

**EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND *Trichoderma*
spp. ON NUTRIENT UPTAKE AND WATER STRESS TOLERANCE IN
Vigna unguiculata (L.) Walp. (COWPEA)**

**JUMA DANIEL WANJALA (B. Ed)
I56/28453/2018**

**“A Thesis submitted in Partial Fulfillment of the Requirements for the Award
of the Degree of Master of Science (Plant Physiology and Biochemistry) in the
School of Pure and Applied Sciences of Kenyatta University”**

JULY 2024

DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other University or for any award.

.....

.....

Daniel Wanjala Juma
I56/28453/2018
Department of Plant Sciences

Date

SUPERVISORS

We confirm that the candidate under our supervision carried out the work reported in this thesis.

.....

.....

Dr. Adelaide Mutune
Department of Plant Sciences
Kenyatta University

Date

.....

.....

Dr. Ezekiel Mugendi Njeru
Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

Date

DEDICATION

I dedicate this work to my loving parents, Mary and Maurice, to my daughters Danelle and Danette and to my supportive wife, Aldrine.

ACKNOWLEDGEMENTS

Firstly, I thank the Almighty God for the enablement to complete this project successfully. Secondly, I convey my deepest gratitude to my Supervisors; Dr. Adelaide Mutune and Dr. Ezekiel Mugendi Njeru for their availability, support and crucial guidance that enabled me successfully undertake this project. I also thank Morris Muthini of the Tissue Culture laboratory, Kenyatta University, who assisted me in fungal culture preparations, dissemination, green house activities and collection of data in the course of this project.

I also take note of Mercy, Caroline, Richard, Lilian, Kibet, Maureen and Ng'ang'a who provided expertise knowledge and support at the initial and later stages of my project work.

My gratitude also goes to my postgraduate colleagues including Eva, Hilda, Edith, Peter and Titus for their invaluable support.

Finally yet importantly, I thank my entire family for their prayers and moral encouragement for the success of this study. Be blessed.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF PLATES	xii
ACRONYMS AND ABBREVIATIONS	xiii
ABSTRACT	xiv
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background of the study	1
1.2 Statement of the problem	3
1.3 Justification	4
1.4 Research Hypotheses	6
1.5 General objective	6
1.5.1 Specific objectives.....	6
1.6 Significance of the study.....	7
CHAPTER TWO	8
LITERATURE REVIEW	8
2.1 Fungal ecology in the rhizosphere	8
2.1.1. Arbuscular mycorrhizal fungi	8
2.1.2. <i>Trichoderma</i> spp.....	9
2.2 Fungal role in plant growth and alleviation of water stress	10
2.2.1. Arbuscular mycorrhizal fungi	10
2.2.2. <i>Trichoderma</i> spp.....	11
2.2.3. The concept of co-inoculation of fungal strains	13
2.3 Arbuscular mycorrhizal fungi, <i>Trichoderma</i> spp. and cowpea production ..	14

2.4 Cultivation and distribution of cowpea.....	16
2.5 Constraints in cowpea productivity.....	19
2.6 Water stress and nutrition on plant growth.....	21
CHAPTER THREE.....	22
MATERIALS AND METHODS.....	22
3.1 Experimental design and treatments.....	22
3.2 Soil collection, preparation and analysis.....	22
3.3 Soil physicochemical properties.....	23
3.3.1 Soil pH.....	23
3.3.2 Available nutrient elements.....	23
3.3.3 Total soil organic carbon.....	24
3.3.4 Determination of nitrogen.....	24
3.3.5 Exchangeable acidity.....	24
3.3.6 Available trace elements.....	25
3.4 Preparation of fungal inocula.....	25
3.5 Fungal inoculation and plant growth conditions.....	26
3.6 Determination of water deficits in the pots.....	27
3.7 Planting material.....	28
3.8 Chlorophyll estimates.....	29
3.9 Relative Water content of the leaf.....	30
3.10 Leaf area.....	31
3.11 Specific leaf area.....	31
3.12 Plant height and root length.....	31
3.13 Stem diameter.....	32
3.14 Determination of the dry mass.....	32
3.15 Root mycorrhizal colonization.....	32
3.16 Nutrient analysis.....	33
3.17 Data analysis.....	33

CHAPTER FOUR	34
RESULTS	34
4.1 Soil physicochemical analysis	34
4.2 Influence of AMF and <i>Trichoderma</i> spp. on growth and water uptake of cowpea at different soil water status	35
4.2.1 Influence of AMF and <i>Trichoderma</i> spp. on shoot dry weight.....	35
4.2.2. Influence of AMF and <i>Trichoderma</i> spp. on root dry weight	37
4.2.3 Influence of AMF and <i>Trichoderma</i> spp. on root length	39
4.2.4 Influence of AMF and <i>Trichoderma</i> spp. on stem diameter	41
4.2.5 Influence of AMF and <i>Trichoderma</i> spp. on leaf area	45
4.2.6 Influence of AMF and <i>Trichoderma</i> spp. on relative water content	47
4.2.7 Influence of AMF and <i>Trichoderma</i> spp. on specific leaf area.....	50
4.2.8 Influence of AMF and <i>Trichoderma</i> spp. on cowpea height	53
4.2.9 Influence of AMF and <i>Trichoderma</i> spp. on cowpea chlorophyll content	56
4.3 Influence of AMF and <i>Trichoderma</i> spp. on nutrient uptake in cowpea at different soil water status	59
4.3.1 Influence of AMF and <i>Trichoderma</i> spp. on nitrogen content.....	59
4.3.2 Influence of AMF and <i>Trichoderma</i> spp. on phosphorous content	62
4.3.3 Influence of AMF and <i>Trichoderma</i> spp. on the potassium content.....	65
4.4 The effect of different soil water levels on root colonization by AMF	68
CHAPTER FIVE	71
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	71
5.1. Discussion	71
5.1.1. Soil physicochemical analysis.....	71
5.1.2. Influence of AMF and <i>Trichoderma</i> spp. on growth and water uptake of cowpea at different soil water contents	71
5.1.3 Influence of AMF and <i>Trichoderma</i> spp. on nutrient uptake in cowpea at different soil water contents	77
5.1.4 The effect of different soil water contents on root colonization by AMF	80
5.2 Conclusions.....	83

5.3 Recommendation	84
REFERENCES	85
APPENDIX	103
Appendix: Research license	103

LIST OF TABLES

Table 4.1: The physicochemical analysis of soil that was used in the experiment.....	34
Table 4.2: Means of shoot dry weight of two cowpea varieties treated with AMF and <i>Trichoderma</i> at different soil water levels.....	36
Table 4.3: Means of root dry weights of two cowpea varieties treated with AMF and <i>Trichoderma</i> at different soil water levels.....	38
Table 4.4: Means of root lengths of two cowpea varieties treated with AMF and <i>Trichoderma</i> at different soil water levels	40
Table 4.5: Means of stem diameters of two cowpea varieties treated with AMF and <i>Trichoderma</i> at different soil water levels	44
Table 4.6: Means of leaf areas of two cowpea varieties treated with AMF and <i>Trichoderma</i> at different soil water levels	46
Table 4.7: Means of relative leaf water content two cowpea varieties treated with AMF and <i>Trichoderma</i> at different soil water levels.....	49
Table 4.8: Effect of AMF and <i>Trichoderma</i> on the specific leaf area of cowpea at three soil water levels	52
Table 4.9: Effect of AMF and <i>Trichoderma</i> on nitrogen content of cowpea at three soil water levels.....	61
Table 4.10: Effect of AMF and <i>Trichoderma</i> on the phosphorous content of cowpea at three soil water levels.....	64
Table 4.11: Effect of AMF and <i>Trichoderma</i> on the potassium content of cowpea at three soil water levels.....	67
Table 4.12: Effect of the three soil water levels on the mycorrhizal colonization of cowpea	70

LIST OF FIGURES

Figure 4.1: Shoot Dry Weight (g) for two cowpea varieties subjected to 30%, 60% and 90% soil water levels.	35
Figure 4.2: Root Dry Weight (g) means for two cowpea varieties subjected to 30%, 60% and 90% soil water levels.....	37
Figure 4.3: Means of Root Length (cm) for two cowpea varieties under 30%, 60% and 90% soil water levels.....	39
Figure 4.4: Means of stem diameters for two cowpea varieties under 30%, 60% and 90% soil water levels.....	41
Figure 4.5: Means of stem diameters for variety K80 under different fungal inocula..	42
Figure 4.6: Means of Leaf Areas for two cowpea varieties under 30%, 60% and 90% soil water levels.	45
Figure 4.7: Means of relative water content for two cowpea varieties under different fungal inocula.....	47
Figure 4.8: Means of relative water content for both varieties at different fungal inocula.	48
Figure 4.9: Means of specific leaf area for two cowpea varieties under 30%, 60% and 90% soil water levels.....	50
Figure 4.10: Means of specific leaf area for variety KVU 27-1 at different fungal inocula.....	51
Figure 4.11: Means of Heights for two cowpea varieties under 30%, 60% and 90% soil water levels.	53
Figure 4.12: Means of heights for KVU 27-1 and K80 at 30% FC	54
Figure 4.13: Means of heights for KVU 27-1 and K80 at 60% FC.	54
Figure 4.14: Means of height for KVU 27-1 and K80 at 90% FC.....	55
Figure 4.15: Means of chlorophyll content for two cowpea varieties under 30%, 60% and 90% soil water levels.....	56
Figure 4.16: Means of chlorophyll content for KVU 27-1 and K80 at 30% FC with different fungal inoculations.....	57
Figure 4.17: Means of chlorophyll content for KVU 27-1 and K80 at 60% FC with different fungal inoculations.....	57

Figure 4.18: Means of chlorophyll content for KVVU 27-1 and K80 at 90% FC with different fungal inoculations.....	58
Figure 4.19: Nitrogen content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels.....	59
Figure 4.20: Means of nitrogen content at different fungal inocula... ..	60
Figure 4.21: Phosphorous content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels.....	62
Figure 4.22: Means of phosphorous content for both varieties at different fungal inocula.....	63
Figure 4.23: Potassium content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels.....	65
Figure 4.24: Means of potassium content at different fungal inocula.	66
Figure 4.25: Mycorrhizal colonization in the two cowpea varieties at 30%, 60% and 90% soil water levels.....	68
Figure 4.26: Means of mycorrhizal colonization with different fungal inocula.....	69

LIST OF PLATES

Plate 3.1: *Trichoderma harzianum* and *Trichoderma asperellum* cultured on
the PDA26

Plate 3.2: Determination of chlorophyll content.....29

Plate 3.3: Leaves soaked in distilled water in determination of RWC30

ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ABA	Abscisic acid
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of Variance
ASALs	Arid and semi-arid areas
CRD	Completely Randomized Design
DAFFSA	Department of Agriculture, Forestry and Fisheries of South Africa
DAS	Days after sowing
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FC	Field Capacity
ISTA	International Seed Testing Association
KALRO	Kenya Agricultural Livestock and Research Organization
OECD	Organization of Economic Co-operation and Development
PDA	Potato Dextrose Agar
SPAD	Soil Plant Analysis Development
SSA	Sub-Saharan Africa

ABSTRACT

In a bid to explore alternatives that mitigate effects of low water and nutrient supply for crops, the influence of arbuscular mycorrhizal fungi and *Trichoderma* spp. on cowpea growth and nutrient uptake at different soil water levels under greenhouse conditions was undertaken. Cowpea is an essential food crop for both humans and livestock. It's a source of income in marginal areas and also adds nutritive value to the soil by fixing nitrogen. The experiment was arranged on a completely randomized design (CRD) with the three water levels treatment, of 90 % (no stress), 60 % (mild stress), and 30 % (severe stress) of field capacity (FC). Two cowpea varieties including KVVU 27-1 and Katumani 80 (K80) that are commonly grown by farmers were used in each water level. Each water level was subjected to seven fungal inoculation treatments: I- *Funneliformis mosseae* (BEG 12), II- *Rhizophagus irregularis* (BEG 44), III- *Trichoderma harzianum* (Har), IV- *Trichoderma asperellum* (Asp), V- BEG 12 + BEG 44 (BB), VI- Har + Asp (HarS), VII- Har + BEG 12 + BEG 44 (HBB), and VIII- which was un-inoculated as control (C). Each experimental unit was replicated four times resulting in 192 pots. The variables measured included, plant height, chlorophyll content, Shoot Dry Weight, Root Dry Weight, Root Length, Leaf Area, Stem Diameter, Relative Water Content, Specific Leaf Area and root mycorrhizal colonization. The shoot tissue analysis was also done to determine the nitrogen (N), phosphorous (P) and potassium (K) contents. All data were subjected to a two-way ANOVA in R software version 4.2.1 to test levels of significance due to treatments and their interactions. Means that were significant at $p \leq 0.05$ were separated using Bonferroni test. Results showed that all the growth parameters and the shoot nutrient content were statistically significant at ($p \leq 0.05$) with soil water level treatment in both varieties. Water stress had a negative influence on growth and the shoot nutrient content. The fungal inoculants were significant on stem diameter ($p = 0.006$), relative water content ($p = <0.001$) and $p = 0.002$ for KVVU 27-1 and K80 respectively) and Specific Leaf Area ($p = 0.049$) for KVVU 27-1. The widest stem diameter was 0.4 cm in KVVU 27-1 under Har and HarS fungal treatments. The highest Relative Water Content was 94 % in KVVU 27-1 with the inoculation of HBB while the highest SLA was 657 g/cm² in K80 with HarS inoculation. In addition, the interaction between water levels and fungal inoculations were observed in Stem Diameter, Relative Water Content and Specific Leaf Area. The combined inoculation of HarS and HBB had the greatest influence on the shoot nutrient levels for both cowpea varieties. The highest N and P shoot content were 3.7 % (in KVVU 27-1) and 0.12 % (in K80) inoculated with HBB and HarS respectively. The highest K content was 2.7 % in both varieties inoculated with HarS. Mycorrhizal colonization increased with increase in soil water level with the highest root colonization percentage of 37.8 % at 90 % FC. From the study, the co-inoculation of HarS and HBB was the most appropriate to enhance growth and nutrient uptake in the cowpea varieties. Therefore, these two treatments are recommended to be incorporated on agricultural soils to enhance plant growth.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Drought and nutrient deficiency culminate in poor agricultural yields, a defining characteristic of arid and semi-arid lands (Al-kaisi *et al.*, 2015; Abobatta, 2019). Application of chemical fertilizers to ameliorate nutrient deficiency is not economically sustainable for most smallholder farmers and has also intensified pollution (Oruru *et al.*, 2018). Rainfall remains unreliable and water for irrigation unsustainable. Certain microorganisms that have been proposed to promote growth by enhancing nutrient and water uptake provide a viable alternative. Among such microorganisms are arbuscular mycorrhizal fungi and *Trichoderma* spp. (Stewart and Hill, 2014).

Arbuscular mycorrhizal fungi (AMF) are purely endomycorrhizal as they exhibit invasive symbiosis (Pei *et al.*, 2020). They promote uptake of essential nutrients (Chandrasekaran, 2022) by the host plants hastening growth for interchange of plant carbon (Hashem *et al.*, 2018). Through their mutual association with plants roots, AMF's widely spread hyphal web increases acquisition of essential nutrients such as phosphorous from soil (Chandrasekaran, 2022). AMF protect plants against drought injury in various ways such as enhanced root development (Begum *et al.*, 2019). On the other hand, *Trichoderma* spp. are opportunistic endophytes that live

in soil and roots. They stimulate plant growth and pathogen resistance (Alfiky and Weisskopf, 2021). Sustaining plant immunity promotes physiological activity and enhances growth (Alfiky and Weisskopf, 2021). *Trichoderma* spp. also promote growth and development of the root system (Harman *et al.*, 2004). Increased root surface area facilitates water and nutrient uptake especially in the event of water deficit (Azarmi *et al.*, 2011). A mixture of *Trichoderma* isolates is capable of enhancing greater bioactivity than when one isolate is used (Kuzmanovska *et al.*, 2018).

Cowpea (*Vigna unguiculata* (L). Walp), belongs to the family Fabaceae (Agbogidi, 2010) and is the basis of both human and livestock food not only in Kenya but also globally. It is extensively grown in approximately sixty five countries that cut across the 6 continents in the arid zones of the tropical and sub-tropical countries (Singh, 2020). The current cowpea production area globally stands at an estimate of 12.5 hectares with 7 million tons produced yearly (FAOSTAT, 2019). Cowpea seeds and leaves are sold to generate income in marginal areas (ASALs) (Rusike *et al.*, 2013; Owade *et al.*, 2020a). The crop improves soil nutritive status by fixing atmospheric nitrogen in the soil (Kebede and Bekeko, 2020) and is drought tolerant. In spite of its advantages, soil nutrient deficiency and inadequate rainfall limits its production. There is a need to ameliorate soil fertility and improve crop tolerance to drought for higher yields. This project aimed at establishing the influence of AMF and *Trichoderma* spp. on cowpea's nutrient and water uptake and consequent effect on

growth. Results from the research serve to inform use of these inoculants as biofertilizers and improvement production of cowpea and other crops.

1.2 Statement of the problem

Cowpea is a useful crop as it provides food for humans, livestock and other crops (da Silva *et al.*, 2018). Its grains are rich in high-quality protein, carbohydrates and essential minerals (Carvalho *et al.*, 2019). Cowpea is a widely produced legume globally and the first half of the past three decades experienced its increase in terms of area coverage and production potential (Kebede and Bekeko, 2020). However, according to FAOSTAT (2019), there has been a decline in its global production between the year 2012 and 2017. Previous reports indicate that the change in climatic patterns have resulted to water stress leading to the decline in cowpea yields (Larweh *et al.*, 2019).

Despite cowpea being a drought tolerant, severe soil water deficits that occur during the early vegetative stage harm the cowpea's physiological qualities and lowers yields (Ravelombola *et al.*, 2020). Low soil fertility also lowers cowpea yields. In spite of cowpea being tolerant to low soil fertility because of its nitrogen fixing ability, low phosphorous levels in the soil reduce nodule formation and the efficiency to form symbiotic relationships with the soil microbiota (Tharanathan and Mahadevamma, 2003; Sudharani *et al.*, 2020). Extreme droughts also lowers the absorption of phosphorous and nitrogen which interferes with the rhizobial activities at the cowpea roots (Oruru *et al.*, 2018). Low potassium concentration in the soil

reduces the tolerance of cowpea to other abiotic stresses including high temperatures and salinity. The ineffective absorption of other nutrients including Mg, Fe, Mn and Cu due to droughts affects the chlorophyll content and therefore photosynthetic activities are hindered. The pursuit for consistent increase in agricultural production has led to excessive utilization of chemical fertilizers. The later apart from posing environmental pollution, are costly and therefore unaffordable to most of the small holder farmers (Azarmi *et al.*, 2011; Oruru *et al.*, 2018). The alternatives to mitigate soil infertility and water stress proposes utilization of the growth-promoting microorganisms such as arbuscular mycorrhizal fungi and *Trichoderma* spp. that sustain high yields under adverse environmental stresses. Even though these microorganisms are native in most agricultural and desert soils, their precise effect on growth promotion on cowpea has not been clearly addressed. Additionally, soil water management plays a crucial role in optimizing water usage for crop production. There is however no documentation of precise watering requirement for cowpea. Therefore, this research sought to determine the effect of arbuscular mycorrhizal fungi and *Trichoderma* spp. on the nutrient uptake at different soil water levels in cowpea.

1.3 Justification

Cowpea is a legume crop that thrives in dry areas where other legumes and nearly all cereals and tubers do not grow well (Hall, 2004; Dadson *et al.*, 2005). It is a cost-effective substitute for the less affordable food products from livestock and fish (OECD, 2018). It is the most important legume in the tropical Africa with high

levels of energy equal to that of cereals (Njonjo, 2018). Cowpea's high nutritive content comprises of proteins, vitamins and minerals that qualifies it as a food security crop in sub-Saharan Africa (SSA) (Owade *et al.*, 2020b). Conventional cultivation of cowpea involves application of chemical fertilizers and sometimes irrigation. Soil fertility is crucial when making decisions on food security, poverty reduction and environment management (Conceição *et al.*, 2016). Water and soil management play a crucial role in sustaining excellent crop production.

In this study, the incorporation of AMF and *Trichoderma* spp. in cowpea were used to enhance nutrient absorption and growth under water stress. The two fungal inoculants can be used as bio-fertilizers instead of chemical fertilizers (Khoshmanzar *et al.*, 2019). The AMF and *Trichoderma* spp. promote water uptake, and absorption of essential nutrients. Their application will consequently improve growth and yield of cowpea (Giovannetti and Mose, 1980). Their usage is also cost effective, will reduce water and soil contamination resulting in healthy crops.

The use of growth-promoting microorganisms is a worthwhile substitute for chemical fertilizers that are ingredients of water and soil pollution and lately have become unaffordable to the majority of the poor smallholder farmers. Colonization of plant roots by these microorganisms causes secretion of plant defense compounds that are inhibitory to majority of infectious bacteria and fungi. In this case, they can be used for disease control instead of chemical fungicides as they are safer to use,

their disease control effects lasts longer than synthetic chemical pesticides (Kumara *et al.*, 2022).

1.4 Research Hypotheses

- i. Arbuscular mycorrhizal fungi and *Trichoderma* spp. significantly promote growth in cowpea at different water levels.
- ii. Arbuscular mycorrhizal fungi and *Trichoderma* spp. influences nutrient uptake in cowpea under different water levels.
- iii. Different water levels affect arbuscular mycorrhizal fungi and *Trichoderma* spp. root colonization.

1.5 General objective

To determine the influence of arbuscular mycorrhizal fungi and *Trichoderma* spp. on nutrient uptake and water stress tolerance in cowpea.

1.5.1 Specific objectives

- i. To evaluate the effect of arbuscular mycorrhizal fungi and *Trichoderma* spp. on growth of cowpea at different soil water levels.
- ii. To assess the effect of arbuscular mycorrhizal fungi and *Trichoderma* spp. on nutrient uptake in cowpea under different soil water levels.
- iii. To establish the effect of different soil water levels on root colonization by arbuscular mycorrhizal fungi and *Trichoderma* spp.

1.6 Significance of the study

This study aimed at ascertaining the role of the growth promoting microorganisms in enhancing nutrient uptake in cowpea at different water levels. Through promotion of absorption of nitrogen, phosphorous and potassium in cowpea at the three water levels, arbuscular mycorrhizal fungi and *Trichoderma* spp. reduce the use of farm inputs like the high-cost chemical fertilizers. This will lead to a reduction in the cost of production of cowpea especially to the resource-poor farmers in the tropical and sub-tropical African countries. The results of the study add to the knowledge and growing use of these microorganisms to improve crop productivity. It supports the use of these beneficial soil biota to upgrade the nutritive value of soils. This is important as it will lead to utilization of less fertile land that was previously poorly productive. Water availability is important for cowpea cultivation, and water deficit exposes the crop to physiological injury. This study determined the agronomical and physiological parameters presented by cowpea at different soil water levels and the role played by the two fungal inoculants in promoting growth at these levels. These parameters are important for farmers in selecting cowpea germplasm to be grown in specific areas with various water demands for maximization of water resource application. This work also determines the soil water level that is appropriate for root mycorrhizal colonization as this informs the incorporation of arbuscular mycorrhizal fungi in agricultural systems to improve cowpea production.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fungal ecology in the rhizosphere

Plant roots live in association with different microbial communities emanating from the soil with environmental factors and plant characteristics influencing their diversity and composition. Fungi have been in existence with plant roots since the history of plant evolution. The interaction of plants with free-living fungi have been observed to improve on disease suppression, abiotic stress tolerance and nutrient mobilization for sustainable crop yield (Wang *et al.*, 2017). The plant-*Trichoderma* interactions is versatile in plant growth promotion and soil improvement qualities (Halifu *et al.*, 2019). The majority of plant species also form mycorrhizal associations and they depend on mycorrhizal fungal symbionts for health, growth and productivity (Dickie *et al.*, 2013). Amongst many mycorrhizal symbiosis the most common associated with plants is arbuscular mycorrhizal fungi (Brundrett 2009).

2.1.1. Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) evolved as early as terrestrial plants and fossil records prove their fidelity in the success of early terrestrial plants (Muthukumar *et al.*, 2009). They are always in the close association with higher plants (Kehri *et al.*, 2018). Over 80 % of terrestrial plants have symbiotic association with AMF (Khaliq *et al.*, 2022). They influence rhizosphere ecology by forming associations with all

terrestrial ecosystems and agro-systems. They are obligate biotrophs that will only grow and complete their life cycles in plant roots. Kavitha *et al.* (2018) expresses AMF's unique survival and proliferation in the soil as directly pegged on the close association with the plant hosts without which they are exposed to desiccation and parasitism.

