at al 2021). However, moisture stress and low soil fertility enhance the decrease in cowpea grain yield and the overall productivity including grain quality (Boukar et al 2019; Ahmadi et al 2015). Moisture stress has ability to alter plant growth, development and metabolic processes and ultimately affect the quality and yields of crops, thus constituting a threat to food security (Islam et al 2011). It is an important abiotic factor, which limits cowpea growth and yield notwithstanding its drought tolerant ability. In order to address this threat and the resultant problem, we initiate a study to investigate the potential of improved agronomic practice as a management strategy. Mycoroot^{TM}, an inoculant product containing local (South African) strains of AMF reported to possess the potential to enhance crop growth, and yield, is among many inputs used for crop production. AMF are soil microorganisms that participate in water and nutrient acquisition in plants under various stresses such as drought (Lanfranco et al 2018). Similarly, both biochar and compost are considered as excellent soil amendments that improve water holding capacity and plant available water content (Yeboah et al 2020; Lui et al 2012). Given the benefits associated with integrated natural resource use, this study investigated the integrated use of Mycoroot^{TM} inoculation with different ratios of biochar-compost mixtures as an agronomic package as a potential adaptive strategy to assist potential cowpea growers to overcome the challenges of low yields brought by moisture stress under different soil conditions.

6.2 Research organisation

The greenhouse pot trial planted at the UMP experimental farm during January to April 2022, examined the effect of combined Mycoroot^{TM} inoculation with varied biochar-compost mix ratios on the growth, yield and yield attributes of cowpea grown on two soils with distinct textural characteristics and moisture regimes. The mineral, protein, secondary metabolites and total soluble sugars content of the harvested cowpea grains were chemically analysed under laboratory condition.

6.3 Main findings of the study

a. Results of pre-planting soil analysis revealed that the soil textural classes are sandy loam and loamy sand soil. The measured pH value was 6.80 (loamy sand) and 6.27 (sandy loam) suggesting that both soils are slightly acidic. The measured available P level in sandy loam of 13.51 mg kg^{-1} was medium in adequacy (Buchholz et al 2004) while the loamy sand value of 5.86 mg kg^{-1} was below the critical level of 8 mg kg^{-1} considered adequate to support plant growth and development (FSSA 2016).

b. The results of the greenhouse trail revealed the following:

  - Soil textural types exerted a positive ($p<0.05$) on growth parameters. Results revealed that loamy sand soil managed to enhance growth parameters.
  - Sole application of Mycoroot^{TM} inoculation managed to show variation in leaf length at reproductive stage. Moreover, its interaction with moisture levels and soil amendments exerted a positive effect ($p<0.05$) on plant height and chlorophyll content at vegetative and physiological maturity stage.
  - Application of Mycoroot^{TM} inoculation and soil amendments had inconsequential effect on the mean number of days to flowering. However, the interaction between Mycoroot^{TM} inoculation and soil types had significant effect on the mean number of days to flowering of the cowpea plant with the highest number of days to flowering recorded in loamy sand soil following Mycoroot^{TM} inoculation.
  - The variation in soil moisture levels had significant ($p<0.05$) effect on all measured yield parameters except root dry weight while differences in soil textural types significantly ($p<0.05$) influenced all measured yield parameters.
  - Moisture stress condition significantly lowered stomatal conductance resulting in a significant reduction in yield attributes under loamy sand soil.
  - Cowpea grain P, K, Ca, Zn, Cu and Fe content did not respond to any of the Mycoroot^{TM} inoculation, variation in soil textural types and soil amendments treatments. However, moisture levels and its interaction with Mycoroot^{TM} inoculation had a positive effect ($p<0.05$) on P content. Similarly, application of a mixture of 75% Biochar and 25% Compost as soil amendment resulted increases in the Zn content of cowpea grain.

- Both anthocyanin and proteins contents of cowpea grains increased significantly following Mycoroot^{TM} inoculation but lowered the flavonoids contents.

- Integrated use of Mycoroot^{TM} with varied biochar-compost mix ratio (50:50) resulted in positive increase in the mean number of trifoliate leaves and leaf length at the vegetative stage while the integrated Mycoroot^{TM} use with 25Bio75Comp treatment resulted in increased chlorophyll content at physiological stage.

- The different soil amendments exerted significant ($p<0.05$) effect on anthocyanin and flavonoids but had insignificant effects on protein and TSS content. Soil amendments containing 50% and more compost in the mix ratios (i.e. 25:75 and 50:50; biochar: compost) gave elevated amount of anthocyanin and flavonoids, respectively.

- Based on the results of this study, the hypothesis that sole and combined Mycoroot™ inoculation and variable biochar-compost mixtures has no effect on cowpea growth and yield attributes with and without moisture stress is hereby rejected. Results of the laboratory analysis undertaken indeed confirmed that the mineral, protein and secondary metabolites content of cowpea grown on the two soil textural types under different moisture regimes differed significantly.