The AMF are members of the phylum Glomeromycota in the class Glomeromycetes and are characterized by spores, sporocarps, arbuscules and a subtending hyphae (Willis *et al.*, 2013). Colonization in plant roots is caused by compounds exuded from roots (Tamasloukht *et al.*, 2003). The AMF hyphae infiltrate the cell and the arbuscules form contact and exchange sites from the fungus to the host roots cortical cells (Khaliq *et al.*, 2022). The extend of mycelial network in the soil varies with the AMF species. Arbuscules form a large surface area which is determined by fungal species, edaphic factors and soil water status (Posada *et al.*, 2007).

2.1.2. *Trichoderma* spp.

Trichoderma spp. are free-living filamentous dueteromycetes that are distributed globally and form an important part of soil flora (Gams and Bisset, 1998; Sharma *et al.*, 2019). As the most dominant component of the soil microflora, *Trichoderma* are great influencers of rhizospheric ecosystem (Samuels, 2006). They form effective heterotrophic associations including opportunistic endophytism enabling them to thrive in soil and other complex ecological zones. As endophytes they live in healthy

plant parts such as roots and shoots, locally and systematically influencing plant growth and resistance against pathogens (Alfiky and Weisskopf, 2021).

Trichoderma has over 260 species with approximately 35 recognized species used as biocontrol agents (Bissett *et al.*, 2015). They either reproduce asexually through sporulation (Gams and Bisset, 1998) or asexually through ascospores (Samuels, 2006). *Trichoderma* are morphologically characterized by highly branched conidiophores as their conidia is used to colonize host roots (Samuels, 2006). *Trichoderma* associate with roots by invading their superficial layers, however, most species are purely avirulent (Harman *et al.*, 2004). Soil colonization is a very important aspect in establishing *Trichoderma* communities in the soil. Successful colonization depends on the prevailing soil conditions such as pH, organic matter, nutrient content, temperature and moisture content (Carro-Huerta *et al.*, 2021; Kakabouki *et al.*, 2021)

Soil moisture affects availability, intensity and distribution of *Trichoderma* communities in the soil (Bourguignon, 2008) as most *Trichoderma* spp. attain optimum growth at a negative water potential (Clarkson *et al.*, 2004).

2.2 Fungal role in plant growth and alleviation of water stress

2.2.1. Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi promote uptake of large amounts of phosphorous and other nutrients including N, K, Zn, Cu from soil through solubilization (Hashem *et al.*, 2014; Khaliq *et al.*, 2022). The fungi have the capacity to modify soil

physiochemical properties. Firstly, their extra-radical hyphae actively produce large amounts of a glycoprotein called glomalin that aggregates particles hence improving soil structure (Willis *et al.*, 2013). The fungi also modify soil pH which releases phosphorous from bound forms, and other minerals that have low solubility (Cheng *et al.*, 2021).

Mycorrhizal fungi regulate growth of plants in ordinary and adverse environment (Abd_Allah *et al.*, 2017). Inoculation with these fungi protect plants from injury from water deficit in various ways. The capacity to absorb different mineral nutrients promotes enzyme activity and photosynthesis that enables plants to establish early to escape injury from physiological stress such as drought. They sustain leaf water status (Begum *et al.*, 2019) and improve stomata conductance during soil water deficit (Diagne *et al.*, 2020). Arbuscular mycorrhizal fungi promote accumulation of compatible osmolytes that maintain water potential during drought to sustain physiological activity (Ntombela, 2012). Moreover, improvement of the plants' water use efficiency is attributed to AMF's protection of photosynthetic activity and apparatus. This is supported by Ismail *et al.* (2014) who recorded an increase in photosynthetic pigment possibly from enhanced absorption of nitrogen and magnesium after AMF inoculation.

2.2.2. *Trichoderma* spp.

Plant-*Trichoderma* interactions have the ability to enhance growth under both optimal and sub-optimal conditions (Chepsergon *et al.*, 2014). Although

Trichoderma spp. are used as biocontrol agents (Harman *et al.*, 2004; Martínez-medina *et al.*, 2011), colonization of plant tissues with *Trichoderma* spp. establishes plant immunity which leads to increased plant metabolism and promotes plant growth (Mei *et al.*, 2019).

According to Harman *et al.* (2004), *Trichoderma* spp. has the ability to promote formation and enlargement of roots which is a mechanism for drought tolerance in most plants. Stewart and Hill (2014) recorded increased growth of tomato seedling roots after inoculating them with *Trichoderma* strains. An increased root surface area increases uptake and solubilization of nutrients that lead to improved water and nutrient acquisition efficiency (Azarmi *et al.*, 2011; Bader *et al.*, 2019). Khoshmanzar *et al.* (2019) observed that this increases photosynthetic rate and reduces formation of free radicals that cause oxidative stress under water stress thus improving the plant water status.

Inoculation with *Trichoderma* spp. stimulates synthesis of phytohormones and their equivalents that enhance plant performance (Illescas *et al.*, 2021). Different *Trichoderma* strains are used to initiate production of different hormones and their precursors which include ethylene, ABA and Gibberellic Acid-3 in plants roots and shoots translating to higher shoot growth (Kamalov *et al.*, 2018; Zhang *et al.*, 2019). Chagas *et al.* (2016) recorded synthesis of Indoleacetic acid (IAA) after inoculating cowpea with different isolates of *Trichoderma* resulting in higher dry matter

content. According to Chepsergon *et al.* (2014), IAA stimulates adventitious root formation surface area for absorption.

2.2.3. The concept of co-inoculation of fungal strains

The soil is a sink of microbial communities consisting of diverse strains that promote growth in plants. Isolating and merging of these strains in optimal and sub-optimal conditions preserves their utmost utilities in improving growth in plants. Combination of growth-promoting microorganisms has been applied successfully. For example, da Silva *et al.* (2019) co-inoculated *Bradyrhizonium* and *T. asperelloides* on cowpea under salinity stress and recorded increased biomass and height. Stewart and Hill (2014) also observed that a combination of *T. harzianum*, *T. viride* and *T. virens* increased N and P uptake in chick pea. In addition, co-inoculation of *T. harzianum* and *T. asperellum* was successfully used to inhibit growth of *Botrytis cinerea* in tomato (Kuzmanovska *et al.*, 2018). The inoculation of both *T. harzianum* and *T. asperellum* was successfully used to inhibit growth of *Botrytis cinerea* in tomato (Kuzmanovska *et al.*, 2018). Dual inoculation of AMF and *Trichoderma* spp. stimulate synergistic effects that lead to increased growth and drought tolerance. Metwally and Al-Amri (2019) recorded increased biomass and bulb diameter in onions after it was co-inoculated with *Trichoderma viride* and AMF consortium.

Synergy between *Trichoderma* spp. and AMF produce better results than when one type of the isolate was used (Harman *et al.*, 2004; Srivastava *et al.*, 2010). This is whether *Trichoderma* is used to control a pathogen or to enhance growth. The

mechanism behind the *Trichoderma*-AMF interactions is partly based on mycoparasitism whereby according to De Jaeger *et al.* (2010), the *Trichoderma* parasitizes the AMF mycelium and the derived mycoparasitism stress causes increased uptake of nutrients that increases growth. Co-inoculation between *Trichoderma* and AMF have been used severally with different plants producing positive results (Poveda *et al.*, 2019). Among other studies, dual inoculation of *T. aureoviride* and *G. mosseae* promoted greater biomass in marigold (Stewart and Hill, 2014). However, this has been not adequately examined under both field and greenhouse conditions.

Dual or triple inoculations of the fungal strains may in some cases negatively impact plant growth and nutrient uptake. Tchameni *et al.* (2011) proposes that the effectiveness of dual inoculation of *Trichoderma* spp. or mycorrhizal fungus is dependent on the interaction within and between the fungal species, the type of fungal strain and the type of host plants.

2.3 Arbuscular mycorrhizal fungi, *Trichoderma* spp. and cowpea production

Cowpea is a versatile crop in terms of human food, animal fodder and source of income in most of rural populations (Muindi *et al.*, 2023). In spite of its importance its production remains low as it is grown in marginalized regions that are less fertile. Drought episodes in such regions affect the morphological and physiological ability of cowpea that slows down growth and production. One way to improve its

cultivation and production may be through promoting the mutualistic partnerships with beneficial soil microbes like *Trichoderma* spp. and arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi form symbiotic associations with more than 80% of the terrestrial plants primarily for absorption of phosphorous for exchange of plant carbon (Khaliq *et al.*, 2022). The mutualistic association between AMF and cowpea makes other nutrients including calcium, potassium, and manganese available which are the drivers of the biological nitrogen fixation that maintains soil fertility status and high plant productivity (Rochange *et al.*, 2019; Muindi *et al.*, 2023). The enhanced nutrients uptake protect the plants against abiotic stresses like drought (Begum *et al.*, 2019). Use of many chemical fertilizers in the soil affect the proliferation of AMF and it is costly to many farmers. Higher mycorrhizal colonization in cowpea is an indicator of fungal contact and more uptake of nutrients hence enhanced growth (Ranadev *et al.*, 2022). Though cowpea is drought tolerant, advanced water stress affects its physiology. The early vegetative stage, flowering, and pod filing stages are very sensitive to drought effects and will reduce cowpea yields (Jemo *et al.*, 2017; Ravelombola *et al.*, 2020). Negative effects of drought stress on cowpea's physiology include decreased biomass, leaf area, chlorophyll content, plant height and yield (Cardona-Ayala *et al.*, 2020). Previous studies have revealed the enhancement effect of AMF on growth and productivity in cowpea. Ranadev *et al.* (2022) recorded an increase in cowpea stem height, stem diameter, and shoot dry mass after inoculating with *F. mosseae*. According to Chandrasekaran

(2022) AMF extend their extraradicle hyphae in the soil enabling plant roots to access more water and nutrients.

On the other hand, *Trichoderma* promote growth and yield of cowpea through production of growth stimulating hormones and phosphorous mobilization (Rodrigues *et al.*, 2020). Hormones like IAA induce formation of adventitious roots which increase the plant's absorptive area that enhances greater absorption of water and nutrients (Chagas *et al.*, 2016). *Trichoderma* increases efficiency of nodulation and growth in cowpea. This simulates the action of nitrogen fixing bacteria that increases the efficiency of using nitrogen in cowpea. Rodrigues *et al.* (2022) observed increased shoot biomass and grain yield in cowpea after co-inoculation of *T. asperellum* with rhizobia. *Trichoderma* spp. are able to decompose nutrients, and cause their flow in the development of pods and filling of cowpea grains (Haddad *et al.*, 2017). *Trichoderma* are biocontrol agents that pave way to growth and productivity in plants. Kumar *et al.* (2020) observed that *T. viride* was able to successfully control *Sclerotium rolfsii* in cowpea and recorded an increase in plant height, stem girth and number of leaves in cowpea that led to their higher productivity.

2.4 Cultivation and distribution of cowpea

Cowpea was first domesticated in the African continent between 1700 and 1500 (Singh, 2014; DAFFSA, 2011). The West, Central and Southern parts of Africa are

perceived to be probable centers of origin of cowpea (Padulosi and Ng, 1997). These regions display a blend of the wild undomesticated and the modern cultivated cowpea. *Vigna unguiculata*, a warm-seasoned annual, flourishes in this region due to its diverse climatic zones (OECD, 2016). Among these regions, South Africa has the greatest genetic diversity of cowpea and retains the most primitive sub-species (Padulosi and Ng, 1997). Cultivation of *Vigna unguiculata* has since spread widely including South America, Central America, the United States and the Caribbean (OECD, 2016) forming an integral part of the diet of approximately 110 million people (Edeh and Igberi, 2012).

Cowpea leaf is one of the chief traditional vegetables in sub-Saharan Africa (SSA) (Ekesa *et al.*, 2009; Kiremire *et al.*, 2010). It is grown in approximately 14.5 M hectares globally with grain production of 6 million metric tons per annum (Kebede and Bekeko, 2020). Africa contributes approximately 83 % of the overall production with over 80 % of this yield coming from West Africa (Horn *et al.*, 2022) where Nigeria and Niger have been the first and second highest cowpea producers respectively in the region for 14 consecutive years (Huynh *et al.*, 2016).

In Kenya, the crop is currently grown on 227,807 hectares of land, the largest in East Africa and comes third after beans and pigeon pea in importance (FAOSTAT, 2019). Leaf cowpea is largely produced in Western Kenya whereas Coastal, Central and Eastern regions account for the largest cultivation for grain production (Rusike *et al.*, 2013; Muniu, 2017). The arid and semi-arid areas (ASALs) of Eastern region

account for two thirds of the total mass under cowpea cultivation in Kenya (Rashid *et al.*, 2016; Muli and Saha, 2000). Cultivated varieties include both landraces and cultivars (Mamiro *et al.*, 2011). Quite often, cowpea is inter-cropped with maize, sorghum and cassava because of its nitrogen-fixing property (Owade *et al.*, 2020a). Intercropping may however lower yields owing to shading from taller crops (Sibhatu *et al.*, 2018).

Leaf cowpea is rich in proteins, vitamins, folic acid, and antioxidants and serves as a source of dietary fiber for both humans and livestock (Mamiro *et al.*, 2011). Fresh immature pods and grains are rich in proteins and vitamins for both humans and livestock supplementing the low-protein cereals and tubers (OECD, 2016). Cowpea is also low in cholesterol unlike animal sources of protein, and more affordable. The rich nutritional property attached to cowpea regards it as the ultimate crop in improving food security in SSA (Owade *et al.*, 2020b). The crop residue supplies livestock with hay and fodder (Singh *et al.*, 2003). Dried cowpea haulms used as fodder are rich in calcium, richer than animal sources (Achuba, 2006).

Cowpea is a cover crop that flourishes within a short period averting soil erosion. The symbiotic association between the cowpea root nodules and rhizobia enables the fixing of the atmospheric nitrogen in the soil thereby improving the soil nutrition status (Emmanuel *et al.*, 2021) and thus improves nitrogen status reducing use of chemical nitrogen fertilizers (Tijjani *et al.*, 2015). For cases where drought occurrences persist, the symbiosis between the natural soil *Mycorrhizae* enables

absorption of nutrients. Moreover, the cowpea plant exhibits a deep root system that enables it to tolerate drought although there exists variation in tolerance across genotypes (Fatokun *et al.*, 2009; Woomeer and Mulei, 2015).

2.5 Constraints in cowpea productivity

Africa records low yields as compared to other countries (FAOSTAT, 2014). Similarly, production in Kenya remains at a low level declining from about 173,000 MT in 2015 to about 146,000 MT in 2017 (FAOSTAT, 2019). This has partly been attributed to lack of prioritization of the crop by Research and Development programs (Ojiewo *et al.*, 2019). Cowpea farmers have not also embraced improved cultivars and production systems (Kebede and Bekeko, 2020). According to Hutchinson *et al.* (2017), most farmers highly prefer local landraces compared to improved varieties because they are palatable and adaptable despite their poor yield traits.

Insect pests cause destruction in all stages of cowpea growth (Adelaide *et al.*, 2018). Pests can cause up to 80 % crop loss in cowpea as they occur at pre-flowering, post-flowering and storage (Oyewale and Bamaiyi, 2013; Souleymane., *et al.*, 2013). Pod borers infest flowering structures and young parts of the plant while the hairy caterpillar attack young stages (OECD, 2016). The most important pests are the aphids which attack the vegetative stage of cowpea. Aphids cause damage by sucking plant sap at seedling stage. They also cause curling of leaves, delayed flowering, shriveling of pods and as a result reduced photosynthetic processes

finally leading to reduced yields (Stoddard *et al.*, 2010). Cowpea cultivars with good resistance genes against aphids need to be pursued in breeding new aphid-resistant varieties.

Infestation by fungal, viral, and bacterial pathogens directly cause stem and root rots, seed rot, seedling mortality, and foliar diseases. It is estimated that over twenty (20) virus types globally can cause field losses up to 90% (Mbeyagala *et al.*, 2014).

Abiotic factors including salinity, extreme temperatures, heavy metal toxicity, water stress and soil infertility also affect cowpea productivity. Amongst the factors, water stress is identified as a major limitation to cowpea production (Chand *et al.*, 2020).

Drought stress caused by erratic rainfall in arid and semi-arid areas (ASALs) adversely influences cowpea growth especially at the early stages of establishment, flowering and seed-filling that results in substantial drop in yields (Jemo *et al.*, 2017). It is estimated that cowpea suffers a yield loss of approximately (35-69)% due to drought stress (Krasilnikoff *et al.*, 2003). Soil infertility caused by deficiency of macro and micro-elements reduce cowpea yields. Though cowpea fixes atmospheric nitrogen through symbiotic association with rhizobia, it needs phosphorous and potassium elements for root development, transport of photo-assimilates and tolerance against environmental stresses. Other micro elements including Mg, Mn, Zn, Fe, and Cu are essential for build-up of chlorophyll which enhances photosynthetic processes. Many resource-poor farmers do not afford the farm inputs to complement the soil infertility status.

2.6 Water stress and nutrition on plant growth

Low, short-seasoned and inconsistent rainfall results in soil water deficits, a hindrance to effective plant production worldwide (Hayatu and Mukhtar, 2010). Germination and flower setting are most sensitive to drought stress (Karim *et al.*, 2018; Ntombela, 2012). Water deficit results in lowered vegetative growth (Zhang *et al.*, 2004; Mulyungi, 2014) and leaf area which reduces photosynthetic surface (Kaushal *et al.*, 2016) in crops. Photosynthesis efficiency is further reduced under water stress via impairment of photosynthetic machinery, leaf senescence and leaf abscission (Hayatu and Mukhtar, 2010). Additionally, stomatal closure in response to water stress reduces the rate of photosynthesis (Anjum *et al.*, 2011). Shukla *et al.* (2014) reports that drought impairs major photosynthetic components like the thylakoid electron transport and carbon reduction cycle. It also causes destruction of chlorophyll or simply prevents its synthesis (Kamanga *et al.*, 2018; Anjum *et al.*, 2011) which is considered the main hindrance of photosynthesis efficiency.

Reduced water availability may limit nutrient availability in soil. Most nutrients in dry soils exist in complex-non-absorbable forms with low diffusion rates. Inaccessibility to nutrients like phosphorous by roots highly reduces the capacity of biological nitrogen fixation by legumes. Solubilization and degradation are the processes that are meant to translate such nutrients into absorbable forms by plant roots.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental design and treatments

Growth and water uptake experiment was arranged in a complete randomized design (CRD). The treatments were three water levels of 90 %, 60 %, and 30 % of field capacity (FC) that represented no soil water stress, mild soil water stress and severe soil water stress respectively. In each water level, there were two cowpea varieties that included KVVU 27-1 and Katumani 80 (K80). The two cowpea varieties were chosen because they are locally available and are mostly grown by farmers. Each cowpea variety was treated with seven fungal inoculation treatments namely: I- *Funneliformis mosseae* (BEG 12), II- *Rhizophagus irregularis* (BEG 44), III- *Trichoderma harzianum* (Har), IV- *Trichoderma asperellum* (Asp), V- BEG 12 + BEG 44 (BB), VI- Har + Asp (HarS), VII- Har + BEG 12 + BEG 44 (HBB), and VIII- which was un-inoculated as control (C). Each experimental unit had four replicates resulting in 192 pots. The pots measurements were determined by using a tape measure where 17 cm diameter and 16 cm height were established.

3.2 Soil collection, preparation and analysis

Soil for greenhouse experiment was collected from six locations in South Kinangop in the Aberdare Forest Reserve that is located on latitude S 00° 43'28.4" , longitude E036° 40'51.7" and 2545 m above sea level. The soil samples were mixed to obtain a homogenous soil sample. The soil was thoroughly mixed with sand in the ratio

3:1. The substrate was left to air-dry (26-28)°C for seven days after which it was crushed and passed through a 2 mm pore sieve to remove large particles, broken sticks and other fragments. The substrate was then sterilized through autoclaving at 121°C, 1.5 psi for 1 hour. Shortly after autoclaving, a uniform quantity of approximately 1.5 kg of autoclaved soil was potted. A sample of approximately 1kg soil was taken to the soil-testing laboratory of Kenya Agricultural and Livestock Research Organization (KALRO), Nairobi for physicochemical properties analysis.

3.3 Soil physicochemical properties

3.3.1 Soil pH

A 50 g soil sample was weighed into a 100 ml polythene bottle. Fifty ml of 1.0 M KCl solution was added to the bottle, then it was closed and vortexed for 2 hours. The electrode of a pH meter was immersed in the clear suspension till reading stabilized, and the figure with accuracy of ± 0.1 recorded (Van Reeuwijk, 2002).

3.3.2 Available nutrient elements

The elements P, K, Na, Ca, Mg, and Mn were extracted by the Mehlich Double Acid method (Mehlich *et al.*, 1962). The oven-dry soil samples were extracted with a mixture of 0.1 N HCl and 0.025 N H₂SO₄ in the ratio of 1:5 (w/v). Flame photometry was used to determine Ca and K in the filtrate. Magnesium, P and Mn were determined using Atomic Absorption Spectrophotometer (AAS) (210 VGP Buck Scientific).

3.3.3 Total soil organic carbon

Colorimetric method (Anderson and Ingram, 1993) was used to determine soil organic carbon. One gram of finely ground soil (0.5 mm mesh) was oven-dried at 40°C. Acidified dichromate was added to the soil at 150°C for 30 minutes. The mixture was cooled then barium chloride added followed by thorough mixing. After allowing the mixture to stand, carbon was measured using a 21D spectrometer at 600 nm wavelengths.

3.3.4 Determination of nitrogen

Available nitrogen was determined using Kjeldahl method (Page *et al.*, 1982). The soil sample used was sieved using a 5 mm mesh followed and dried at 40°C. A mixture of concentrated H₂SO₄, K₂SO₄, CuSO₄.H₂O and selenium was used to decompose the organic nitrogen. Digestion was followed by distillation with NaOH and the ammonia absorbed by HCl. Volumetric analysis of N was done by titration with standardized HCl.

3.3.5 Exchangeable acidity

This procedure documented by Okalebo *et al.* (2002) was done because the soil pH was less than 5.5. Five grams of soil was passed through a 2 mm mesh before drying it at 40°C. The soil was mixed with 12.5 ml of 1 M KCl, stirred then let to stand for half an hour. Filtration of the mixture was done and further leached by adding five aliquots of 12.5 ml of 1 M KCl. The filtrate was titrated with 0.1 M NaOH in the presence of phenolphthalein indicator. The titration readings were corrected by use

of a blank titration of 75 ml KCl solution. The acidity was analyzed as the amount of base used.

3.3.6 Available trace elements

Zinc, copper and iron were determined following the procedure described by Mehlich *et al.* (1962). The available elements were extracted from the oven-dried soil using 0.1 M HCl and the filtrate analyzed in Atomic Absorption Spectrophotometer.

3.4 Preparation of fungal inocula

The AMF inocula consisted of *Funneliformis mosseae* and *Rhizophagus irregularis* that were obtained from the International Bank of the Glomeromycota INRA, France and bulked in pot cultures at Kenyatta University greenhouse using Bermuda grass (*Cynodon dactylon*) as the host plant.

Two *Trichoderma* isolates namely, *T. harzianum* and *T. asperellum* were used in this study. The preserved *Trichoderma* spp. were obtained from the repository of Kenyatta University in the Biochemistry, Microbiology and Biotechnology Department. The isolates were rejuvenated by growing them on fresh PDA media (potato extract 12 g/L, dextrose 20 g/L, agar 14 g/L; Haibo Biotechnology, China) at pH of 6.0, incubated in the dark at a routine 28°C (Halifu *et al.*, 2019) (Plate 3.1). Five days after culturing, the culture was cut with a sterile puncher ($\varnothing = 5$ mm). The mycelium inoculum was transferred to liquid potato dextrose medium to obtain a suspension culture then stored in the dark at 28°C up to the 7th day. Thirty milliliters

of sterile distilled water was added and the culture agitated at 150 rpm for 30 minutes. The concentration of *Trichoderma* spores was counted using a hemocytometer and determined to be 10^6 spores/ml through serial dilution. This suspension was used to inoculate the seeds.



Plate 3.1: *Trichoderma harzianum* and *Trichoderma asperellum* cultured on the PDA

3.5 Fungal inoculation and plant growth conditions

For inoculation with AMF, crude AMF inoculant consisting colonized Bermuda grass roots, spores, fungal hyphae and soil medium was crushed into smaller particles. Five grams of AMF inoculant were placed in 3 cm diameter holes made in the pot and mixed thoroughly with soil prior to sowing. Where dual inoculation of *F. mosseae* and *R. irregularis* was involved, 2.5 g of each species were mixed thoroughly with soil in a 3 cm diameter the hole.

For *Trichoderma* inoculation, seed lots were placed in a *Trichoderma* spore suspension of concentration 10^6 spores/ml. Three grams of Gum Arabica were

mixed with two spoonfuls of distilled water to form a solution. Two table spoonfuls of Gum Arabic solution were added to the seed-*Trichoderma* mixture to reinforce the attachment of fungi on the seeds (Nyoki and Ndakidemi, 2014). The treatment was left for 24 hours after which the seeds were ready for sowing.

Each pot was sown with five cowpea seeds at a depth of (2.5 – 6) cm, and a spacing of about 6 cm (Dugje *et al.*, 2009). The pots were immediately watered with tap water and the water content in the pots was maintained at 90 % FC by using the HydroSense II (HS2 and HS2P, Campbell Scientific, Inc). All seeds germinated between 4 to 7 days after sowing (DAS) and the seedlings were allowed for establishment up to the 14th day after sowing (Dugje *et al.*, 2009). Fifteen (15) DAS, the newly germinated seedlings were thinned to retain two cowpea plants per pot (Karim *et al.*, 2018). Pots were also subjected to respective water levels of; 90% (no stress), 60 % (moderate stress) and 30 % (severe stress) FC until the 49th day.

3.6 Determination of water deficits in the pots

Soil water status was maintained using HydroSense II (HS2 and HS2P, Campbell Scientific, Inc). Three pots with uniform volume capacity were filled with forest soil at a $\frac{3}{4}$ mark. Pots were filled with water and excess water was allowed to drain through the pots' drainage holes. Pots were covered with a black polythene bag and left for 48 hours for enough saturation to take place (Karim *et al.*, 2018). The HydroSense II was then calibrated at 90 %, 60 % and 30 % FC and was used to determine the volumetric water deficits in the pots as described in its Product

Manual. Measurements using the HydroSense II were done between 6.00 am and 8.00 am daily after water stress was introduced in order to determine the volumetric content of water deficit that was needed to be added in the pots to maintain the pots at their respective water levels. To ensure uniformity in the water level deficits in the pots, the HydroSense II probes were inserted at different points in the respective pots and the average values were obtained.

3.7 Planting material

Seeds of two cowpea cultivars (KVU 27-1 and K80) were purchased from Kenya Agricultural Livestock and Research Organization (KALRO). The varieties are some of the most commonly grown by farmers. Surface sterilization was done by washing the seeds in 70 % ethanol followed by addition of 2 % NaOCl for two minutes before they were rinsed severally with sterile water (Njonjo, 2018).

Seeds with deformities were removed and visually good ones surface sterilized with 2 % sodium hypochlorite for 2 minutes then washed seven times with sterile distilled water before the germination test. The seeds were selected and tested for viability (ISTA, 2015). Four replicates of 50 seeds each were placed on moist paper towels spread in petri-dishes of 90 mm diameter and incubated at 28°C (Njonjo, 2018). A seed was considered to have germinated when a radicle protruded outside the seed coat by at least 0.2 cm (Covell *et al.*, 1986). Germination was scored daily from 4th to 7th (DAS) and this data used to calculate germination percentage (Njonjo, 2018) as follows:

$$\text{Germination \% (GP)} = \frac{\text{number of emerged seedlings at the final count}}{\text{Total number of seeds planted}} \times 100$$

3.8 Chlorophyll estimates

The chlorophyll content (the equivalent of leaf greenness) was determined non-destructively by use of a Soil Plant Analysis Development Meter (SPAD-502 Plus; Konica Minolta, Inc, Japan) according to Colla *et al.* (2015). Readings were taken from a fully developed leaf on the second node from the apex in each pot (Plate 3.2). Three measurements were made at different positions of the leaf lamina (Dong *et al.*, 2019). Three leaves were used per plant and their average value was treated as a single SPAD value. The SPAD values were repeated twice at the interval of two weeks.



Plate 3.2: Determination of chlorophyll content

3.9 Relative Water content of the leaf

Two fully developed leaves were randomly excised from the cowpea plants in each pot and their fresh weight (FW) taken immediately. Afterwards they were soaked in distilled water (Plate 3.3) for 4 hours after which they were blotted dry with filter paper (Hayatu *et al.*, 2014).



Plate 3.3: Leaves soaked in distilled water in determination of RWC

The leaves were re-weighed to determine their Turgid weight (TW) then placed in the oven at 70°C for 12 hours to obtain their dry weight. The Relative Water Content (RWC) was determined by the following formula (Anyia and Herzog, 2004; Pirzad *et al.*, 2011).

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where;

RWC-relative water content

FW-sample fresh weight

DW-sample dry weight

TW-sample turgid weight

3.10 Leaf area

The leaf area was determined destructively just before harvesting. In each pot a fully developed leaflet was excised and its margins traced on a graph paper. The area was calculated by multiplying the total number of full squares by 1 cm². The partially filled squares were multiplied by 0.5 cm² and was added to the total (Agbogidi, 2010). Three leaves per pot were used and the area of the leaf in each replicate was the mean of the three leaves.

3.11 Specific leaf area

The specific leaf area was calculated by dividing the leaf area (in cm²) by its dry weight (in g) (Anyia and Herzog, 2004).

$$\text{Specific leaf area} = \frac{\text{Area of the leaf sample (cm}^2\text{)}}{\text{Dry weight of leaf sample (g)}}$$

3.12 Plant height and root length

The height of the shoot and the length of roots in each pot were determined using a measuring tape. The height from the base of the stem was taken at a seven days interval from the 21st DAS week up to 49th day. At harvesting, the root length (RL)

was determined using a meter rule and was measured from the base of the root to the tip.

3.13 Stem diameter

Immediately before harvesting, the stem diameter (SD) at 2 cm above the soil surface was measured for all plants using a digital caliper (Agbogidi, 2010).

3.14 Determination of the dry mass

After harvesting, all roots and shoots in each of the treatments were carefully separated. Soil was washed with mesh to remove tiny roots from soil. All the shoots and roots from each treatment were dried in the oven at 72°C till constant weights were attained (Yaseen *et al.*, 2011) to obtain the shoot and root dry masses. A sensitive balance at a resolution of ± 0.000 g was used measure the dry weights.

3.15 Mycorrhizal root colonization

At the end of the growth experiments, the roots of all plants inoculated with BEG 12, BEG 44, BB, HBB and Control in all the three water levels were carefully washed with water, separated into small pieces of 10 mm length and mixed thoroughly. The root pieces were then cleared with KOH (10%) in a water bath at 80°C for 10 minutes, followed by treatment with 2% HCl solution for 5 minutes and stained with 0.05% trypan blue in lactic acid (Philips and Hayman, 1970). Mycorrhizal colonization on the roots was observed using a dissecting microscope at $\times 40$ magnification using gridline intersect method as described by Giovannetti and Mosse (1980).

3.16 Nutrient analysis

Dry shoot samples were ground to a homogenous composite and ashed at 400°C for 1 hour. Five grams of each sample were chemically digested using a mixture of salicylic acid, H₂SO₄, H₂O₂ and selenium (Novozamsky *et al.*, 1983). Hydrogen peroxide breaks down the organic matter at 100°C. The excess H₂O₂ naturally decomposed and the water produced evaporated. Completion of organic matter breakdown was done using H₂SO₄ at 330°C with Selenium catalysing the reactions. Potassium content was established through Flame Photometry, Phosphorus was calorimetrically determined on a spectrophotometer, while Nitrogen was determined calorimetrically using Segmented Flow Analyser (Walinga *et al.*, 1989).

3.17 Data analysis

Data on shoot dry weight, root dry weight, root length, leaf area, plant height, stem diameter, relative water content, specific leaf area, shoot N, P, K and mycorrhizal root colonization were checked for homogeneity of variance using Bartlett test followed by a two-way ANOVA in R software version 4.2.1. The factors were fungal inoculum and soil water level. Data of cowpea varieties were analyzed separately. Where variations of means were significant at $p = 0.05$, multiple mean comparison was done using Bonferroni.

CHAPTER FOUR

RESULTS

4.1 Soil physicochemical analysis

The soil that was used for the experiment was medium acidic with a pH of 5.22. The soil total organic carbon was adequate. Most of the available elements including K, Ca, Mg, Mn, Cu and Fe were also within the limits apart from P and Zn that were in low quantity (Table 4.1).

Table 4.1. The physicochemical analysis of soil that was used in the experiment

Soil properties	Value	Class
Soil pH	5.22	Medium acid
Exchange acidity (meq %)	0.3	Adequate
Total nitrogen %	0.8	High
Total Organic Carbon %	8.32	High
Phosphorous (ppm)	15	Low
Potassium (meq %)	0.64	Adequate
Calcium (meq %)	3.20	Adequate
Magnesium (meq %)	1.41	Adequate
Manganese (meq %)	0.79	Adequate
Copper (ppm)	1.00	Adequate
Iron (ppm)	59.4	Adequate
Zinc (ppm)	3.60	Low
Sodium (meq %)	0.28	Adequate

4.2 Influence of AMF and *Trichoderma* spp. on growth and water uptake of cowpea at different soil water status

The effect of single and co-inoculation of four fungal inoculants on growth and water uptake were tested of two cowpea varieties and each variety was treated with 3 different soil water levels. The fungal inoculants were *F. mosseae*, *R. irregularis*, *T. harzianum* and *T. asperellum*.

4.2.1 Influence of AMF and *Trichoderma* spp. on shoot dry weight

Results showed that water level had a significant ($p \leq 0.05$) influence on the shoot dry weight (SDW) for both K80 and K80 cowpea varieties (Figure 4.1). The SDW increased with increase in soil water from 30 to 90 % FC. For both varieties, 30 % recorded a significantly less SDW than 60 and 90 % FC.

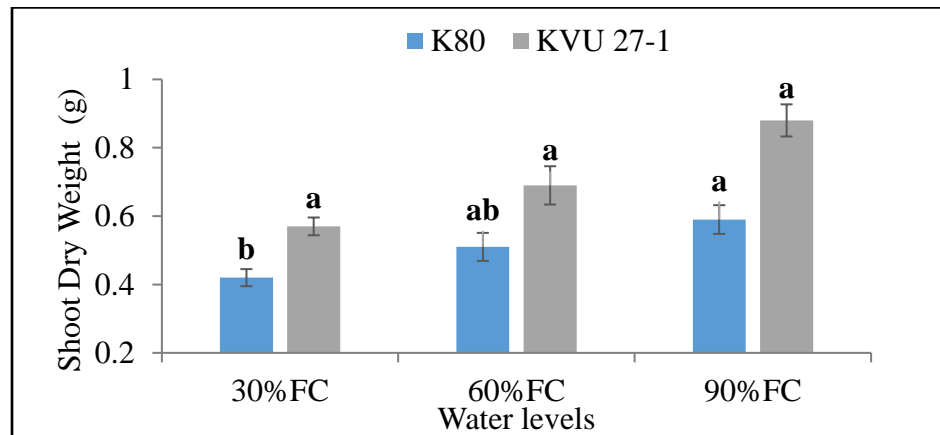


Figure 4.1. Shoot Dry Weight (g) for two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

Inoculants did not have a significant effect on SDW for both varieties. Additionally, the interaction between water level and fungal inoculants did not differ significantly (Table 4.2).

Table 4.2: Means of shoot dry weight of two cowpea varieties treated with AMF and *Trichoderma* at different soil water levels

Cowpea Varieties	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%C	30%FC	60%FC	90%C
Fungal inoculant						
BEG 12	0.67±0.05 ^a	0.68±0.15 ^a	0.98±0.08 ^a	0.42 ±0.05 ^a	0.53 ±0.15 ^a	0.57 ±0.05 ^a
BEG44	0.60±0.05 ^a	0.71±0.10 ^a	0.84±0.32 ^a	0.47 ±0.11 ^a	0.52 ±0.19 ^a	0.54 ±0.19 ^a
Har	0.60±0.04 ^a	0.67±0.23 ^a	0.96±0.14 ^a	0.39 ±0.05 ^a	0.58 ±0.12 ^a	0.65 ±0.14 ^a
Asp	0.58±0.06 ^a	0.75±0.20 ^a	0.84±0.21 ^a	0.45 ±0.04 ^a	0.59 ±0.13 ^a	0.70 ±0.09 ^a
BB	0.60±0.31 ^a	0.65±0.27 ^a	0.86±0.17 ^a	0.45 ±0.10 ^a	0.44 ±0.12 ^a	0.63 ±0.10 ^a
HarS	0.42±0.09 ^a	0.69±0.16 ^a	0.91±0.11 ^a	0.43 ±0.08 ^a	0.56 ±0.17 ^a	0.65 ±0.07 ^a
HBB	0.49±0.06 ^a	0.67±0.13 ^a	1.04±0.20 ^a	0.45 ±0.08 ^a	0.50 ±0.06 ^a	0.58 ±0.15 ^a
Control	0.51±0.05 ^a	0.58±0.21 ^a	0.81±0.07 ^a	0.32 ±0.06 ^a	0.32 ±0.04 ^a	0.41 ±0.09 ^a
P values						
Inoculant		0.976			0.673	
Water level		0.001			0.016	
Inoculant*Water level		0.901			0.953	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.2. Influence of AMF and *Trichoderma* spp. on root dry weight

Increased soil water caused a significant ($p \leq 0.05$) increase on the root dry weight for KVVU 27-1 but not for K80 (Figure 4.2). For KVVU 27-1, RDW at 60 and 90 % was not significantly different.

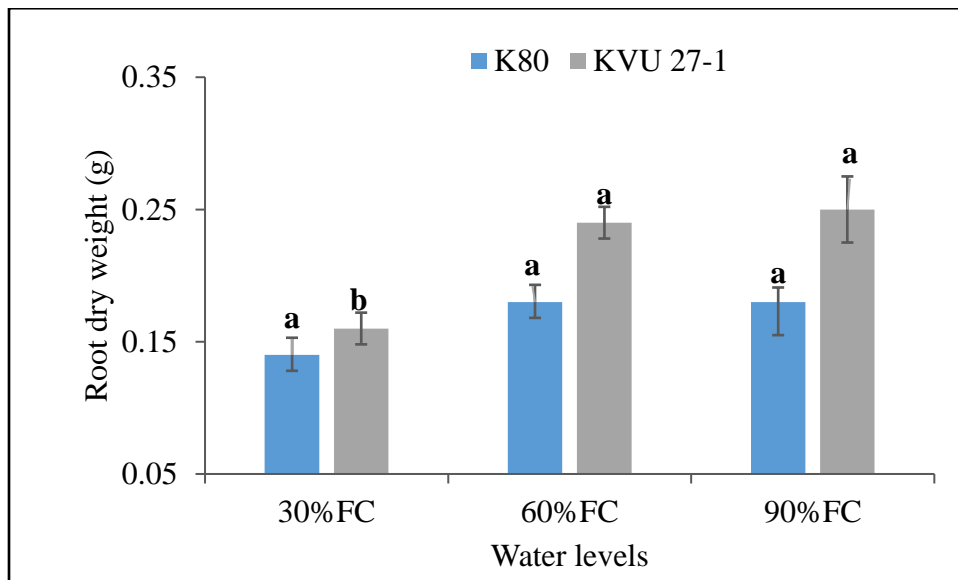


Figure 4.2. Root Dry Weight (g) means for two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

The effect of inoculants was not significant for both varieties, neither was the interaction between water levels and inoculants at $p \leq 0.05$ (Table 4.3).

Table 4.3: Means of root dry weights of two cowpea varieties treated with AMF and *Trichoderma* at different soil water levels

Cowpea varieties	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%FC	30%FC	60%FC	90%FC
Fungal inoculant						
BEG 12	0.17±0.03 ^a	0.27±0.01 ^a	0.39±0.18 ^a	0.12±0.06 ^a	0.18±0.03 ^a	0.22±0.03 ^a
BEG44	0.18±0.03 ^a	0.19±0.04 ^a	0.23±0.03 ^a	0.14±0.04 ^a	0.15±0.04 ^a	0.19±0.02 ^a
Har	0.19±0.03 ^a	0.19±0.05 ^a	0.29±0.03 ^a	0.16±0.04 ^a	0.17±0.01 ^a	0.22±0.04 ^a
Asp	0.14±0.01 ^a	0.25±0.02 ^a	0.28±0.03 ^a	0.12±0.02 ^a	0.14±0.04 ^a	0.25±0.07 ^a
BB	0.16±0.02 ^a	0.20±0.05 ^a	0.22±0.05 ^a	0.12±0.07 ^a	0.17±0.05 ^a	0.19±0.17 ^a
HarS	0.12±0.02 ^a	0.24±0.03 ^a	0.25±0.04 ^a	0.13±0.03 ^a	0.18±0.16 ^a	0.19±0.03 ^a
HBB	0.22±0.06 ^a	0.27±0.02 ^a	0.29±0.03 ^a	0.15±0.02 ^a	0.17±0.02 ^a	0.23±0.06 ^a
Control	0.08±0.02 ^a	0.19±0.02 ^a	0.19±0.03 ^a	0.12±0.05 ^a	0.13±0.17 ^a	0.18±0.01 ^a
P values						
Inoculant		0.810			0.533	
Water level		0.002			0.048	
Inoculant*Water level		0.143			0.127	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.3 Influence of AMF and *Trichoderma* spp. on root length

The increase in soil water caused a significant increase in root length for both cowpea varieties (Figure 4.3).

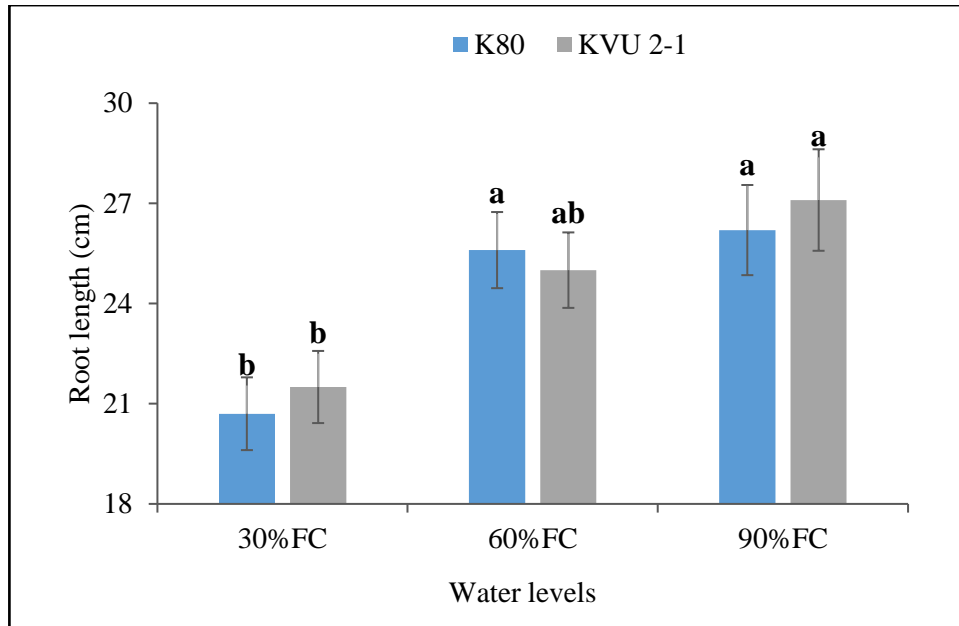


Figure 4.3. Means of Root Length (cm) for two cowpea varieties under 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

The fungal inoculants did not have a significant effect on root length for both varieties, however, the inoculated plants showed higher means as compared to the controls (Table 4.3). The interaction between the two factors did not also show significant differences at $p \leq 0.05$ (Table 4.4).

Table 4.4: Means of root lengths of two cowpea varieties treated with AMF and *Trichoderma* at different soil water levels

Cowpea variety	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%FC	30%FC	60%FC	90%FC
Fungal inoculant						
BEG 12	22.2±0.9 ^a	24.6±3.8 ^a	29.3±6.0 ^a	23.8±4.4 ^a	25.3±3.3 ^a	29.8±5.4 ^a
BEG44	25.6±2.8 ^a	27.6±2.7 ^a	29.9±4.6 ^a	20.9±2.9 ^a	22.5±1.2 ^a	23.0±2.1 ^a
Har	21.2±2.0 ^a	24.5±2.7 ^a	27.0±3.4 ^a	19.4±3.9 ^a	30.9±2.3 ^a	31.5±3.6 ^a
Asp	21.6±4.5 ^a	26.2±3.0 ^a	26.8±3.5 ^a	20.6±3.6 ^a	25.0±1.7 ^a	27.0±2.0 ^a
BB	21.6±2.4 ^a	24.0±1.5 ^a	24.6±5.5 ^a	20.8±3.9 ^a	23.6±7.3 ^a	30.4±4.4 ^a
HarS	22.9±3.3 ^a	26.7±1.4 ^a	36.1±3.6 ^a	22.7±2.5 ^a	25.8±1.5 ^a	27.8±4.1 ^a
HBB	19.5±2.9 ^a	20.8±0.7 ^a	28.1±2.1 ^a	21.4±1.7 ^a	24.4±1.7 ^a	25.8±3.9 ^a
Control	15.3±3.6 ^a	20.3±3.6 ^a	22.8±2.9 ^a	14.5±2.2 ^a	21.6±2.7 ^a	23.1±3.5 ^a
P values						
Inoculant		0.367			0.464	
Water level		0.009			0.004	
Inoculant*Water level		0.436			0.678	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.4 Influence of AMF and *Trichoderma* spp. on stem diameter

Stem diameter increased significantly ($p \leq 0.05$) with the increase in soil water level for both varieties (Figure 4.4).

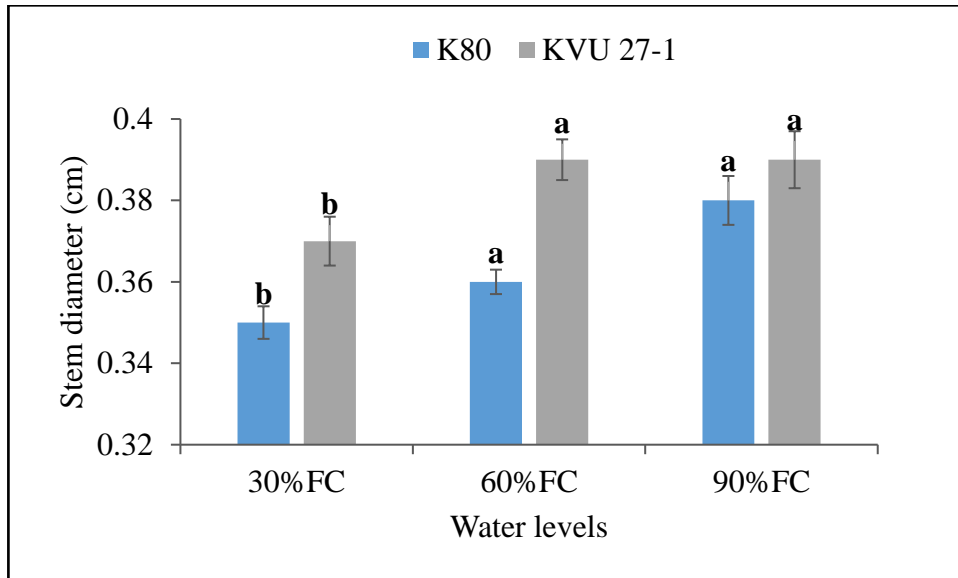


Figure 4.4: Means of stem diameters for two cowpea varieties under 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

Unlike in previous parameters where fungal inoculation elicited no differences, their effect on stem diameter of K80 was significant (Figure 4.5). All inocula increased stem diameter significantly compared to the control. Treatment with *Trichoderma harzianum* and *Trichoderma asperellum* (HarS) elicited the widest stem diameter (Figure 4.5).