6.4 General conclusion

- This study revealed that extended period of moisture stress affected cowpea growth, yield and yield attributes under diverse soil conditions suggesting that managing moisture stress through appropriate agronomic practice is critical to promoting increased and sustainable cowpea production.
- Interaction between Mycoroot^{TM} inoculation and moisture levels had a significant effect on growth parameters. Under moisture stress, Mycoroot^{TM} inoculation increased leaf length, plant height and number of trifoliate leaves. Moreover, protein and anthocyanin grain contents increased by Mycoroot^{TM} inoculation under moisture stress conditions.

- Although Mycoroot^{TM} inoculation had inconsequential effect on cowpea grain yield, enhanced such yield attributes as pod length, number of cavities per pod, pod dry weight, haulm weight and fodder weight.

- Soil amendment with 75% biochar:25% compost mix ratio produced the tallest plants and highest leaf length under loamy sand soil as well as improved Zn grain content in loamy sand soil. The 50% biochar:50% compost mix ratio promoted increase flavonoid content in cowpea grain.

- Integrated use of Mycoroot^{TM} inoculation with appropriate biochar and compost mix ratio offers an effective technique that can relieve cowpea moisture stress and, prevent excessive use of expensive synthetic fertilizer. Additionally, such integrated soil-crop management practice if adopted by small-scale farmers will constitute an improved agronomic technique that can assist small-scale farmers to guarantee increase and sustainable cowpea production under dryland and nutrient-deficient soil.
- Improving poor soil fertility and alleviating moisture stress through the integrated use of biochar, compost and local strain of AMF (e.g. Mycoroot™) enhanced cowpea growth, yield, protein, secondary metabolites and mineral composition.

6. 5 Recommendations

The results of the study indicate that the use of Mycoroot™ inoculation with variable biocharcompost mix ratios can be an effective and inexpensive technique to enhance cowpea growth and yield attributes such as protein and anthocyanin. Furthermore, the various soil amendments evaluated were able to improve mineral and Zn as well as anthocyanin and flavonoid contents under limited soil moisture conditions. The results underscore the potential use of biocharcompost mix to promote profitable pot production of high value vegetable and grain legume crops such as cowpea. However, we recommend a field validation of these findings under different soil and climatic conditions as part of the process for upscaling the practice. The study illustrates the need for identification of more local AMF strains available on diverse soil textural types and climatic conditions in South Africa to assess the efficiency as a cost-effective and low-input agricultural product for promoting increase and sustainable cowpea production under diverse soil and growing conditions.

References

Ahmadi, S.A.K., Ebadi, A., Daneshian, J., Jahanbakhsh, S., Siadat, S.A., and Tavakoli, H., (2015). Effects of irrigation deficit and application of some growth regulators on defense mechanisms of canola. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 43:124-130.

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., and Fatokun, C., (2019). Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding* 138:415-424.

Buchholz, D.D., Brown, J.R., Garret, J., Hanson, R., and Wheaton, H., (2004). Soil test interpretations and recommendations handbook. Columbia, United States: University of Missouri-College of Agriculture, Division of Plant Sciences.

Fertilizer Association of Southern Africa Handbook (FSSA)., (2016). Seventh revised edition. ISBN0-909071-86-1 471.

Ibro, G., Sorgho, M.C., Idris, A.A., Moussa, B., Baributsa, D. and Lowenberg-DeBoer, J., (2014). Adoption of cowpea hermetic storage by women in Nigeria, Niger and Burkina Faso. *Journal of Stored Products Research* 58:87-96.

Islam, M.S., Akhter, M.M., EL Sabagh, A., Liu, L.Y., Nguyen, N.T., Ueda, A., and Saneoka, H., (2011): Comparative studies on growth and physiological responses to saline and alkaline stresses of Foxtail millet (*Setaria italica L.*) and Proso millet (*Panicum miliaceum L.*). *Australian Journal of Crop Science* 5:1269-1277.

Lanfranco, L., Fiorilli, V., and Gutjahr, C., (2018). Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. *New Phytologist* 220:1031-1046.

Lui, J.H., Schulz, S., Brandl, H,M., Miehtke, B., Huwe, B., and Glaser, B., (2012). Short-term effect of biochar and compost on soil fertility and water status of a Dystric cambisol in NE Germany under field conditions. *Journal of Plant Nutrition and Soil Science* 10:698-707.

Olorunwa, O.J., Shi, A. and Barickman, T.C., (2021). Varying drought stress induces morphophysiological changes in cowpea (*Vigna unguiculata L.*) genotypes inoculated with *Bradyrhizobium japonicum*. *Plant Stress* 2:100033.

Owade, J.O., Abong, G., Okoth, M., and Mwang'ombe, A.W., (2020). A review of the contribution of cowpeas leaves to food and nutrition security in East Africa. *Food Science and Nutrition* 8:36-47.

Weng, Y., Qin, J., Eaton, S., Yang, Y., Ravelombola, W.S. and Shi, A., (2019). Evaluation of seed protein content in USDA cowpea germplasm. *HortScience* 54:814-817.

Yeboah, E., Asamoah, G., Ofori, P., Amoah, B., and Agyeman, K.O.A., (2020). Method of biochar application affects growth, yield and nutrient uptake of cowpea. *Open Agriculture* 1:352-360.