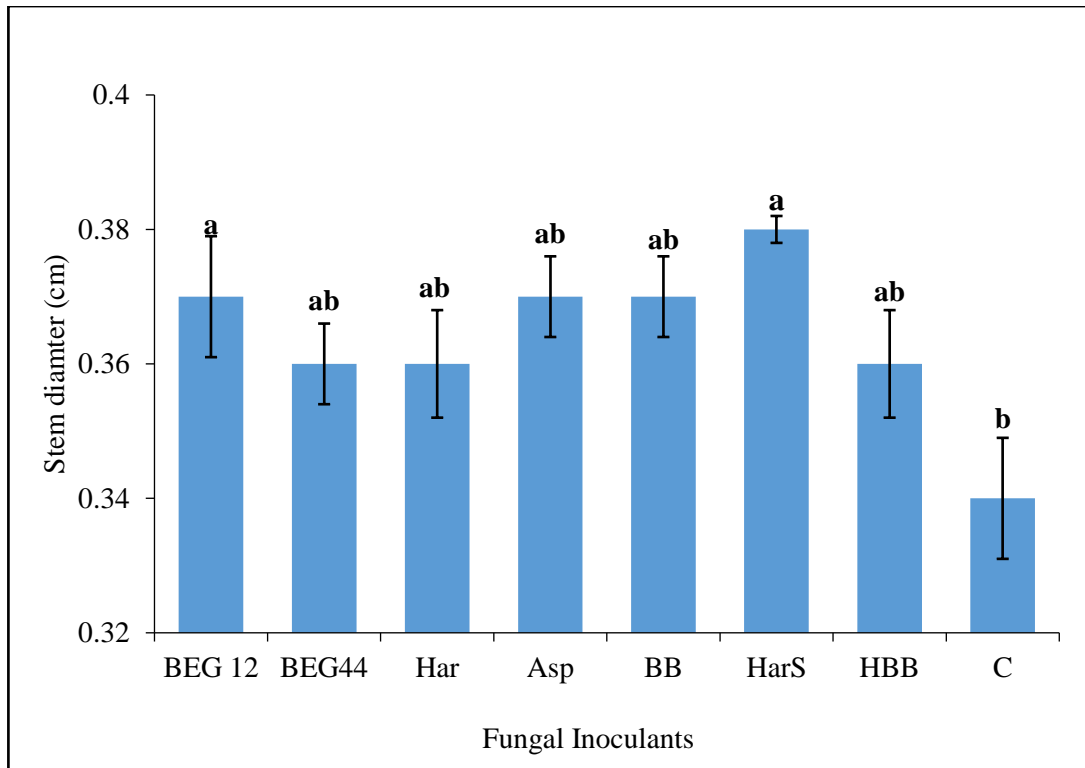


Figure 4.5: Means of stem diameters for variety K80 under different fungal inocula. Bars with different letters indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control (un-inoculated).

The interaction between water levels and inocula was also significant at $p = 0.002$ for KVU 27-1 and $p = 0.003$ for K80 (Table 4.5). Variety KVU 27-1 had widest stems in HarS and *Rhizophagus irregularis* (BEG44) inoculation, both at 90 % FC, and the thinnest were in control at 30 % FC. Similarly, in K80, HarS had the widest stems followed by *Funneliformis mosseae*

(BEG12), both at 90 % water level, while the thinnest stems were recorded in control at 30 % FC.

Table 4.5: Means of stem diameters of two cowpea varieties treated with AMF and *Trichoderma* at different soil water levels

Cowpea variety	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%FC	30%FC	60%FC	90%FC
Fungal inoculant						
BEG 12	0.36±0.02 ^{ab}	0.38±0.01 ^{ab}	0.40±0.01 ^{ab}	0.35±0.0 ^{ab}	0.36±0.0 ^{abc}	0.41±0.0 ^{bc}
BEG44	0.37±0.02 ^{ab}	0.39±0.01 ^{ab}	0.43±0.01 ^b	0.35±0.0 ^{ab}	0.36±0.0 ^{abc}	0.36±0.0 ^{abc}
Har	0.38±0.00 ^{ab}	0.39±0.01 ^{ab}	0.41±0.01 ^{ab}	0.34±0.0 ^a	0.36±0.0 ^{abc}	0.37±0.0 ^{abc}
Asp	0.38±0.01 ^{ab}	0.38±0.01 ^{ab}	0.40±0.02 ^{ab}	0.35±0.0 ^{ab}	0.38±0.0 ^{abc}	0.39±0.0 ^{abc}
BB	0.36±0.01 ^{ab}	0.40±0.02 ^{ab}	0.41±0.01 ^{ab}	0.36±0.0 ^{ab}	0.36±0.0 ^{abc}	0.39±0.0 ^{abc}
HarS	0.37±0.02 ^{ab}	0.39±0.02 ^{ab}	0.44±0.02 ^b	0.35±0.0 ^{abc}	0.37±0.0 ^{ab}	0.42±0.0 ^c
HBB	0.37±0.02 ^{ab}	0.37±0.01 ^{ab}	0.41±0.02 ^{ab}	0.36±0.0 ^{abc}	0.36±0.0 ^{abc}	0.36±0.0 ^{abc}
Control	0.33±0.01 ^a	0.35±0.01 ^{ab}	0.36±0.02 ^{ab}	0.33±0.0 ^a	0.33±0.0 ^a	0.37±0.0 ^a
P values						
Inoculant		0.452			0.006	
Water level		0.006			<0.001	
Inoculant*Water level		0.002			0.003	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.5 Influence of AMF and *Trichoderma* spp. on leaf area

Analysis showed that leaf area significantly ($p \leq 0.05$) increased with increase in water for both cowpea varieties (Figure 4.6).

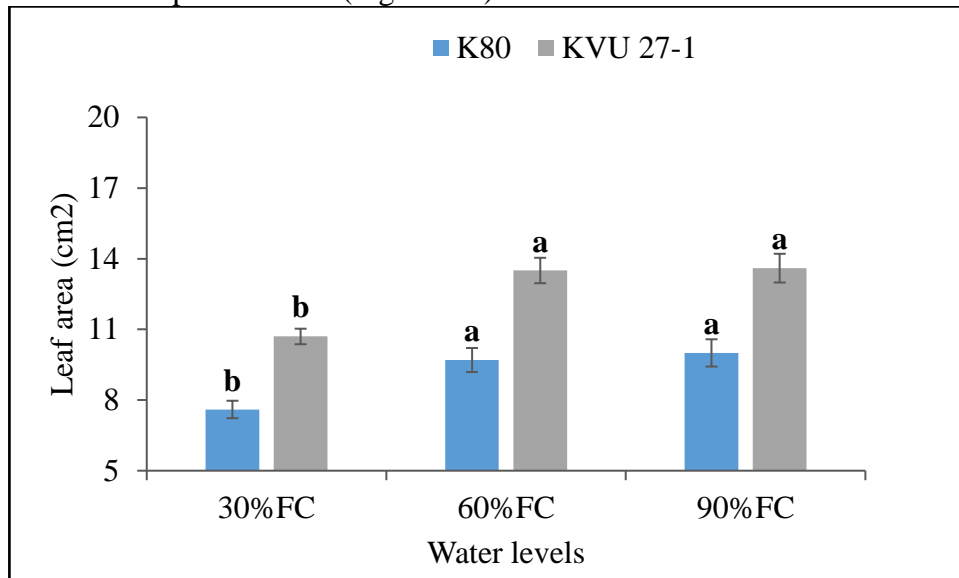


Figure 4.6: Means of Leaf Areas for two cowpea varieties under 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

The fungal inoculants did not significantly at $p \leq 0.05$ influence the leaf area though the inoculated plants exhibited higher leaf areas than their controls at each water level (Table 4.6). The interaction between the fungal inoculants and soil water levels did not also significantly determine the leaf area for both varieties ($p = 0.088$ for KVU 27-1 and $p = 0.634$ for K80) (Table 4.6).

Table 4.6: Means of leaf areas of two cowpea varieties treated with AMF and *Trichoderma* at different soil water levels

Cowpea variety	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%C	30%FC	60%FC	90%C
Fungal inoculant						
BEG 12	12.3±0.7 ^a	12.7±0.5 ^a	15.1±0.7 ^a	8.9±0.4 ^a	10.6±0.7 ^a	13.4±0.7 ^a
BEG44	10.6±0.6 ^a	15.9±2.1 ^a	16.6±2.0 ^a	7.6±1.6 ^a	10.4±2.0 ^a	12.0±2.8 ^a
Har	10.5±0.6 ^a	13.4±0.9 ^a	13.6±1.5 ^a	6.4±1.2 ^a	8.9±1.5 ^a	10.6±1.9 ^a
Asp	10.4±0.9 ^a	13.8±2.0 ^a	14.2±0.3 ^a	7.9±0.3 ^a	9.5±0.9 ^a	9.7±1.5 ^a
BB	11.0±1.8 ^a	12.6±1.8 ^a	16.9±0.6 ^a	8.9±2.1 ^a	9.4±1.0 ^a	10.6±0.8 ^a
HarS	11.2±1.2 ^a	11.7±2.2 ^a	14.4±1.7 ^a	7.6±0.9 ^a	8.3±1.0 ^a	8.5±1.5 ^a
HBB	9.8±2.0 ^a	11.6±1.2 ^a	12.3±1.1 ^a	7.3±1.0 ^a	9.1±1.4 ^a	11.6±0.6 ^a
Control	9.7±0.6 ^a	10.6±1.7 ^a	12.0±2.2 ^a	5.4±0.6 ^a	7.6±1.6 ^a	7.9±1.4 ^a
P values						
Inoculant		0.536			0.051	
Water level		<0.001			0.001	
Inoculant*Water level		0.088			0.634	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.6 Influence of AMF and *Trichoderma* spp. on relative water content

The relative water content determined in leaves increased significantly ($p \leq 0.05$) with increase in the soil water level for both varieties (Figure 4.7).

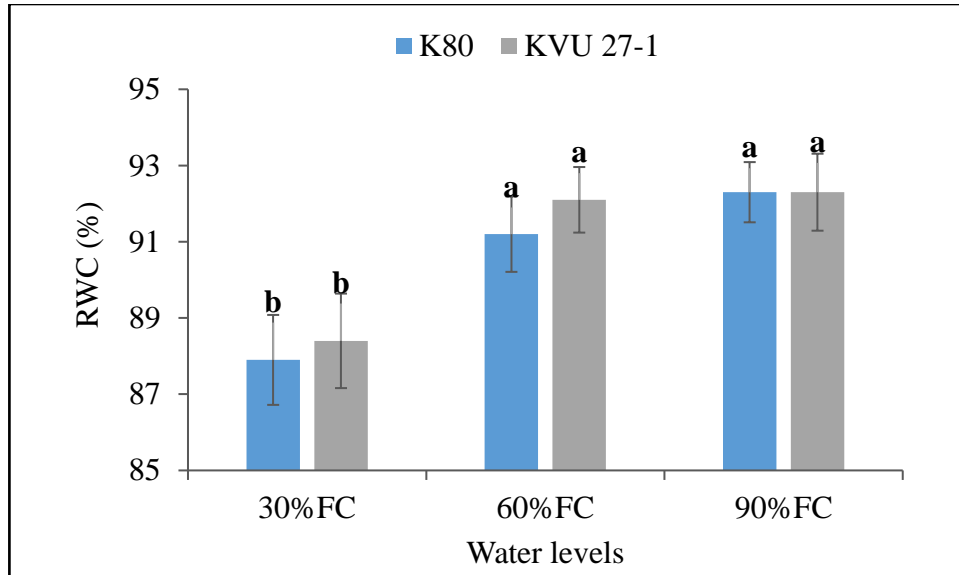


Figure 4.7. Means of relative water content for two cowpea varieties under different fungal inocula. Bars with different letters within each variety indicate that the means are significantly different. RWC- relative water content.

As noted in most parameters, only 30 % had significantly lower RWC for both varieties.

Likewise, inoculants had a significant effect on the RWC for both cowpea varieties (Figure 4.8). In both varieties, the control had a significantly lower RWC compared to all the inocula.

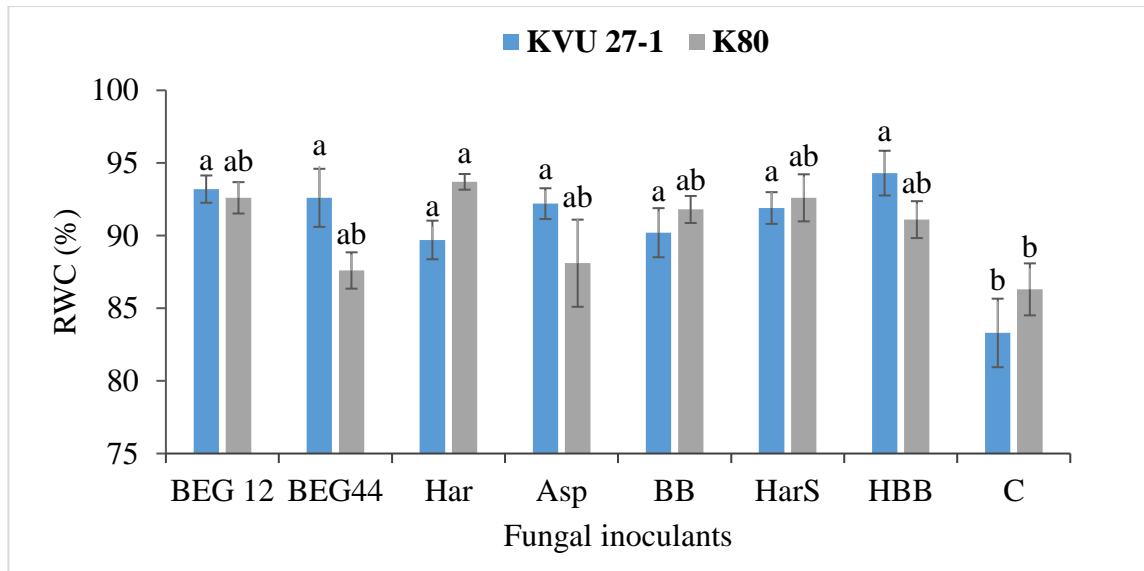


Figure 4.8. Means of relative water content for both varieties at different fungal inocula. Bars with different letters within each variety indicate that the means are significantly different. RWC- relative water content, BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

In K80, RWC was highest in the dual inoculation of *T. harzianum* + *T. asperellum* (HarS) with 97.3 % at 90 % FC. The lowest RWC in K80 was control at 80.3% at the lowest soil water level. On the other hand, the interaction between the two factors had no significant effect at $p \leq 0.05$ for K80, but effect was significant for K80 27-1 ($p = 0.005$) where the lowest value was in control at 30 % FC and the highest were recorded in all inocula except the control under 90 % FC (Table 4.7).

Table 4.7: Means of relative leaf water content two cowpea varieties treated with AMF and *Trichoderma* at different soil water levels

Cowpea variety	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%FC	30%FC	60%FC	90%FC
Fungal inoculant						
BEG 12	90.3±1.1 ^{abc}	93.5±1.6 ^{bc}	95.9±0.6 ^c	91.9±2.2 ^{ab}	92.7±1.8 ^{ab}	93.3±2.1 ^{ab}
BEG44	86.5±4.9 ^{abc}	94.4±0.4 ^{bc}	96.8±0.6 ^c	84.5±2.6 ^{ab}	88.8±1.8 ^{ab}	89.4±1.6 ^{ab}
Har	86.3±2.6 ^{abc}	89.3±1.2 ^{abc}	93.6±1.6 ^{bc}	92.8±0.8 ^{ab}	93.0±0.9 ^b	95.3±0.9 ^{ab}
Asp	90.2±1.6 ^{abc}	91.7±2.1 ^{bc}	94.6±1.4 ^c	83.7±3.2 ^{ab}	91.2±5.0 ^{ab}	92.9±1.8 ^{ab}
BB	86.7±2.9 ^{abc}	89.0±2.9 ^{abc}	94.8±1.6 ^c	89.0±1.5 ^{ab}	92.9±0.8 ^{ab}	93.4±1.5 ^{ab}
HarS	89.3±2.5 ^{abc}	92.7±1.2 ^{bc}	93.5±1.5 ^{bc}	87.2±2.8 ^{ab}	93.3±1.7 ^{ab}	97.3±0.7 ^b
HBB	88.7±2.9 ^{abc}	95.8±0.9 ^c	98.4±0.7 ^c	88.3±2.6 ^{ab}	91.8±2.2 ^{ab}	93.3±1.4 ^{ab}
Control	78.6±4.3 ^a	81.6±2.9 ^{ab}	89.9±4.3 ^{abc}	80.3±6.3 ^a	85.9±3.3 ^{ab}	89.4±2.9 ^{ab}
P values						
Inoculant		<0.001			0.002	
Water level		0.001			0.002	
Inoculant*Water level		0.005			0.180	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.7 Influence of AMF and *Trichoderma* spp. on specific leaf area

Specific Leaf Area (SLA) of both cowpea varieties increased with increase in water level.

Effects of all three water levels on SLA differed significantly ($p \leq 0.05$) for both varieties

(Figure 4.9).

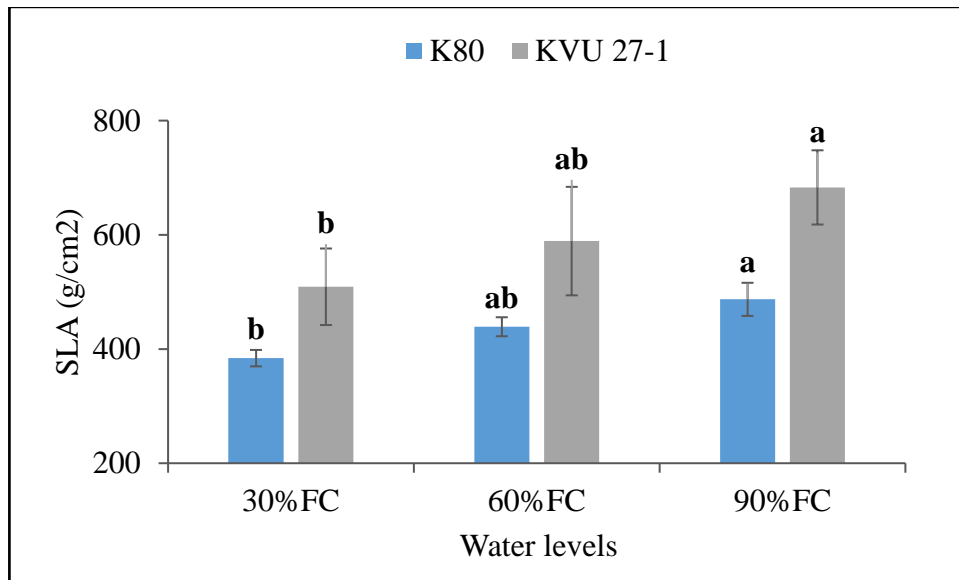


Figure 4.9. Means of specific leaf area for two cowpea varieties under 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

Fungal inoculants had a significant effect for KVU 27-1 but not K80 (Figure 4.10).

Combinations of *T. harzianum* + *T. asperellum* (HarS) was significantly the highest as

compared to the control that had the lowest SLA.

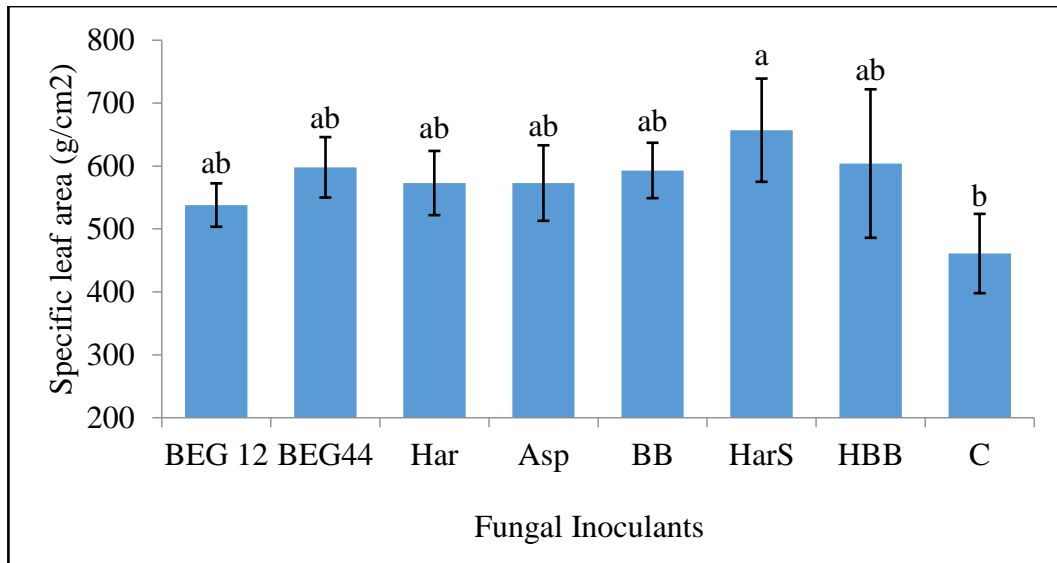


Figure 4.10. Means of specific leaf area for variety KVVU 27-1 at different fungal inocula. Bars with different letters indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

Further, the interaction of the two factors gave a significantly higher SLA only in KVVU 27-1 under inoculants HarS and BB, both at 90 % FC ($p \leq 0.001$) (Table 4.8).

Table 4.8: Effect of AMF and *Trichoderma* on the specific leaf area of cowpea at three soil water levels

Cowpea variety	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%FC	30%FC	60%FC	90%FC
Fungal inoculant						
BEG 12	459±57 ^a	540±155 ^a	616±56 ^a	448±19.1 ^{ab}	495±28.0 ^{ab}	505±77.1 ^{ab}
BEG44	564±77 ^a	566±48 ^a	663±123 ^a	421±58.8 ^{ab}	486±51.1 ^{ab}	504±30.4 ^{ab}
Har	506±39 ^a	561±108 ^a	652±109 ^a	326±27.4 ^a	412±24.3 ^{ab}	471±33.0 ^{ab}
Asp	439±101 ^a	572±75 ^a	723±131 ^a	338±26.3 ^{ab}	375±51.2 ^{ab}	477±92.4 ^{ab}
BB	415±45 ^a	536±59 ^a	828±67 ^{ab}	395±48.3 ^{ab}	494±40.0 ^{ab}	665.±177.8 ^b
HarS	498±133 ^a	623±80 ^a	849±118 ^{ab}	405±3.8 ^{ab}	451±62.5 ^{ab}	486±53.6 ^{ab}
HBB	454±24 ^a	536±85 ^a	821±89 ^b	432±21.3 ^{ab}	434±61.4 ^{ab}	450±25.6 ^{ab}
Control	359±31 ^a	472±29 ^a	552±97 ^a	308±22.3 ^a	363±39.2 ^{ab}	447±19.7 ^{ab}
P values						
Inoculant		0.049			0.142	
Water level		0.010			0.003	
Inoculant*Water level		<0.001			0.275	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.2.8 Influence of AMF and *Trichoderma* spp. on cowpea height

Results indicated that plant height increased progressively from 21 DAS to 49 DAS in both KVVU 27-1 and K80 cowpea varieties (Figures 4.11). It was also shown that for the two varieties, the height increased from 21 DAS to 49 DAS for each soil water level of 30, 60 and 90 %. Shorter plants were observed at 30 % while 90 % FC recorded the tallest plants for both varieties.

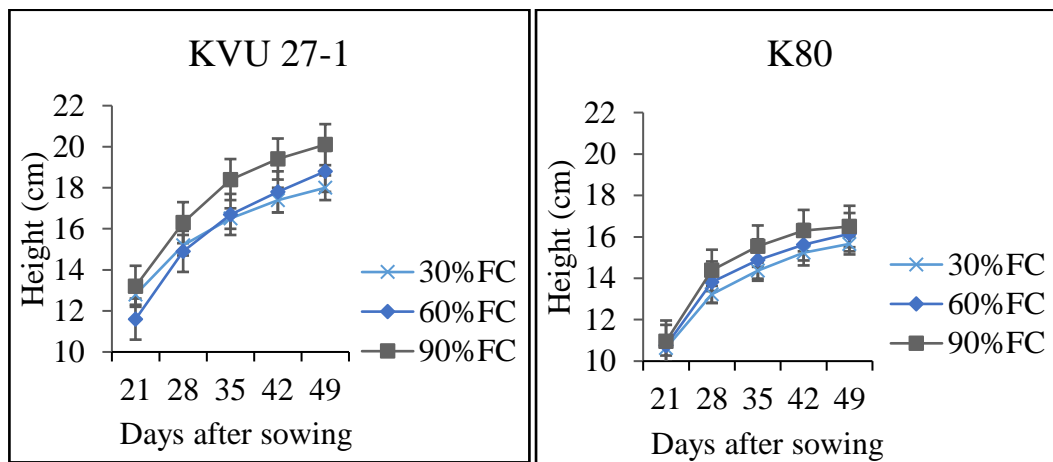


Figure 4.11. Means of Heights for two cowpea varieties under 30%, 60% and 90% soil water levels.

In each water level, KVVU 27-1 had higher average means than K80. *Funneliformis mosseae* (BEG12) influenced the highest average height means at 30%FC and *T. asperellum* (Asp) influencing the highest means at both 60% and 90%FC (Figure 4.12, 4.13 and 4.14).

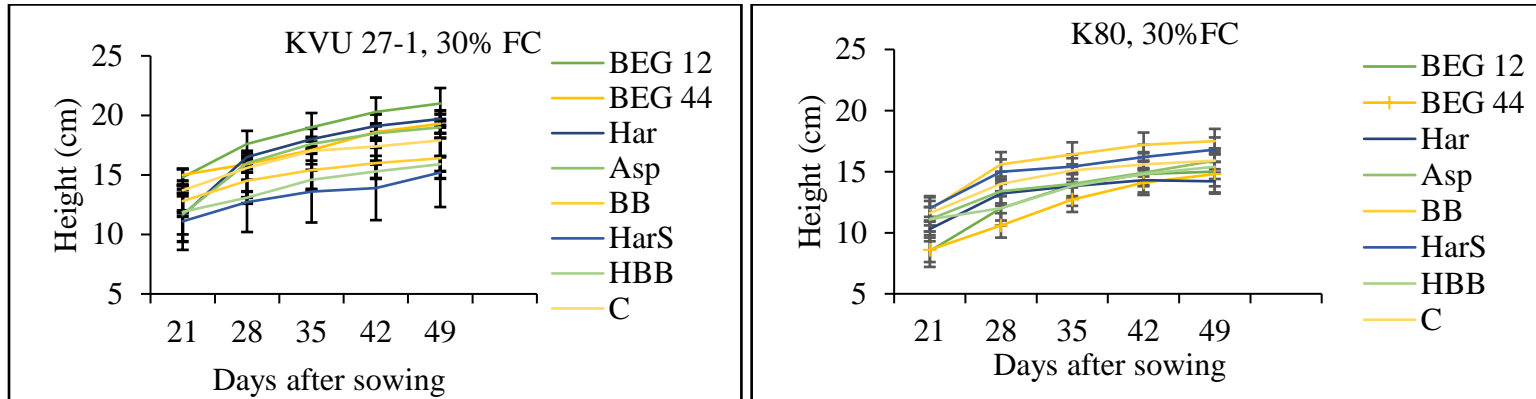


Figure 4.12. Means of heights for KVVU 27-1 and K80 at 30% FC

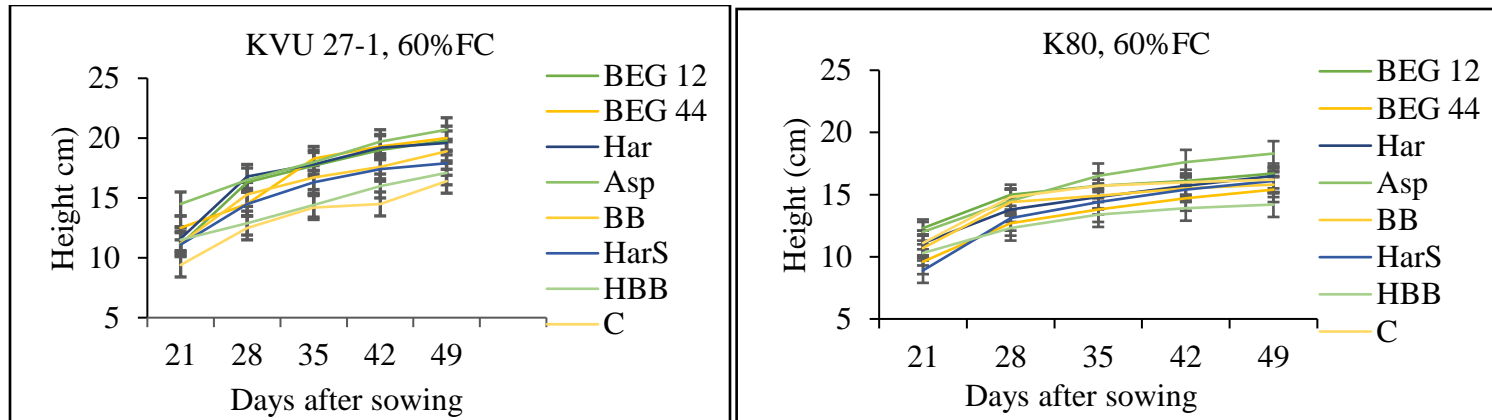


Figure 4.13. Means of heights for KVVU 27-1 and K80 at 60% FC.

Key: BEG12 (*Funneliformis mosseae*), BEG44 (*Rhizophagus irregularis*), Har (*Trichoderma harzianum*), Asp (*Trichoderma asperellum*), BB (BEG12+BEG44), HarS (Har+Asp), HBB (Har+BEG12+BEG44), C (Control).

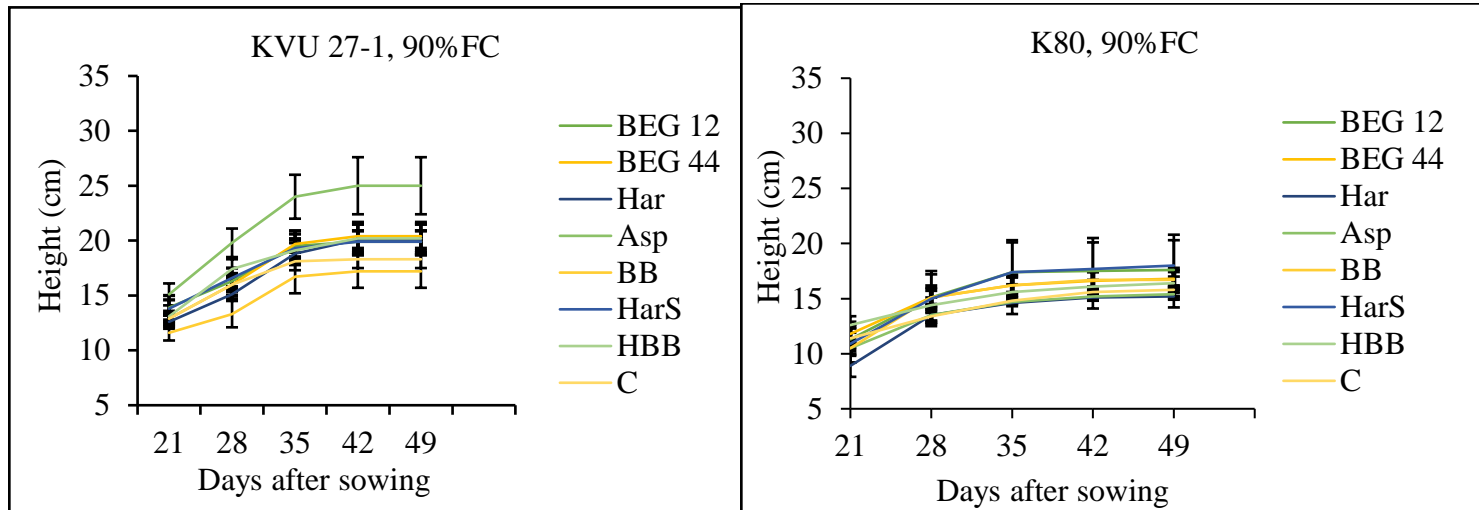


Figure 4.14. Means of height for KVVU 27-1 and K80 at 90% FC. Key: BEG12 (*Funneliformis mosseae*), BEG44 (*Rhizopagus irregularis*), Har (*Trichoderma harzianum*), Asp (*Trichoderma asperellum*), BB (BEG12+BEG44), HarS (Har+Asp), HBB (Har+BEG12+BEG44), C (Control).

4.2.9 Influence of AMF and *Trichoderma* spp. on cowpea chlorophyll content

There was an increase in chlorophyll content in the course of growth for the two cowpea varieties. It was also shown that for the two varieties, chlorophyll content increased at each water level of 30 %, 60 % and 90 % FC. Low chlorophyll content was recorded at 30 % FC while the high chlorophyll content was at 90 % FC for both varieties. The K80 recorded higher chlorophyll content values at 30% and 60% than K80 (Figure 4.15).

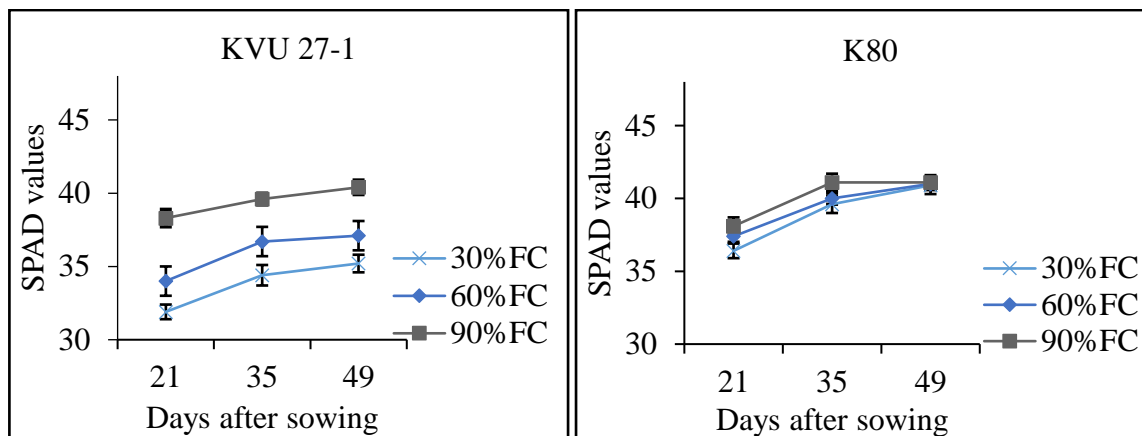


Figure 4.15. Means of chlorophyll content for two cowpea varieties under 30%, 60% and 90% soil water levels

At 30% FC, the inoculation of BB and *T. asperellum* (Asp) influenced the highest chlorophyll content in KVVU 27-1 and K80 respectively (Figure 4.16). The inoculation of HBB influenced the highest average chlorophyll content in both varieties at 60% FC. At 90% FC, *T. harzianum* (Har) and HBB elicited the highest average chlorophyll contents in KVVU 27-1 and K80 respectively.

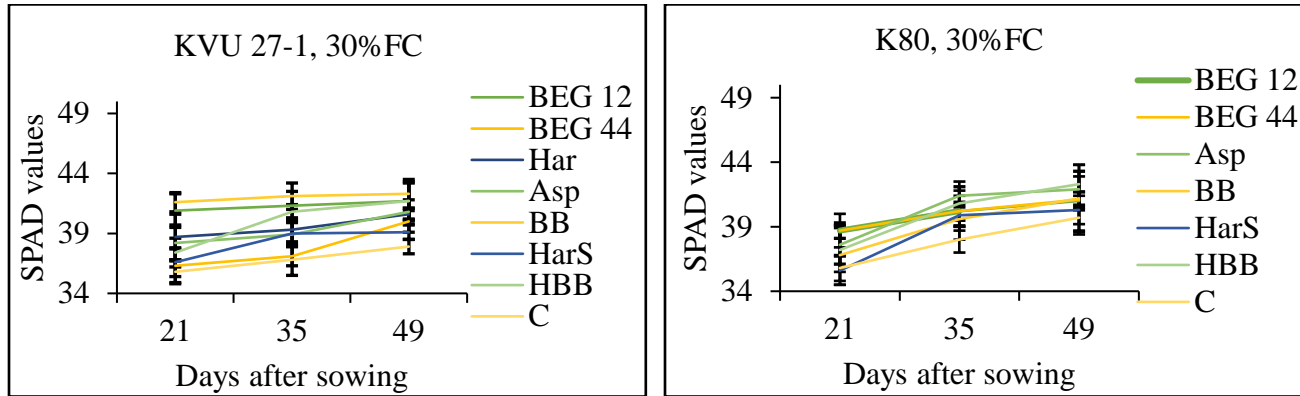


Figure 4.16. Means of chlorophyll content for KVVU 27-1 and K80 at 30% FC with different fungal inoculations.

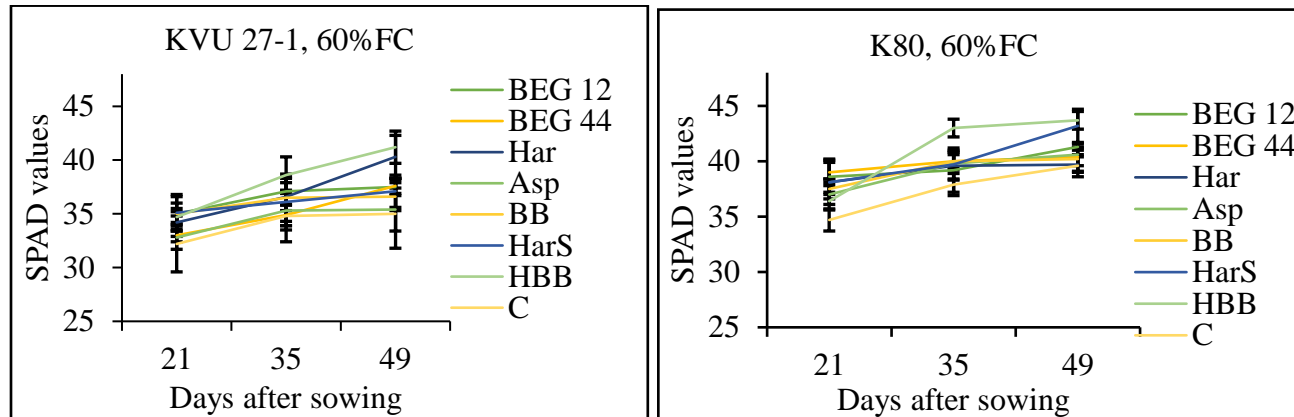


Figure 4.17. Means of chlorophyll content for KVVU 27-1 and K80 at 60% FC with different fungal inoculations.

Key: BEG12 (*Funneliformis mosseae*), BEG44 (*Rhizophagus irregularis*), Har (*Trichoderma harzianum*), Asp (*Trichoderma asperellum*), BB (BEG12+BEG44), HarS (Har+Asp), HBB (Har+BEG12+BEG44), C (Control).

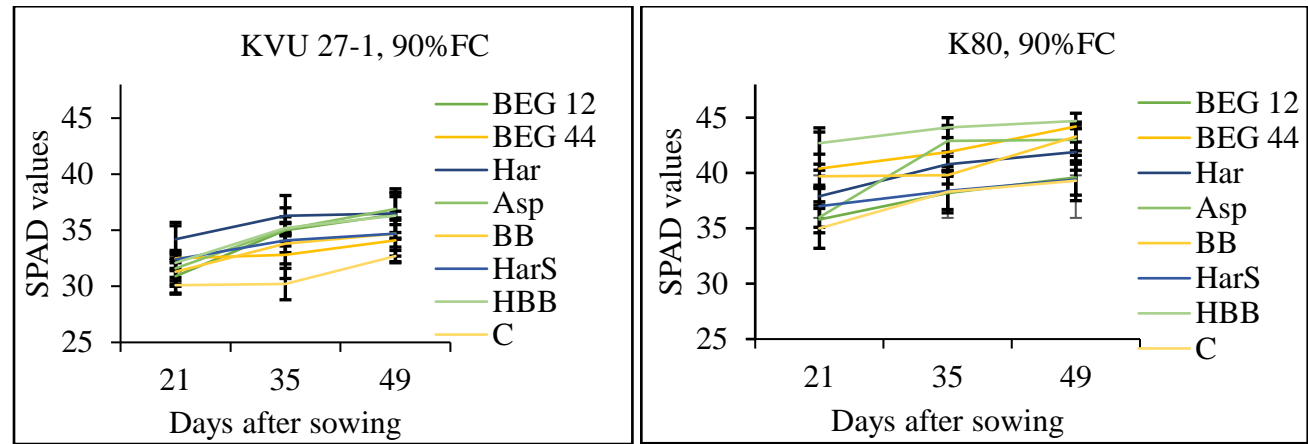


Figure 4.18. Means of chlorophyll content for KVVU 27-1 and K80 at 90% FC with different fungal inoculations. BEG12 (*Funneliformis mosseae*), BEG44 (*Rhizophagus irregularis*), Har (*Trichoderma harzianum*), Asp (*Trichoderma asperellum*), BB (BEG12+BEG44), HarS (Har+Asp), HBB (Har+BEG12+BEG44), C (Control).

4.3 Influence of AMF and *Trichoderma* spp. on nutrient uptake in cowpea at different soil water status

The effect of fungal inoculants on uptake of nitrogen (N), phosphorous (P) and potassium (K) was tested on two cowpea varieties at three different soil water levels. The interaction between water levels and the fungal inoculants was statistically significant on nutrient uptake in both cowpea varieties except for P in KVVU 27-1 at $p = 0.721$.

4.3.1 Influence of AMF and *Trichoderma* spp. on nitrogen content

There was a significant increase ($p \leq 0.05$) in nitrogen content in the shoots of both KVVU 27-1 and K80 cowpea varieties (Figure 4.19). Nitrogen content increased significantly with increase in water level from 30 to 90 %. At 30% FC, K80 recorded a lower nitrogen content while at 90% FC, KVVU 27-1 recorded a higher nitrogen content (Figure 4.19).

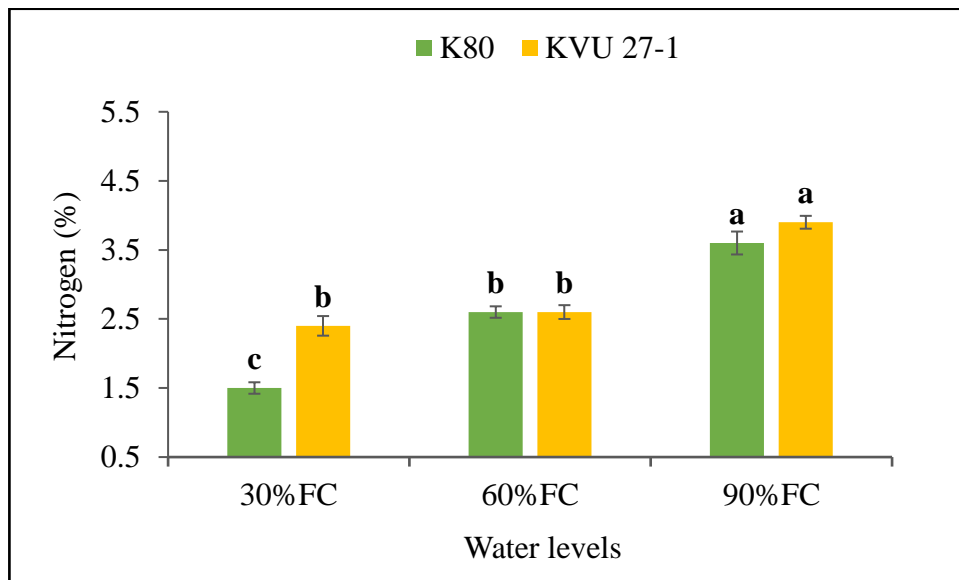


Figure 4.19. Nitrogen content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

The fungal inoculants had a significant effect on the nitrogen content in both cowpea varieties (Figure 4.20). The controls in both varieties had the lowest nitrogen content while HBB in K80 and HarS in K80 had the highest N uptake.

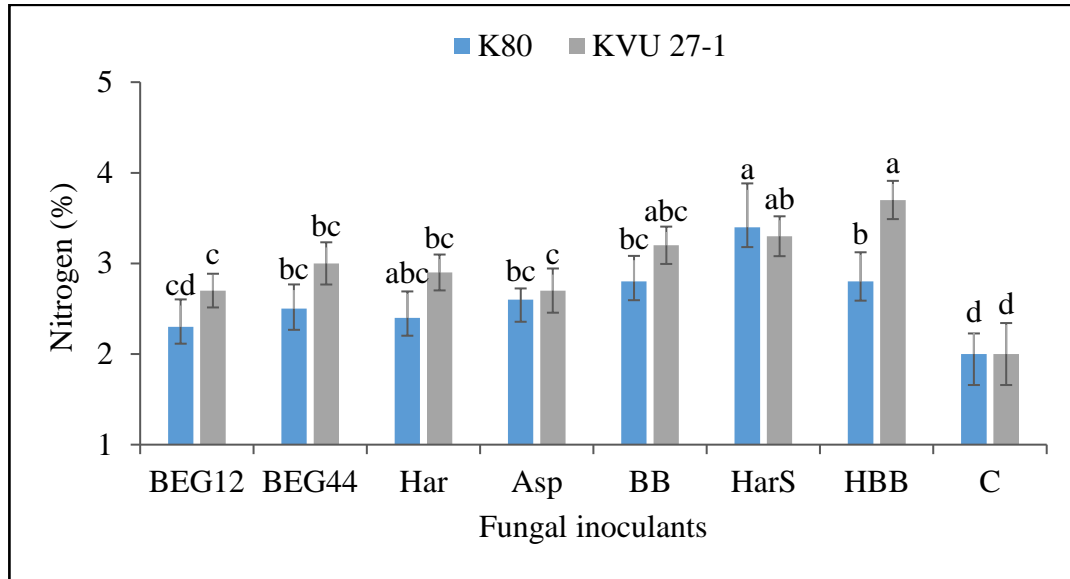


Figure 4.20. Means of nitrogen content at different fungal inocula. Bars with different letters within each variety indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

The interaction between water levels and the fungal inocula was also significant at $p = 0.001$ for K80 27-1 and $p < 0.001$ for K80 (Table 4.9). Co-inoculation of *T. harzianum* + *T. asperellum* (HarS) in K80 and *T. harzianum* + *F. mosseae* + *R. irregularis* (HBB) in K80 27-1 recorded the highest nitrogen content at 5.56 % and 4.56 % respectively, both at 90 % FC.

Table 4.9: Effect of AMF and *Trichoderma* on nitrogen content of cowpea at three soil water levels

Cowpea Varieties	KVU 27-1 Soil water level			K80 Soil water level		
	30%FC	60%FC	90% C	30%FC	60%FC	90% C
Fungal inoculant						
BEG 12	2.27±0.21 ^{bcd}	2.38±0.07 ^{bcde}	3.51±0.14 ^{efghij}	1.00 ±0.76 ^a	2.65 ±0.20 ^{efghij}	3.30 ±0.14 ^{ghijk}
BEG44	2.25±0.29 ^{bcd}	3.01±0.15 ^{cdefgh}	3.88±0.22 ^{hij}	1.46 ±0.10 ^{abc}	2.52 ±0.27 ^{efghi}	3.46 ±0.19 ^{ijk}
Har	2.40±0.17 ^{bcde}	2.65±0.17 ^{bcdefg}	3.64±0.30 ^{fghij}	1.34 ±0.09 ^{ab}	2.34 ±0.23 ^{cdefg}	3.57 ±0.19 ^{jk}
Asp	2.00±0.13 ^{abc}	2.39±0.14 ^{bcde}	3.77±0.21 ^{ghij}	2.04 ±0.11 ^{bcdef}	2.77 ±0.08 ^{efghij}	2.91 ±0.07 ^{fghij}
BB	3.21±0.22 ^{defghi}	2.60±0.25 ^{bcdef}	3.91±0.26 ^{hij}	1.51 ±0.18 ^{abcd}	3.38 ±0.16 ^{hijk}	3.43 ±0.15 ^{hijk}
HarS	2.71±0.23 ^{cdefg}	2.94±0.17 ^{cdefgh}	4.22±0.09 ^{ij}	2.21 ±0.14 ^{bcdef}	2.46 ±0.08 ^{defgh}	5.56 ±0.46 ^l
HBB	3.41±0.26 ^{efghi}	3.18±0.11 ^{defghi}	4.56±0.22 ^j	1.84 ±0.07 ^{abcde}	2.30 ±0.11 ^{bcdef}	4.21 ±0.28 ^k
Control	0.98±0.08 ^a	1.52±0.15 ^{ab}	3.50±0.29 ^{efghij}	1.02 ±0.06 ^a	2.18 ±0.12 ^{b^{cdef}}	2.75 ±0.17 ^{efghij}
P values						
Inoculant		<0.001			<0.001	
Water level		<0.001			<0.001	
Inoculant*Water level		0.001			<0.001	

Means followed by different letters within each variety are significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.3.2 Influence of AMF and *Trichoderma* spp. on phosphorous content

The phosphorous content in both varieties increased significantly with increase in water level for both varieties (Figure 4.21). At 30% FC, low phosphorous content was observed for both varieties though K80 had a lower phosphorous content than K80 (Figure 4.21).

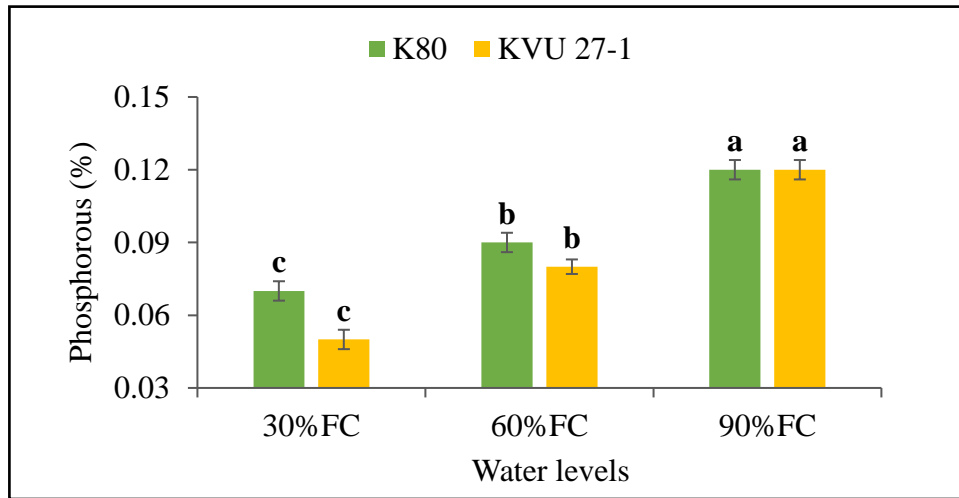


Figure 4.21. Phosphorous content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

Just like for nitrogen, the effect of fungal inoculants was statistically different for both varieties at $p \leq 0.05$ (Figure 4.22). Both HBB and HarS had the highest values for both varieties. The controls likewise recorded significantly lower phosphorous content in both varieties.

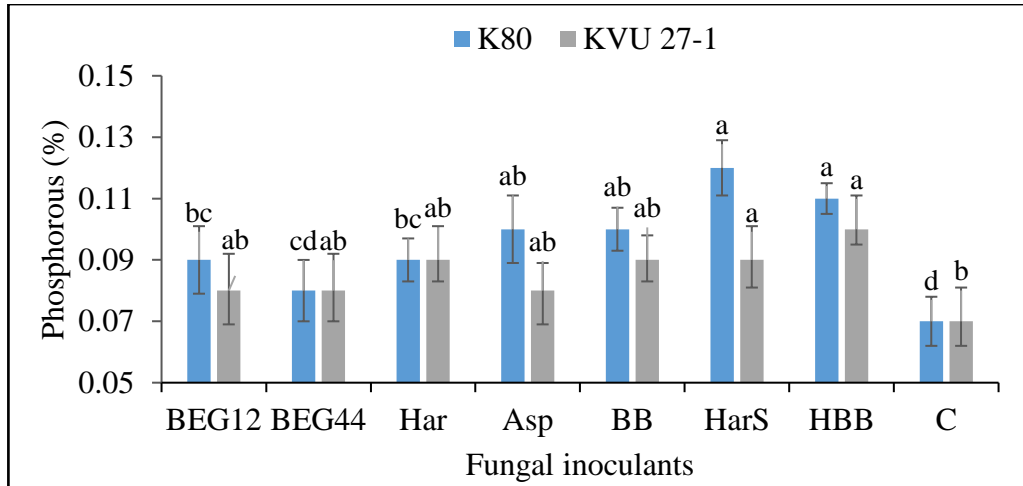


Figure 4.22. Means of phosphorous content for both varieties at different fungal inocula. Bars with different letters within each variety indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS-(Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

The interaction between the two factors differed significantly at $p = 0.002$ in K80, but not in KVU 27-1 (Table 4.10). In both KVU 27-1 and K80, the co-inoculation of *T. harzianum* + *T. asperellum* (HarS) and *T. harzianum* + *F. mosseae* + *R. irregularis* (HBB) elicited the highest phosphorous content both at 90 % soil water level.

Table 4.10: Effect of AMF and *Trichoderma* on the phosphorous content of cowpea at three soil water levels

Cowpea Varieties	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90% C	30%FC	60%FC	90% C
Fungal inoculant						
BEG 12	0.04±0.02 ^{ab}	0.08±0.01 ^{abcde fgh}	0.12±0.00 ^{fgh}	0.05 ±0.01 ^{ab}	0.10 ±0.00 ^{cdefg}	0.13 ±0.01 ^{ghi}
BEG44	0.04±0.01 ^a	0.08±0.00 ^{abcde fgh}	0.13±0.01 ^{fgh}	0.04±0.00 ^a	0.08 ±0.01 ^{abcde}	0.12 ±0.00 ^{fghi}
Har	0.05±0.01 ^{abc}	0.08±0.01 ^{abcde fgh}	0.13±0.00 ^{gh}	0.07 ±0.01 ^{abcd}	0.10 ±0.01 ^{cdefg}	0.12 ±0.00 ^{fghi}
Asp	0.06±0.01 ^{abcde}	0.07±0.01 ^{abcde f}	0.12±0.01 ^{defgh}	0.06 ±0.01 ^{abc}	0.10 ±0.00 ^{defgh}	0.14 ±0.00 ^{hi}
BB	0.06±0.01 ^{abcde}	0.08±0.00 ^{abcde fgh}	0.12±0.01 ^{efgh}	0.09 ±0.01 ^{bcdef}	0.10 ±0.02 ^{defgh}	0.12 ±0.00 ^{fghi}
HarS	0.07±0.01 ^{abcde f}	0.08±0.01 ^{abcde f}	0.14±0.01 ^h	0.09 ±0.00 ^{bcdefg}	0.11 ±0.00 ^{defgh}	0.15 ±0.01 ⁱ
HBB	0.06±0.01 ^{abcde}	0.10±0.00 ^{bcde fgh}	0.14±0.02 ^h	0.11 ±0.00 ^{defgh}	0.12 ±0.01 ^{efghi}	0.12 ±0.01 ^{fghi}
Control	0.04±0.01 ^{ab}	0.06±0.01 ^{abcd}	0.10±0.02 ^{cde fgh}	0.04 ±0.00 ^a	0.07±0.01 ^{abcd}	0.10 ±0.00 ^{defg}
P values						
Inoculant		0.020			<0.001	
Water level		<0.001			<0.001	
Inoculant*Water level		0.721			0.002	

Means followed by different letters within each variety are significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.3.3 Influence of AMF and *Trichoderma* spp. on the potassium content

Results showed that potassium content in the shoots increased significantly ($p \leq 0.05$) with increase in water level (Figure 4.23).

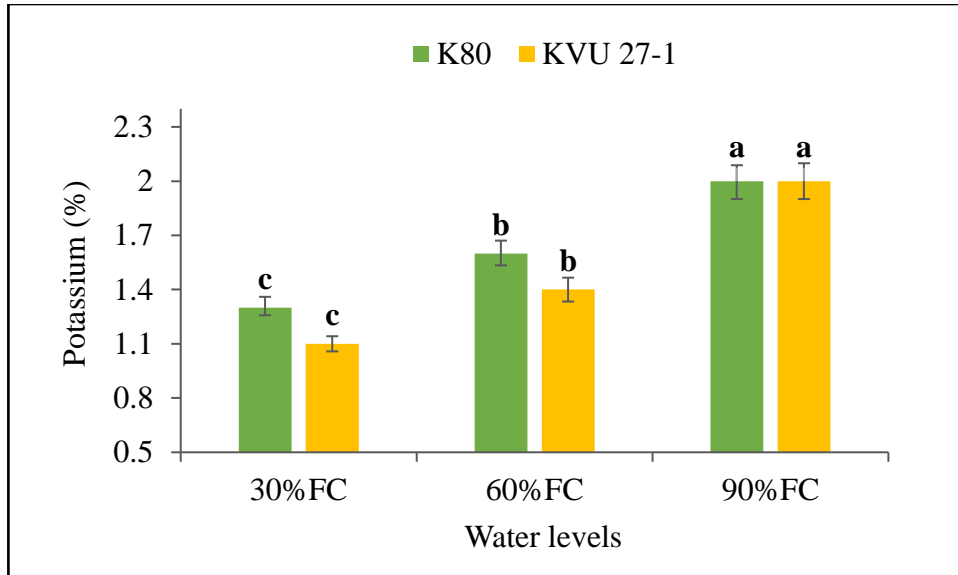


Figure 4.23. Potassium content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

The effect of inoculants on potassium content was also statistically significant at $p \leq 0.05$

For K80, HarS and HBB resulted in the highest potassium uptake (Figure 4.24).

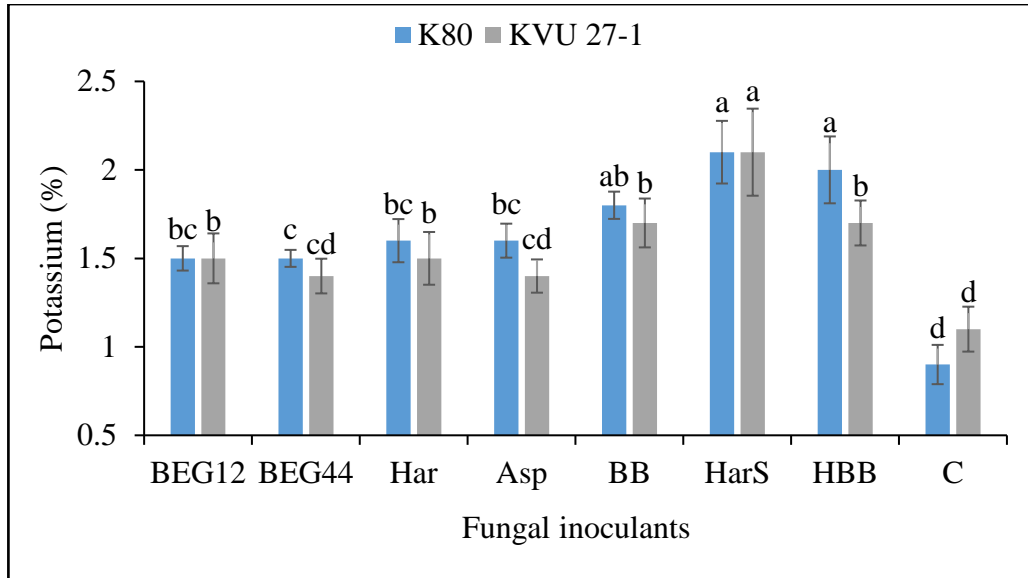


Figure 4.24. Means of potassium content at different fungal inocula. Bars with different letters within indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizopagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

Interaction between the fungal inoculants and soil water levels were statistically significant at $p = <0.001$ for KVVU 27-1 and $p = 0.003$ for K80. In KVVU 27-1, the dual inoculation of *T. harzianum* and *T. asperellum* (HarS) recorded the highest potassium content at 90% soil water level. (Table 4.11).

Table 4.11: Effect of AMF and *Trichoderma* on the potassium content of cowpea at three soil water levels

Cowpea Varieties	KVU 27-1			K80		
	Soil water level			Soil water level		
	30%FC	60%FC	90%C	30%FC	60%FC	90%C
Fungal inoculant						
BEG 12	1.11±0.04 ^{abcde}	1.22±0.06 ^{abcdef}	2.06±0.18 ^{ghi}	1.27 ±0.08 ^{bc}	1.63 ±0.08 ^{cdef}	1.72 ±0.04 ^{cdef}
BEG44	1.06±0.06 ^{abcd}	1.37±0.12 ^{abcdefg}	1.67±0.16 ^{defghi}	1.40 ±0.09 ^{bcde}	1.53 ±0.05 ^{bcdef}	1.61 ±0.09 ^{cdef}
Har	0.97±0.04 ^{abc}	1.66±0.22 ^{cdefghi}	1.78±0.26 ^{efghi}	1.33 ±0.08 ^{bcd}	1.46 ±0.05 ^{bcde}	2.16 ±0.15 ^{fg}
Asp	1.11±0.10 ^{abcde}	1.33±0.11 ^{abcdef}	1.66±0.16 ^{cdefghi}	1.34 ±0.05 ^{bcd}	1.61 ±0.05 ^{cdef}	1.96 ±0.18 ^{def}
BB	1.23±0.13 ^{abcdef}	1.59±0.06 ^{bcdefghi}	2.25±0.09 ⁱ	1.55 ±0.06 ^{bcdef}	1.93 ±0.09 ^{cdef}	2.04 ±0.11 ^{efg}
HarS	1.22±0.10 ^{abcdef}	1.85±0.08 ^{fghi}	3.15±0.07 ^j	1.61 ±0.08 ^{cdef}	1.88 ±0.34 ^{cdef}	2.69 ±0.04 ^g
HBB	1.40±0.18 ^{abcdefg}	1.45±0.07 ^{abcdefg}	2.18±0.10 ^{hi}	1.52 ±0.06 ^{bcdef}	1.79 ±0.17 ^{cdef}	2.68 ±0.03 ^g
Control	0.91±0.10 ^a	0.77±0.12 ^{ab}	1.55±0.20 ^{bcdefgh}	0.56 ±0.08 ^a	0.93 ±0.15 ^{ab}	1.32 ±0.10 ^{bcd}
P values						
Inoculant		<0.001			<0.001	
Water level		<0.001			<0.001	
Inoculant*Water level		<0.001			0.003	

Means followed by different letters within each variety are significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control.

4.4 The effect of different soil water levels on root colonization by AMF

Four inoculants that included BEG12, BEG44, BB and HBB were used to determine the extent of mycorrhizal colonization in cowpea roots. Un-inoculated plants recorded zero colonization. For plants inoculated with AMF, colonization increased significantly ($p \leq 0.05$) with decrease in water stress for both cowpea varieties (Figure 4.25). At each soil water level KVVU 27-1 roots exhibited higher mycorrhizal colonization than in K80.

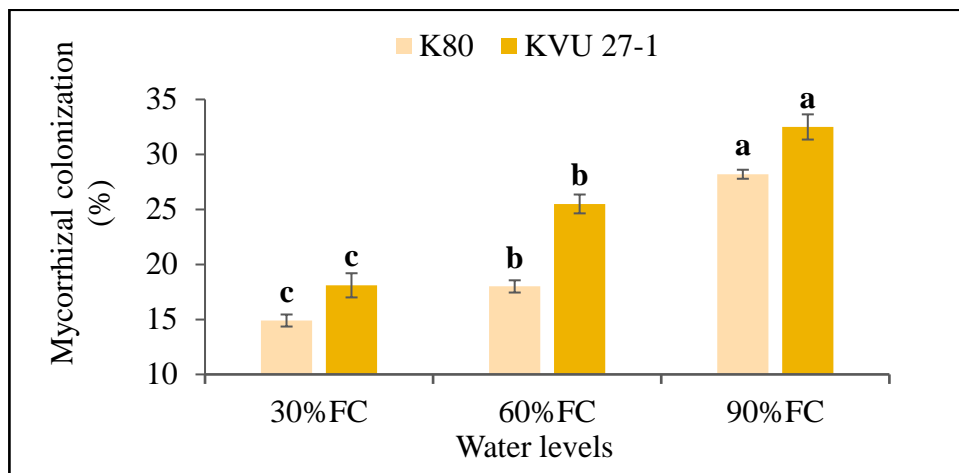


Figure 4.25. Mycorrhizal colonization in the two cowpea varieties at 30%, 60% and 90% soil water levels. Bars with different letters within each variety indicate that the means are significantly different.

Comparison between the fungal inoculants showed that BB and HBB colonized plants in significantly higher numbers than the other inoculants. This was true for both cowpea varieties (Figure 4.26).

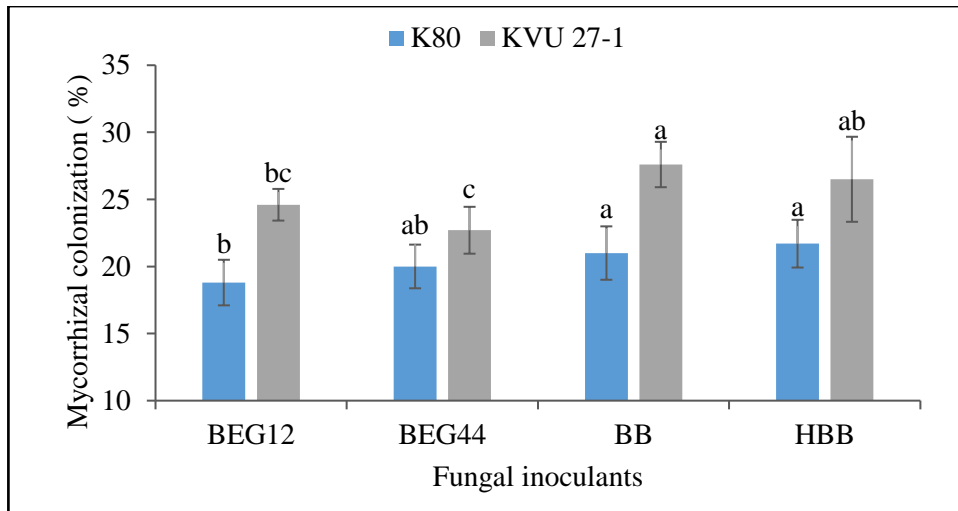


Figure 4.26. Means of mycorrhizal colonization with different fungal inocula. Bars with different letters within each variety indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, BB-(BEG12+BEG44), HBB-(Har+BEG12+BEG44)

The interaction between the fungal inoculants and the water levels was significant at $p = <0.001$ in KVU 27-1 (Table 4.12). The highest colonization means in KVU 27-1 were of the co-inoculation of *T. harzianum* + *F. mosseae* + *R. irregularis* (BB), while in K80 the highest values were from the combined inoculation of *F. mosseae* + *R. irregularis* (BB) at 90 % FC. In K80, all inoculants performed the same at 90 % FC.

Table 4.12: Effect of the three soil water levels on the mycorrhizal colonization of cowpea

Cowpea Varieties	KVU 27-1 Soil water level			K80 Soil water level		
	30%FC	60%FC	90%C	30%FC	60%FC	90%C
Fungal inoculant						
BEG 12	20.8±1.7 ^{bc}	25.1±1.2 ^{cde}	28.0±1.5 ^{def}	13.7 ±1.1 ^a	16.2 ±0.2 ^{abc}	26.4 ±0.6 ^d
BEG44	16.3±0.8 ^{ab}	21.8±1.0 ^{bcd}	30.0±0.8 ^{ef}	16.0 ±1.1 ^{abc}	16.7 ±0.2 ^{abc}	27.5 ±0.6 ^d
BB	22.1±1.5 ^{bcd}	26.3±0.5 ^{cde}	34.3±1.8 ^{fg}	14.4 ±1.2 ^a	19.0 ±1.2 ^{bc}	29.6 ±0.4 ^d
HBB	13.0±0.7 ^a	28.8±1.9 ^{ef}	37.8±0.9 ^g	15.5 ±1.1 ^{ab}	20.3 ±1.0 ^c	29.3 ±0.5 ^d
P values						
Inoculant		<0.001			0.001	
Water level		<0.001			<0.001	
Inoculant*Water level		<0.001			0.191	

Means followed by the same letter within each variety are not significantly different at $p \leq 0.05$ (Bonferroni). BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, BB-(BEG12+BEG44), HBB-(Har+BEG12+BEG44).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

5.1.1. Soil physicochemical analysis

In this study the pH of the experimental soil was 5.22 meaning it was acidic. The lower pH increases the solubility of Mn and Fe that slightly increases the toxicity to plants by slowing root growth and microbial activities (Ste-Marie and Houle, 2006). According to Alkobaisy (2022) and Kawaka *et al.* (2014) for successful symbiosis with cowpea, fungi require either neutral or alkaline soil conditions. The soil nitrogen and organic matter were high and within the recommended values by Okalebo *et al.* (2002) therefore considered fertile.

5.1.2. Influence of AMF and *Trichoderma* spp. on growth and water uptake of cowpea at different soil water contents

The influence of arbuscular mycorrhizal fungi and *Trichoderma* spp. on growth of two cowpea varieties at the three water levels was determined in this study. Parameters measured included shoot dry weight, root dry weight, root length, leaf area, plant height, stem diameter, relative water content, and specific leaf area. In most of the parameters, the interaction between water levels and fungal inocula was not statistically different. The effect of soil water content was significant in all parameters. Although AMF and *Trichoderma* spp. promoted growth compared to the control, not all parameters recorded significant differences between the inocula.

As soil water content increased from 30 % to 90 % field capacity (FC) all parameters of growth increased significantly. Ninety percent water content produced significantly higher growth than the other levels. This implies that although cowpea still yields at low soil water content, maximum foliage yields can be obtained with high supply of water. These results are in conformity with the findings of Ntombela (2012) who reported a significant reduction in growth of cowpea due to water deficits. Al Hassan *et al.* (2015) also observed that the vegetative growth of tomatoes was inhibited when tomato plants were subjected to different levels of water stress in the field conditions. Water stress restricts the growth of roots in the soil reducing the absorptive area for water and nutrients that leads to reduced growth. Low soil moisture content reduces cell turgidity, causes stomatal closure and destruction of photosynthetic pigments impairing the photosynthetic machinery of plants which decreases biomass accumulation (Anjum *et al.*, 2011). Optimal turgor leads to cell elongation culminating in overall plant growth. Increased surface area for carbon IV oxide exchange improves photosynthetic efficiency. Increased soil water status increases solubilization, absorption and transportation of nutrients up the plant as well as mass flow of nutrients which leads to increased growth (Ortas, 2010).

Both AMF and *Trichoderma* spp. promoted increase in root length which led to the buildup of the root mass. Symbiosis between the fungi and cowpea induced development of roots. Though the means for root length were low at severe water stress (30 % FC), the inoculated plants showed greater root length than the controls. These results concur with the findings of Colla *et al.* (2015) who recorded that a mixture of *Glomus intraradices*

(AMF) and *Trichoderma atroviride* on lettuce and melon led to the increase in root length therefore increasing their absorptive area. Other studies that support these findings are Harman *et al.* (2004) and Stewart and Hill (2014) who observed the increased root length in maize and tomato respectively after inoculating them with *T. harzianum*. The fungi, in particular *Trichoderma* induces growth-promoting substances including phytohormones like IAA, gibberellins and cytokinins that regulate root development (Martínez-medina *et al.*, 2011; Chagas *et al.*, 2016).

Plant height, leaf area and stem diameter increased with decrease in soil water stress. Inoculated plants had higher means as compared to the un-inoculated controls that led to increase in total shoot dry weight for both cowpea varieties. These results are consistent with those of Hashem *et al.* (2018), who recorded increased shoot elongation and leaf area after inoculating cowpea with *Trichoderma* spp. This led to the increase in the total shoot mass. This study recorded lower shoot dry weight at severe water stress than at higher water levels probably because of lower bulking of photoassimilates due to reduced photosynthetic area.

This study observed a low leaf area at severe water stress for both cowpea varieties. This is consistent with Olorunwa *et al.* (2021) who observed reduced leaf area in cowpea under water stress. The plants' productivity is pegged on the leaf area (Souza *et al.*, 2017). Though a small leaf area is a water conservation strategy to minimize water loss through evaporative surface, it reduces the photosynthetic area of the plant. In most cases at severe

soil water stress, stomatal conductivity reduces to minimize water loss hindering photosynthetic activities thus low bulk of plants biomass. This explains why in this study the specific leaf area (SLA), a ratio between the leaf area and the leaf biomass progressively reduced with increase in water deficits in both cowpea varieties. This phenomenon was also observed by Melo *et al.* (2021) who found that reduction of SLA is an adaptive mechanism that reduces water loss from evaporative leaf surfaces. According to Zhou *et al.* (2020), under water deficits plants reduce SLA by increasing the leaf thickness which results in thicker cell walls and tighter connections to prevent water loss by evaporation to achieve higher water use efficiency.

Enhancement of plant water status influenced by AMF and *Trichoderma* was demonstrated in increased leaf relative water content (RWC) under different fungal inocula treatments. Inoculated cowpea showed enhanced RWC than their controls. The highest RWC was obtained through the inoculation of HBB in KVVU 27-1 and Har in K80. *Trichoderma* and AMF are known to increase root length (Colla *et al.*, 2015). Higher RWC with the two fungal inocula can be explained by AMF hyphae spreading over large volumes of soil enhancing surface area for absorption of water. *Trichoderma* and AMF also sustain stomatal opening which promotes water status of the leaves and their relative water content (Begum *et al.*, 2019). The fungi also regulate the function of phytohormones like abscisic acid (ABA) to improve stomata conductance during soil water deficit (Diagne *et al.*, 2020). Lastly, they improve synthesis of metabolites like jasmonic acid and

strigolactones, which alleviate moisture stress via osmotic adjustment enabling plants to maintain turgor.

Similar results were recorded by Hayatu and Muhammad (2014). Results show that cowpea maintained high water levels at all soil water levels compared. According to Pirzad *et al.* (2011), plants that are most resistant to drought have higher RWC. These results reveal that both KVVU 27-1 and K80 are able to perform better under drought stress due to their high RWC.

This study demonstrated the role of soil water levels on chlorophyll content in cowpea. It was observed that the increase in soil water stress reduced the chlorophyll content in both cowpea varieties. This observation concurs with the findings of Bolat *et al.* (2014) who recorded a decline in chlorophyll index in apple and quince root stalks by 46% and 51% respectively than their controls. Water deficits lower the chlorophyll content by destroying chlorophyll or preventing its synthesis (Kamanga *et al.*, 2018). However much a decline in chlorophyll content is an adaptive strategy by plants to reduce the absorption of excess energy, it reduces the photosynthetic capacity of the plant.

This study also showed the role of mycorrhizal fungi and *Trichoderma* spp. in enhancing the chlorophyll content in the two cowpea varieties. It was observed that AMF and *Trichoderma* inoculated plants had higher chlorophyll contents than the un-inoculated plants at each of 30%, 60% and 90% FC water levels. The results concur with the findings of Chandrasekaran *et al.* (2019) who reported an increase in total chlorophyll contents

after inoculating AMF with different C3 and C4 plants. Furthermore, *T. harzianum* increased the chlorophyll content in maize seedlings (Akladios and Abbas, 2012) and *Triticum aestivum* (Shukla *et al.*, 2014). Arbuscular mycorrhizal fungi and *Trichoderma* influences the increased uptake of phosphorous and magnesium that enhances increase in chlorophyll content (Chandrasekaran *et al.*, 2019).

Amongst the inoculations, HBB produced the highest chlorophyll content as compared to other inoculations including single inoculations. The co-inoculation between AMF and *Trichoderma* is also supported by Metwally and Abdelhameed (2018) who observed an increase in chlorophyll content in onion leaves after inoculating with a mixture of AMF strains and *T. viride*. According to Mwangi *et al.* (2009), the synergy between *Trichoderma* and AMF increases the solubilization of vital minerals like phosphorous to enable them be absorbed by AM fungi. Availability of these minerals enhances synthesis of chlorophyll. Chlorophylls are the chief photosynthetic pigments that enable growth and development in plants. High chlorophyll contents sustained by the fungal inoculations represents higher rates of photosynthesis which increases the plants biomass.

Higher chlorophyll content recorded at severe water stress in inoculated plants than in controls showed how the fungal inoculants cushioned the photosynthetic apparatus from oxidative damage. It also emerged that chlorophyll content increased progressively from 21st DAS to 49th DAS meaning that the chlorophyll content increased with the maturity of cowpea leaves. This occurrence was also observed by Kamble *et al.* (2015) who recorded

an increase in chlorophyll concentration in ten different leaf species as they progressed to maturity. It is possible that adult leaves accumulate more chlorophyll than younger leaves and it is an indicator of increased photosynthetic activities and metabolism of plant (Siwach and Gill, 2014).

Growth promotion observed in the study can be attributed to increased capacities of inoculated plants to absorb nutrients and water. Arbuscular mycorrhizal fungi greatly influences the surface area of the root system by extending their extraradicle hyphae in the soil forming a mycelial network (Chandrasekaran, 2022). The hyphae can explore a larger volume of soil than plant roots alone, accessing nutrients that may otherwise be inaccessible to the plant. At the same time, extensive fungal hyphae can access water from greater soil depths. Root development supported by *Trichoderma* enhances water uptake conferring advantage to plants during drought stress. During drought stress the less soluble nutrients are not accessed by the plant easily. *Trichoderma* spp. is believed to produce organic acids and enzymes that solubilizes and mobilizes nutrients especially phosphorous making them available for plant uptake in water stressed conditions.

5.1.3 Influence of AMF and *Trichoderma* spp. on nutrient uptake in cowpea at different soil water contents

This study recorded progressive increase in shoot nutrient contents from 30 % FC to 90 % FC in two cowpea varieties, KVU 27-1 and K80. Nitrogen (N), phosphorous (P) and

potassium (K) contents were significantly higher in the shoots of inoculated plants than in the un-inoculated controls irrespective of the water stress level. This indicated promotory impact of the two fungi in nutrient uptake in the two cowpea varieties. It also indicated that nutrient acquisition is reliant on water availability. The positive effect of AMF and *Trichoderma* spp. on plant nutrient acquisition is increased by availability of water.

At severe water stress, low nitrogen content was recorded in plant shoots. The inoculated plants had higher levels of nitrogen contents than the controls. In support of the findings of this study, increased rhizosphere N was recorded after the dual inoculation of *T. harzianum* and *T. virens* on *Pinus sylvestris* seedlings (Halifu *et al.*, 2019). Increased nitrogen levels in plants enhance enzyme activity, transport of nutrients resulting in plant growth and development (Torres-olivar *et al.*, 2014). The symbiosis between the plants' roots and *Trichoderma* spp. induces the release of hydrolase enzymes that degrade rhizospheric proteins and chitin into simpler compounds. These simple compounds are easily absorbed by the rhizospheric nitrogen-fixing microorganisms that probably lead to increased nitrogen levels in shoots (Khoshmanzar *et al.*, 2019). The colonization of roots by AM fungi might have led to formation of arbuscules, the point contacts for nutrient and water absorption that increased surface area of the root system that provided more sites for nutrient exchange that included nitrogen. Regardless of the soil water status, AMF caused the extension of their extra-radicle hyphae beyond the roots depletion zone which also enabled them to enhance uptake of nutrients including nitrogen and transporting them up the plant.

Phosphorous is insoluble in most of the soils therefore inaccessible for absorption by most plants (Zhang *et al.*, 2014). Phosphorous exists in complex forms and chemically bound to other elements making them less soluble and less accessed by plants. This study recorded higher shoot phosphorous contents in inoculated plants than the controls illustrating the role of AMF and *Trichoderma* isolates in its uptake. Combined inoculation of AMF and *Trichoderma* spp. (HBB) elicited the highest P shoot contents than when the inoculants were used separately in KVVU 27-1 variety. These findings concur with the results of Tchameni *et al.* (2011) on cocoa plants. They indicated that a high AMF and *Trichoderma* diversity in the soil creates greater fungal communities with broader benefits that complement each other benefiting the plant in terms of growth. The primary role of AM fungi in phosphorous absorption is their ability to enhance the uptake and transfer of phosphorous from the soil to the plant (Oruru *et al.*, 2018). The high affinity of AMF for P enables them to access P than plants alone. The AMF secrete phosphatase enzymes which break down P-containing compounds and release P to the most absorbable forms. *Trichoderma* spp. also secretes phosphate solubilizing enzymes and low molecular acids to solubilize inorganic phosphate (P_i) from their complex cationic forms into simple absorbable forms (Richardson and Simpson, 2011).

There was a progressive increase in potassium contents in the shoot of cowpea with decrease in water stress. Inoculated plants had higher shoot potassium contents than the

un-inoculated plants which indicated the positive impact of the fungi in increasing potassium uptake. Co-inoculation of *Trichoderma* spp. (*T. harzianum* and *T. asperellum*-HarS) had the highest potassium levels in both cowpea varieties. These results concur with Kundu *et al.* (2013) who noted increase in potassium levels after inoculating a mixture of four AMF isolates on sweet potatoes. According to Khoshmanzar *et al.* (2019), soil potassium exists in complex silicate-bonded form which cannot be absorbed directly by plant roots. *Trichoderma* produce organic acids that weather these minerals to release potassium to be easily absorbed by plant roots. The AMF hyphae colonize the root cells and form elaborate networks that extend into the soil to access potassium sinks that are further away from plant roots. The increase in shoot potassium level is vital for drought tolerance in plants. According to Soriano *et al.* (2009) and Bagheri *et al.* (2012), potassium regulates stomatal conductance hence controls the water status and photosynthetic activity of the plant. Under water-deficit conditions, there is low water absorption by plant roots. This is attributed to lower diffusion of potassium in drier soils.

5.1.4 The effect of different soil water contents on root colonization by AMF

This study demonstrated that the three soil water levels that included highly stressed (30 % FC), mildly stressed (60 % FC) and well-watered (90 % FC) affected the AMF root colonization differently in both KVU 27-1 and K80 varieties. There were four inoculations namely *F. mosseae* (BEG12), and *R. irregularis* (BEG44) both as individual fungi, and *F. mosseae* + *R. irregularis* (BB) and *T. harzianum* + *F. mosseae* + *R. irregularis* (HBB) as

combinations. A root section was considered colonized by AMF when mycorrhizal vesicles or arbuscules were clearly observed (Bourles *et al.*, 2020). Arbuscules and vesicles are structures formed within the roots of the host plants as a result of a symbiotic association between a fungus and a plant. These structures are contact points for the exchange of materials between the fungus and the host plant.

Generally, the mycorrhizal colonization percentage of the fungi on cowpea roots was relatively low as compared Tsikou *et al.* (2022) where the percentage colonization went up to 71. However, these results are in line with that of Pons and Muller (2022) who observed the highest total root length colonization ranging between (13-20)% on wheat. The low root mycorrhizal colonization may have been caused by edaphic factors including low soil pH. It's most probable that the low soil pH of the experimental soil might have affected the germination and growth of the AMF. This is in line with the study performed by Alkobaisy (2022) on *P. lanceolate* who observed that the highest root length mycorrhizal colonization took place in neutral and alkaline media. Katumani 80 had lower mycorrhizal colonization values as compared to KVU 27-1. This is because the colonization levels in plants is species dependent and KVU 27-1 roots proved to interact well with the AMF.

Cowpea being a legume is a mycorrhizal plant and easily formed a symbiotic relationship with the AM fungi that led to the colonization of its roots. Apart from the plant species, colonization of the host roots by mycorrhizae may depend on the availability of the AMF spores in the soil and the soil stress levels. The soil sterilization eliminated all the native

AMF spores that were present in the soil. Mycorrhizal colonization test on the uninoculated plants showed zero colonization. At severe water stress (30% FC), low mycorrhizal colonization was recorded for all inoculants and in both varieties. These results are in agreement with the findings of Pons and Müller (2022) who also observed low AMF colonization in wheat cultivars at drought stressed conditions. Under drought stress the germination and growth of hyphae are highly inhibited which reduces the proliferation of AMF in the roots (Huang *et al.*, 2011; Gong *et al.*, 2013). Furthermore, drought conditions restrict the distribution of carbon to AMF reducing the successful development and establishment of AMF in plant roots. In this study, the consequences of low mycorrhizal colonization were quite evident through reduced nutrient absorption which reduced growth in cowpea.

Mycorrhizal colonization increased with decrease in water stress levels. At higher water levels (60% and 90% FC), AMF root colonization increased significantly. These results concur with those of Zhang *et al.* (2014) who recorded increased levels of mycorrhizal colonization in *Cyclobalanopsis glauca* seedling roots at well-watered conditions after inoculating with a mixture of AMF isolates. The availability of moisture enhances colonization although excess soil water causes anaerobic environment that reduces the efficiency of AMF in colonization (Bhardwaj *et al.*, 2023).

Amongst the four inoculations, the combined inoculations of HBB and BB led to higher mycorrhizal colonization than the single inoculations of BEG12 and BEG44 in both cowpea varieties irrespective of the soil water levels. The highest mycorrhizal colonization was 37.8% observed in HBB for KVVU 27-1 and 29.6% in BB for K80. Various studies have proved compatibility between various inoculants that leads to a higher mycorrhizal colonization as opposed to when they are used singly. Pons and Müller (2022) recorded similar results with a mixture of AMF isolates on wheat cultivars. Metwally (2020) also recorded increased root mycorrhizal colonization after combining a mixture of AMF species with *Trichoderma* strains on onions. The increase in colonization is caused by increased intensity of hyphae penetration in plants roots.

5.2 Conclusions

- i. Arbuscular mycorrhizal fungi and *Trichoderma* spp. enhanced the increase in shoot dry weight, root dry weight, root length, stem diameter, leaf area, plant height, specific leaf area, chlorophyll content and relative water content in both KVVU 27-1 and K80 cowpea varieties at the three water levels. The highest growth was observed at 90% FC (no soil water stress). Combined inoculations including HarS and HBB enhanced more growth as compared to single inoculations. It is concluded that combined fungi promote growth and increase water uptake.

- ii. Arbuscular mycorrhizal fungi and *Trichoderma* spp. promoted uptake of nitrogen, phosphorous and potassium at the three water levels for both cowpea varieties. Combined inoculation involving HBB and HarS enhanced the highest nutrient content at 90% FC.
- iii. Root colonization by AMF increased with availability of soil water. Combined inoculations of BB and HBB had higher colonization of 29.6% and 37.8% in K80 and KVU 27-1 respectively at 90% FC than single AMF inoculations.

5.3 Recommendations

- i. Arbuscular mycorrhizal fungi and *Trichoderma* spp. are recommended for use in agricultural soils to enhance growth of crops. In addition, their influence to nutrients absorption in water stressed soils can be utilized to successfully cultivate crops in drought vulnerable lands.
- ii. Through this study, AMF and *Trichoderma* spp. have been identified to improve nutrient uptake and are therefore recommended for use on agricultural soils instead of chemical fertilizers, which have become a threat to environmental pollution.
- iii. The fungi AMF and *Trichoderma* colonize cowpea effectively. More studies can explore colonization in other crop plants.

REFERENCES

- Abd Allah, E. F., Hashem, A., Alqarawi, A. A., Wirth, S., and Egamberdieva, D. (2017).** Calcium application enhances growth and alleviates the damaging effects induced by Cd stress in sesame (*Sesamum indicum* L.). *Journal of Plant Interactions*, 12(1), 237–243. <https://doi.org/10.1080/17429145.2017.1319500>.
- Abobatta, W. F. (2019).** Drought adaptive mechanisms of plants – a review. *Advances in Agriculture and Environmental Science*, 2(1), 62–65. <https://doi.org/10.30881/aeoa.00022>.
- Achuba, F. I. (2006).** The effect of sub-lethal concentration of crude oil on the growth and metabolism of cowpea (*Vigna unguiculata*) seedlings. *The Environmentalist*, 21(1):17 – 20.
- Adelaïde, P. O., Batiemo, B. J., Traore, F., Jean-Baptiste, T., Huynh, B., Roberts, P. A., Close, T., and Ouédraogo, J. T. (2018).** Screening of cowpea [*Vigna unguiculata* (L.) Walp.] lines for resistance to three Aphids (*Aphis craccivora* Koch) strains in Burkina Faso. *African Journal of Agricultural Research*, 13, 1487–1495.
- Agbogidi, O. M. (2010).** Screening six cultivars of cowpea (*Vigna unguiculata* L.) walp for adaptation to soil contaminated with spent engine oil. *Journal of Environmental Chemistry and Ecotoxicology*, 2, 103–109.
- Akladios, S. A., and Abbas, S. M. (2012).** Application of *Trichoderma harziunum* T22 as a biofertilizer supporting maize growth. *African Journal of Biotechnology*, 11(35), 8672–8683. <https://doi.org/10.5897/AJB11.4323>
- Alfiky, A. A., and Weisskopf, L. (2021).** Deciphering *Trichoderma* – Plant – Pathogen Interactions for Better Development of Biocontrol Applications. *Journal of Fungi*, 7(61), 1–18.
- Al Hassan, M., Fuertes, M. M., Sánchez, F. J. R., Vicente, O., and Boscaiu, M. (2015).** Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43(1), 1-11.
- Al-kaisi, M. M., Elmore, R. W., Guzman, J. G., Hanna, H. M., Hart, C. E., Helmers, M. J., Hodgson, E. W., Lenssen, A. W., Mallarino, A. P., Robertson, A. E., and Sawyer, J. E. (2015).** Drought Impact on Crop Production and the Soil Environment: 2012 experiences from Iowa. *Journal of Soil and Water Conservation*, 68(1), 19–24. <https://doi.org/10.2489/jswc.68.1.19A>

- Alkobaisy, J. S. (2022).** Factors Affecting Mycorrhizal Activity. *Mycorrhiza - New Insights*, (October), 1–14. <https://doi.org/10.5772/intechopen.108099>
- Anderson, J. M. and Ingram, J. S. I. (1993).** Tropical soil biology and fertility: A handbook of methods. CAB International, Wallingford, Oxon, UK
- Anjum, S. A., Xie, X., Wang, L., Saleem, M. F., Man, C., and Lei, W. (2011).** Morphological , physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6(9), 2026–2032. <https://doi.org/10.5897/AJAR10.027>
- Anyia, A. O., and Herzog, H. (2004).** Water-use efficiency , leaf area and leaf gas exchange of cowpeas under mid-season drought. *European Journal of Agronomy*, 20, 327–339. [https://doi.org/10.1016/S1161-0301\(03\)00038-8](https://doi.org/10.1016/S1161-0301(03)00038-8)
- Azarmi, R., Hajieghrari, B., and Giglou, A. (2011).** Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology*, 10(31), 5850–5855. <https://doi.org/10.5897/AJB10.1600>.
- Bader, A. N., Salerno, G. L., Covacevich, F., and Consolo, V. F. (2019).** Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). *Journal of King. Saud University- Science*, <https://doi.org/10.1016/j.jksus.2019.04.002>.
- Bagheri, V., Shamshiri, M. H., Shirani, H., and Roosta, H. R. (2012).** Nutrient Uptake and Distribution in Mycorrhizal Pistachio Seedlings under Drought Stress. *Journal of Agriculture Science and Technology*, 14, 1591–1604.
- Begum, N., Ahanger, M. A., Su, Y., Lei, Y., Mustafa, N. S. A., Ahmad, P., and Zhang, L. (2019).** Improved Drought Tolerance by AMF Inoculation in Maize (*Zea mays*) Involves Physiological and Biochemical Implications. *Plants*, 8(579), 1–20.
- Bhardwaj, A. K., Chandra, K. K., and Kumar, R. (2023).** Water stress changes on AMF colonization , stomatal conductance and photosynthesis of *Dalbergia sissoo* seedlings grown in entisol soil under nursery condition. *Forest Science and Technology*, 19(1), 47–58. <https://doi.org/10.1080/21580103.2023.2167873>.
- Bissett, J., Gams, W., Jaklitsch, W., and Samuels, G. J. (2015).** Accepted *Trichoderma* names in the year 2015. *IMA Fungus*, 6(2), 263–295. <https://doi.org/10.5598/imafungus.2015.06.02.02>.

- Bolat, I., Dikilitas, M., Ercisli, S., Ikinci, A., and Tonkaz, T. (2014).** The Effect of Water Stress on Some Morphological , Physiological , and Biochemical Characteristics and Bud Success on Apple and Quince Rootstocks. *Scientific World Journal*, 2014, 1–9.
- Bourguignon, E. (2008).** Ecology and diversity of indigenous *Trichoderma* species in vegetable cropping systems. *PhD Thesis. Lincoln University, Canterbury New Zealand.*
- Bourles, A., Guentas, L., Charvis, C., Gensous, S., Majorel, C., Crossay, T., Cavaloc, Y., Burtet-sarramegna, V., Jourand, P., and Amir, H. (2020).** Co-inoculation with a bacterium and arbuscular mycorrhizal fungi improves root colonization , plant mineral nutrition , and plant growth of a Cyperaceae plant in an ultramafic soil. *Mycorrhiza*, (1–11).
- Brundrett, M. C. (2009).** Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil*, 320, 37–77
- Cardona-Ayala, C., Cardona-Villadiego, C., Peñate-Pacheco, C., Araméndiz-Tatis, H., Espitia- Camacho, M. M. (2020).** Growth, biomass distribution, gas exchange and chlorophyll fluorescence in cowpea (*Vigna unguiculata* (L.) Walp.) under drought conditions. *Australian Journal of Crop Science* 14, 371–381.
- Carro-Huerta, G., Mayo-Prieto, S., Rodríguez-González, A., Álvarez-García, S., Gutiérrez, S., Casquero, P. A. (2021).** The Influence of Temperature on the Growth, Sporulation, Colonization, and Survival of *Trichoderma* spp. in Grapevine Pruning Wounds. *Agronomy*, 11(1771).
- Carvalho, M., Castro, I., Moutinho-Pereira, J., Correia, C., Egea-Cortines, M., Matos, M., Rosa, E., Carnide, V., Lino-Neto, T. (2019).** Evaluating stress responses in cowpea under drought stress. *Journal of Plant Physiology*. 241, 153001. <https://doi.org/10.1016/j.jplph.2019.153001>
- Chagas, L. F. B., De Castro, H. G., Colonia, B. S. O., Filho, M. R. C., Miller, L. O and Chagas, A. F. J. (2016).** Efficiency of *Trichoderma* spp . as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis. *Brazilian Journal of Botany*, 39(2), 437–445. <https://doi.org/10.1007/s40415-015-0247-6>.
- Chand, U., Harsh, J., Rintu, N., and Pronob, J. (2020).** Heat stress and cowpea: Genetics, breeding and modern tools for improving genetic gains. *Plant Physiology Reports*. 25, 645–653.

- Chandrasekaran, M., Chanratana, M., Kim, K., Seshadri, S., and Sa, T. (2019).** Impact of arbuscular mycorrhizal fungi on photosynthesis , water status , and gas exchange of plants under salt stress – A meta-analysis. *Frontiers in Plant Science*, *10*, 1–10. <https://doi.org/10.3389/fpls.2019.00457>.
- Chandrasekaran, M. (2022).** Arbuscular mycorrhizal fungi mediated enhanced biomass, root morphological traits and nutrient uptake under drought stress : A meta-analysis. *Journal of Fungi*, (8), 1–8.
- Cheng, H., Giri, B., Wu, Q., Zou, Y., and Kuča, K. (2021).** Arbuscular mycorrhizal fungi mitigate drought stress in citrus by modulating root microenvironment. *Archives of Agronomy and Soil Science*, 1–12. <https://doi.org/10.1080/03650340.2021.1878497>
- Chepserson, J., Mwamburi, L., and Kassim, M. K. (2014).** Mechanism of Drought Tolerance in Plants Using *Trichoderma* spp. *International Journal of Science and Research*, *3*(11), 2012–2015.
- Clarkson, J. P., Mead, A., Payne, T., and Whipps, J. M. (2004).** Effect of environmental factors and *Sclerotium cepivorum* isolate on sclerotial degradation and biological control of white rot by *Trichoderma*. 353–362. <https://doi.org/10.1046/j.1365-3059.2004.01013.x>.
- Colla, G., Roupael, Y., Mattia, Di., El-nakhel, C., and Cardarelli, M. (2015).** Co-inoculation of *Glomus intraradices* and *Trichoderma atroviride* acts as a biostimulant to promote growth , yield and nutrient uptake of vegetable crops. *Journal of Science, Food and Agriculture*, *95*, 1706–1715. <https://doi.org/10.1002/jsfa.6875>.
- Conceição, P., Levine, S., Lipton, M., and Warren-Rodríguez, A. (2016).** Toward a food secure future: Ensuring food security for sustainable human development in Sub-Saharan Africa. *Food Policy*, *60*, 1-9.
- Covell, S., Ellis, R. H., Roberts, E H., and Summerfield, R. J. (1986).** The Influence of Temperature on Seed Germination Rate in Grain Legumes. *Journal of Experimental Botany*, *37*(178), 705–715.
- da Silva, L. V., De Oliveira, S. B. R., De, Azevedo, A. C. R., and Bonifacio, A. (2019).** Coinoculation with Bradyrhizobium and *Trichoderma* Alleviates the Effects of Salt Stress in Cowpea. *Rev. Caatinga*, *32*(2), 336–344.
- da Silva, A. C., da Costa Santos, D., Junior, D. L. T., da Silva, P. B., dos Santos, R. C., Siviero, A. (2018).** Cowpea: A Strategic Legume Species for Food Security and Health,

in: Legume Seed Nutraceutical Research. IntechOpen.

- Dadson, R. B., Hashem, F. M., Javaid, I., Joshi, J., Allen, A. L., and Devine, T. E. (2005).** Effect of Water Stress on the Yield of Cowpea (*Vigna unguiculata* (L.) Walp. Genotypes in the Delmarva Region of the United States. *Journal of Agronomy and Crop Science*, 217, 210–217.
- De Jaeger, N., Declerck, S., and De la Providencia., I. E. (2010).** Mycoparasitism of arbuscular mycorrhizal fungi: a pathway for the entry of saprotrophic fungi into roots. *FEMS Microbial Ecology*. 73(2), 312–322. doi:10.1111/j.1574-6941.2010.00903.x.
- Department of Agriculture, Forestry and Fisheries of South Africa (DAFFSA). (2011).** *Production guidelines for Cowpeas*.
- Diagne, N., Ngom, M., Djighaly, P. I., Fall, D., Hocher, V., and Svistoonoff, S. (2020).** Roles of arbuscular mycorrhizal fungi on plant growth and performance : Importance in Biotic and Abiotic Stressed Regulation. *Diversity*, 12, 1–25.
- Dickie, I. A., Martínez-garcía, L. B., Koele, N., Grelet, G. A., Tyljanakis, J. M., Peltzer, D. A., and Richardson, S. J. (2013).** Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. *Plant Soil*, 367, 11–39. <https://doi.org/10.1007/s11104-013-1609-0>
- Dong, L., Ravelombola, W., Weng, Y., Qin, J., Bhattarai, G., Zia, B., Zhou, W., Wang, Y., Mou, B., and Shi, A. (2019).** Seedling salt tolerance for above ground-related traits in cowpea (*Vigna unguiculata* (L.) Walp). *Euphytica*, 215(53), 1–22. <https://doi.org/10.1007/s10681-019-2379-4>
- Dugje, I. Y., Omoigui, L. O., Ekeleme, F., Kamara, A. Y., and Ajeigbe, H. (2009).** Farmers' Guide to Cowpea Production in West Africa. IITA, Ibadan, Nigeria. 20 pages
- Edeh, H. O., and Igberi, C. O. (2012).** Assessment of vegetable cowpea production among small holder farmers in Ebonyi State, Nigeria. *ARPN Journal of Agricultural and Biological Science*, 7, 215–222.
- Ekesa, B. N., Walingo, M. K. and Abukutsa-Onyango M. O. (2009).** Accessibility to and consumption of indigenous vegetables and fruits by rural households in Matungu Division, Western Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, 9(8):1725-1738.
- Emmanuel, O. C., Akintola, O. A., Tetteh, F. M., and Babalola, O. O. (2021).** Combined

Application of Inoculant, Phosphorus and Potassium Enhances Cowpea Yield in Savanna Soils. *Agronomy*, 11(15), 1–10.

FAOSTAT (2014). FAO Statistics online database, “Production/crops – ‘cow peas, dry’, year 2014”, Food and Agriculture Organization, Rome, <http://faostat3.fao.org/home/E> (accessed 15 February 2016).

FAOSTAT (2019). FAOSTAT [WWW Document]. FAOSTAT. Retrieved from <http://www.fao.org/faostat/en/#data/QC>

Fatokun, C., Boukar, O., Muranaka, S., and Chikoye, D. (2009). Enhancing drought tolerance in cowpea, p 531-536. In 9th African Crop Science, Conference Proceedings, 28 September-2 October 2009, Cape Town, South Africa.

Fatokun, C.A., Boukar, O., Kamara, A., Coulibaly, O., Alene, A., and Boahen, S. (2012). Enhancing cowpea productivity and production in drought-prone areas of Sub-Saharan Africa. Four Seasons of Learning and Engaging Smallholder Farmers: Progress of Phase, 1: 81-112.

Gams, W., and Bissett, J. (1998). Morphology and identification of *Trichoderma*. Pp 1-34 in: *Trichoderma and Gliocladium*. Vol. 1. C. P. Kubicek and G. E. Harman, eds. Taylor & Francis, London.

Giovannetti, M & Mosse, B. (1980). An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *New Phytologist*, 84, 489–500.

Gong, M. G., Tang, M., Chen, H., Zhang, Q. M., and Feng, X. 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.

Guimarães, A. A., Jaramillo, P. M. D., Nóbrega, R. S. A., Florentino, L. A., Silva, K. B., and de Souza Moreira, F. M. (2012). Genetic and symbiotic diversity of nitrogen-fixing bacteria isolated from agricultural soils in the western Amazon by using cowpea as the trap plant. *Applied and Environmental Microbiology*, 78(18), 6726-6733.

Haddad, P. E., Leite, L. G., Lucon, C. M. M., and Harakava, R. (2017). Selection of *Trichoderma* spp. strains for the control of *Sclerotinia sclerotiorum* in soybean. *Pesquisa Agropecuária Brasileira*, 52(12), 1140-1148, <https://doi.org/10.1590/s0100-204x2017001200002>.

Halifu, S., Deng, X., Song, X., and Song, R. (2019). Effects of Two *Trichoderma* Strains on

Plant Growth , Rhizosphere Soil Nutrients , and Fungal Community of *Pinus sylvestris* var . mongolica Annual Seedlings. *Forests*, 10(758), 1–17.

Hall, A. E. (2004). Breeding for adaptation to drought and heat in cowpea. *European Journal of Agronomy*, 21, 447–454. <https://doi.org/10.1016/j.eja.2004.07.005>.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. (2004). *Trichoderma* species - Opportunistic, Avirulent Plant Symbionts. *Microbiology*, 2, 43–56. <https://doi.org/10.1038/nrmicro797>.

Hashem, A., Abd_Allah E. F., Alqarawi, A. A., El-Didamony, G., Alwhibi Mona, S., Egamberdieva, D., and Ahmad, P. (2014). Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. *Pakistan Journal of Botany*, 46:2003–2020. [https://www.pakbs.org/pjbot/PDFs/46\(6\)/10.pdf](https://www.pakbs.org/pjbot/PDFs/46(6)/10.pdf).

Hashem, A., Abd_Allah, E. F., Alqarawi, A. A., and Egamberdieva, D. (2018). Arbuscular mycorrhizal fungi and Plant Stress Tolerance. In P. Egamberdieva, D., Ahmad (Ed.), *Microorganisms for Sustainability* (pp. 81–103). Springer Nature Singapore Pte Ltd.

Hayatu, M and Mukhtar, F. B. (2010). Physiological Responses of some Drought Resistant Cowpea Genotypes (*Vigna unguiculata* (L.) Walp to Water Stress. *Bayero Journal of Pure and Applied Sciences*, 3(2), 69–75.

Hayatu, M., Muhammad, S. Y., and 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 of Scientific & Technology Research*, 3(7), 148–152.

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. <https://doi.org/10.4236/as.2022.131003>

Huang, Z., Zou, Z. R., He, C. X., He, Z. Q., Zhang, Z.B., and Li, J. M (2011). Physiological and photosynthetic responses of melon (*Cucumis melo* L.) seedlings to three glomus species under water deficit. *Plant Soil* 339:391–399.

Hutchinson, M. J., Muniu, F. K., Ambuko, J. Mwalangalu, M., Mwang’ombe, A. W., Okello, J., and Olubayam, F. (2016). Effect of cattle manure and calcium ammonium nitrogen on growth and leaf yield of local cowpea accessions in coastal Kenya. *Journal of Plant and Soil Science*, 12(3), 1-12

Huynh, B. L., Matthews, W. C., Ehlers, J. D., Lucas, M. R., Santos, J. R., Ndeve, A., Close,

- T. J., and Roberts, P. A. (2016).** A Major QTL Corresponding to the Rk Locus for Resistance to Root-Knot Nematodes in Cowpea (*Vigna unguiculata* (L.) Walp.) *Theoretical and Applied Genetics*, 129, 87-95. <https://doi.org/10.1007/s00122-015-2611-0>
- Illescas, M., Pedrero-Méndez, A., Pitorini-Bovolini, M., Hermosa, R., and Monte, E. (2021).** Phytohormone Production Profiles in *Trichoderma* Species and Their Relationship to Wheat Plant Responses to Water Stress. *Pathogens*, 10, 1–18.
- Ismail, I. M., Basahi, J. M., and Hassan, I. A. (2014).** Science of the Total Environment Gas exchange and chlorophyll fluorescence of pea (*Pisum sativum* L.) plants in response to ambient ozone at a rural site in Egypt. *Science of the Total Environment*, 497–498, 585–593. <https://doi.org/10.1016/j.scitotenv.2014.06.047>.
- International Seed Testing Association (ISTA) (2015).** Handbook on Seed Health Testing. Zürich, Switzerland.
- Jemo, M., Sulieman, S., Bekkaoui, F., Olomide, O. A. K., Hashem, A., Abd Allah, E. F., Alqarawi, A. A., and Tran, L. S. P. (2017).** Comparative analysis of the combined effects of different water and phosphate levels on growth and biological nitrogen fixation of nine cowpea varieties. *Frontiers in Plant Science*, 8, 2111.
- Kakabouki, I., Tataridas, A., Mavroeidis, A., Kousta, A., Karydogianni, S., Zisi, C., Kouneli, V., Konstantinou, A., Folina, A., Konstantas, A., and Papastylianou, P. (2021).** Effect of Colonization of *Trichoderma harzianum* on Growth Development and CBD Content of Hemp (*Cannabis sativa* L.). *Microorganisms*, 9(518).
- Kamalov, L. S., Turgunov, K. K., Aripova, S. F., and Abdilalimov, O. (2018).** Gibberillin A-3 from the microscopic fungus *Trichoderma harzianum*. *Chemistry of Natural Compounds*, 54, 421–422. doi: 10.1007/s10600-018-2368-1
- Kamanga, R. M., Mbega, E., and Ndakidemi, P. (2018).** Drought Tolerance Mechanisms in Plants: Physiological Responses Associated with Water Deficit Stress in *Solanum lycopersicum*. *Advances in Crop Science and Technology*, 6(3), 1–8. <https://doi.org/10.4172/2329-8863.1000362>.
- Kamble, P. N., Giri, S. P., Mane, R. S., and Tiwana, A. (2015).** Estimation of Chlorophyll Content in Young and Adult Leaves of Some Selected Plants Abstract : Universal Journal of Environmental Research and Technology. *Universal Journal of Environmental Research and Technology*, 5(6), 306–310.
- Karim, T. D. A., Sanoussi, A., Maârrouhi, I. M., Falalou, H., and Yacoubou, B. (2018).**

Effect of water deficit at different stages of development on the yield components of cowpea (*Vigna unguiculata* (L.) Walp) genotypes. *African Journal of Biotechnology*, 17(9), 279–287. <https://doi.org/10.5897/AJB2017.16347>.

Kaushal, N., Bhandari, K., Siddique, K. H. M., and Nayyar, H. (2016). Food crops face rising temperatures : An overview of responses , adaptive mechanisms , and approaches to improve heat tolerance Food crops face rising temperatures : An overview of responses , adaptive mechanisms , and approaches. *Cogent Food and Agriculture*, 28(1), 1–42. <https://doi.org/10.1080/23311932.2015.1134380>.

Kavitha M. J., Marimuthu, P., Kumutha, K., and Sivakumar, U. (2018). Seed priming effect of arbuscular mycorrhizal fungi against induced drought in rice. *Journal of Pharmacognosy and Phytochemistry*, 7(2), 1742–1746.

Kawaka, F., Mathews, M. D., Peter, A. O., Omwoyo, O., John, M., Morris, M., Alice, A., Dative, M., and John, M. (2014). Symbiotic efficiency of rhizobia nodulating common bean (*Phaseolus vulgaris* L.) in soils of Western Kenya. *International Schorlarly Research Notes*, 2014, 8. <https://doi.org/10.1155/2014/258497>

Kebede, E and Bekeko, Z. (2020). Expounding the production and importance of cowpea (*Vigna unguiculata* (L.) Walp .) in Ethiopia. *Cogent Food and Agriculture*, 6(1), 1–21. <https://doi.org/10.1080/23311932.2020.1769805>.

Kehri, H. K., Akhtar, O., Zoomi, I., and Pandey, D. (2018). Arbuscular mycorrhizal fungi : Taxonomy and its Systematics. *International Journal of Life Sciences Research*, 6(4), 58–71.

Khaliq, A., Perveen, S., Alamer, K. H., Zia Ul Haq, M., Rafique, Z., Alsudays, I. M., Althobaiti., A. T., Saleh, M. A., Hussain, S., and Attia, H. (2022). Arbuscular mycorrhizal fungi Symbiosis to Enhance Plant – Soil Interaction. *Sustainability*, 14(7840).

Khoshmanzar, E., Aliasghar zad, N., Neyshabouri, M. R., Khoshru, B., Arzanlou, M., & Lajayer, A. B. (2019). Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water - deficit stress. *International Journal of Environmental Science and Technology*, 1–10. <https://doi.org/10.1007/s13762-019-02405-4>

Kiremire, B. T., Musinguzi, E., Kikafunda, J. K. and Lukwago, F. B. (2010). Effects of vegetable drying techniques on nutrient content: a case study of south-western Uganda. *African Journal of Food, Agriculture, Nutrition and Development*, 10(5): 2587-2600.

Klein, D. & Eveleigh, E. (1998). Ecology of *Trichoderma*. In *Trichoderma* and *Gliocladium*,

pp. 57-69. Edited by C. P. Kubicek & G. E. Harman. London; Bristol, PA: Taylor & Francis.

- Krasilnikoff, G., Gahoonia, T., and Nielsen, N. E. (2003).** Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere processes. *Plant Soil*, 251, 83–91.
- Kumar, R., Karn, M., A., and Bhargava, P. (2020).** Enhanced plant growth of cowpea supplemented with *Trichoderma* under biotic stress of *Sclerotium rolfsii*. *International Journal of Chemical Studies*, 8(4), 127–132.
- Kumara, U. M. A., Cooray, P. L. V. N., Ambanpola, N and Thiruchchelvan, N. (2022).** Plant-pathogen interaction: Mechanisms and evolution. In R. Soni, R., Suyal, D. C., Yadav, A. N and Goel (Ed.), *Trends of Applied Microbiology for Sustainable Economy* (pp. 655–679). London: Elsevier.
- Kundu, C. A., Karanja, N. K., Jefwa, J., Ndolo, P. J., and Mwangi, E. (2013).** Response of orange fleshed sweetpotato to arbuscular mycorrhizal fungi inoculation and fertilizer application in western Kenya. *Joint Proceedings of the 27th Soil Science of East Africa and the 6th African Soil Science Society*, 1–11.
- Kuzmanovska, B., Rusevski, R., & Jankulovska, M., and Oreshkovikj, K. B. (2018).** Antagonistic activity of *Trichoderma asperellum* and *Trichoderma harzianum* against genetically diverse *Botrytis cinerea* isolates. *Chilean Journal of Agricultural Research*, 78(September), 391–399. <https://doi.org/10.4067/S0718-58392018000300391>.
- Larweh, V., Akromah, R., Amoah, S., Asibuo, J. Y., Kusi, F. and Prempeh, R. (2019).** Effect of *Striga gesnerioides* on Cowpea (*Vigna unguiculata* (L.) Walp) Yield Components. *Research Square*. <https://doi.org/10.21203/rs.2.11106/v1>
- Mamiro, P. S., Mbwaga, A. M., Mamiro, D. P., Mwanri, A. W., and Kinabo, J. L. (2011).** Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. *African Journal of Food Agriculture Nutrition and Development*, 11(1), 4490–4506. <https://doi.org/10.4314/ajfand.v11i1.65876>.
- Martínez-medina, A., Roldán, A., Albacete, A., & Pascual, J. A. (2011).** Phytochemistry The interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in melon plants. *Phytochemistry*, 72, 223–229. <https://doi.org/10.1016/j.phytochem.2010.11.008>.
- Mbeyagala, E. k., Mukasa, B. S., Tukamuhabwa, P., and Bisikwa J. (2014).** Evaluation of cowpea genotypes for virus resistance under natural conditions in Uganda. *Journal of*

Agricultural Science, 6(10), 176-187. <http://dx.doi.org/10.5539/jas.v6n10p176>.

- Mehlich, A., Pinkerton, A., Robertson, W. and Kepton, R. (1962).** Mass analysis methods for soil fertility evaluation. Cyclostyled Paper, National Agric. Laboratories, Nairobi.
- Mei, L. I., Guang-shu, M. A., Hua, L., Xiao-lin, S. U., Ying, T., Wen-kun, H., Jie, M., & Xi-lian, J. (2019).** The effects of *Trichoderma* on preventing cucumber fusarium wilt and regulating cucumber physiology. *Journal of Integrative Agriculture*, 18(3), 607–617. [https://doi.org/10.1016/S2095-3119\(18\)62057-X](https://doi.org/10.1016/S2095-3119(18)62057-X)
- Melo, A. S., Yule, T. S., Barros, V. A., Rivas, R., and Santos, M. G. (2021).** C3-species *Calotropis procera* increase specific leaf area and decrease stomatal pore size, alleviating gas exchange under drought and salinity. *Acta Physiologiae Plantarum*, 43(11). <https://doi.org/10.1007/s11738-021-03312-3>
- Metwally, R. A. and Abdelhameed, R.E. (2018).** Synergistic effect of arbuscular mycorrhizal fungi in growth and physiology of salt-stressed *Trigonella foenum-graecum* plants. *Biocatal Agric Biotechnol* 16, 538–544.
- Metwally, R. A and Al-Amri, S. M. (2019).** Individual and interactive role of *Trichoderma viride* and arbuscular mycorrhizal fungi on growth and pigment content of onion plants. *Letters in Applied Microbiology*, 70, 79–86. <https://doi.org/10.1111/lam.13246>
- Metwally, R. A. (2020).** Arbuscular mycorrhizal fungi and *Trichoderma viride* cooperative effect on biochemical , mineral content , and protein pattern of onion plants. *Journal of Basic Microbiology*, 1–10. <https://doi.org/10.1002/jobm.202000087>
- Muindi, M. M., Muthini, M., Njeru, E. M., and Maingi, J. (2023).** Greenhouse studies showing arbuscular mycorrhizal fungi and rhizobia as potential tools for improving cowpea (*Vigna unguiculata*) production in lower Eastern Kenya. *Tropical Agriculture (Trinidad)*, 100(3), 136–148.
- Muli, M. B and Saha, H. (2000).** Participatory evaluation of Cowpea Cultivars for Adaptation and Yield Performance in Coastal Kenya. *Proceedings of 2nd Scientific Conference of the Soil Management and Legume Research Network Projects Mombasa, Kenya*, 267–272.
- Mulyungi, P. S. (2014).** Effects of Calcium, Potassium and Water Stress on Growth, Yield and Quality of different Tomato Varieties in Mandera County, Kenya. *Msc Thesis*, Kenyatta University.
- Muniu, F. K. (2017).** Characterization and Evaluation of Local Cowpea Accessions and Their Response to Organic and Inorganic Nitrogen Fertilizers in Coastal Kenya. *Msc Thesis*,

University of Nairobi.

- Muthukumar, T., Radhika, K. P., Vaingankar, J., D' souza, J., Dessai, S., and Rodrigues, B. F. (2009).** Taxonomy of AM Fungi - An Update. In T. Rodrigues, B. F & Muthukumar (Ed.), *Arbuscular Mycorrhizae of Goa - A Manual of Identification Protocols* (pp. 75–108). Goa University.
- Mwangi, M. W., Monda, E. O., Okoth, S. A and Jefwa, J. M. (2009).** Effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on growth in tomato (*Lycopersicon esculentum* MILL) seedlings, napier (*Pennisetum purpureum* L.) and tea (*Camellia sinensis* L.) cuttings. *Tropical and Subtropical Agroecosystem*, 11, 423–429.
- Ndema, N. E., Etame, J., Taffouo, V. D., and Bilong, P. (2010).** Effects of some physical and chemical characteristics of soil on productivity and yield of cowpea (*Vigna unguiculata* (L.) Walp) in coastal region (Cameroon). *African Journal of Environmental Science and Technology*, 4(3), 108-114. <https://www.ajol.info/index.php/ajest/article/viewFile/56331/44772Ndema>.
- Njonjo, M. W. (2018).** Quality of Cowpea Seed used by Farmers in Makueni and Taita Taveta Counties and its Effect on Crop Performance. *Msc Thesis*, University of Nairobi, Kenya.
- Novozamsky, I., Houba, V. J. G., van Eck, R., and van Vark, W. (1983).** A novel digestion technique for multi-element plant analysis. *Commun. Soil Sci. Plant Anal.* 14:239-248.
- Ntombela, Z. (2012).** Growth and Yield responses of Cowpeas (*Vigna unguiculata* L.) to Water Stress and Defoliation. *Thesis (Msc)*.
- Nyoki, D and Ndakidemi, P. A. (2014).** Effects of Phosphorus and Bradyrhizobium japonicum on Growth and Chlorophyll Content of Cowpea (*Vigna unguiculata* (L.) Walp). *American Journal of Experimental Agriculture*, 4(10), 1120–1136.
- OECD. (2016).** Cowpea (*Vigna unguiculata*). Safety Assessment of Transgenic Organisms in the Environment: *OECD Consensus Documents*.
- Ojiewo, C., Rubyogo, J. C., Wesonga, J., Bishaw, Z., Abang, M., and Gelalcha, S. (2019).** Mainstreaming Efficient Legume Seed Systems in Eastern Africa: Challenges, opportunities and contributions towards improved livelihoods. In *Food and Agriculture Organization of the United Nations*. <https://doi.org/10.18356/ce824af1-en>.
- Okalebo, J. R., Gathua, K. W., and Woome, P. L. (2002).** Laboratory Methods of Soil and Plant Analysis: A Working Manual. Second edition. TSBF-CIAT and SACRED Africa, Nairobi, Kenya.

- Olorunwa, O. J., Shi, A., and Barickman, T. C. (2021).** Varying Drought Stress Induces Morpho-Physiological Changes in Cowpea (*Vigna unguiculata* (L.) Genotypes Inoculated with *Bradyrhizobium japonicum*. *Plant Stress*, 100033. <https://doi.org/10.1016/j.stress.2021.100033>
- Ortas, I. (2010).** Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Spanish Journal of Agricultural Research*, 8(1), 116–122.
- 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(1), 90–101. <https://doi.org/10.1080/01904167.2017.1381728>.
- Owade, J. O., Abong, G., Okoth, M., and Mwang'ombe, A. W. (2020a).** A review of the contribution of cowpea leaves to food and nutrition security in East Africa. *Food Science and Nutrition*, 8, 36–47. <https://doi.org/10.1002/fsn3.1337>.
- Owade, J. O., Abong, G. O., Okoth, M. W., and Mwang'ombe, A. W. (2020b).** Trends and constraints in the production and utilization of cowpea leaves in the arid and semi - arid lands of Kenya. *Open Access*, 5, 325–334.
- Oyewale, R. O., and Bamaiyi, L. J. (2013).** Management of Cowpea Insect Pests. *Scholars Acaemic Journal of Biociences (SAJB)*, 1(5), 217–226.
- Padulosi, S., and Ng, N. Q. (1997).** Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp . In L. E. N. Singh, B. B., Raj, D. R. M., Dashiell, K. E., and Jackai (Ed.), *Advances in Cowpea Research* (pp. 1–12). Ibadan, Nigeria.
- Page, A. L., Miller, R. H. and Keeney, D. R. (eds.) (1982).** Methods of soil analysis. Part 2. Second edition. Amer. Soc. of Agron., Madison, Winconsin, USA. pp 595-622.
- Pei, Y., Siemann, E., Tian, B., & Ding, J. (2020).** Root flavonoids are related to enhanced AMF colonization of an invasive tree. *AoB Plants*, 12(1), 1–7. <https://doi.org/10.1093/aobpla/plaa002>
- Philips, J. M., and Hayman, D. S. (1970).** Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55(1), 159–161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3).

- Pirzad, A., Shakiba, M. R., Zehtab-Salmasi, S., Mohammadi, S. A., Darvishzadeh, R., and Samadi, A. (2011).** Effect of water stress on leaf relative water content , chlorophyll , proline and soluble carbohydrates in *Matricaria chamomilla* L. *Journal of Medicinal Plants Research*, 5(12), 2483–2488.
- Pons, C and Müller, C. (2022).** Impacts of Drought Stress and Mycorrhizal Inoculation on the Performance of Two Spring Wheat Cultivars. *Plants*, 11(2187), 1–18.
- Posada, R.H., Franco, L. A., Ramos, C., Plazas, L. S., Suarez, J. C., and Alvarez, F. (2008).** Effect of physical , chemical and environmental characteristics on arbuscular mycorrhizal fungi in *Brachiaria decumbens* (Stapf) pastures. *Journal of Applied Microbiology*, 104, 132–140. <https://doi.org/10.1111/j.1365-2672.2007.03533.x>.
- Poveda, J., Hermosa, R., Monte, E., & Nicolás, C. (2019).** *Trichoderma harzianum* favours the access of arbuscular mycorrhizal fungi to non-host Brassicaceae roots and increases plant productivity. *Scientific Reports*, 9(11650), 1–11. <https://doi.org/10.1038/s41598-019-48269-z>
- Ranadev, P., Ashwin, R., Anuroopa, N., and Bagyaraj, D. J. (2022).** Symbiotic response of fodder cowpea (*Vigna unguiculata* L.) and field bean (*Lablab purpureus* L.) with different arbuscular mycorrhizal fungi. *Kavaka*, 58(3), 34–38. <https://doi.org/10.36460/Kavaka/58/3/2022/34-38>
- Rashid, M. A., Hussain, M. I., Rahman, A., Khatun, M. K., A., and Sattar, M. A. (2016).** The Effect of Microwave Cooking on Nutrient Value of Fresh Vegetables. *International Journal of Nutrition and Food Sciences*, 5(4), 273–277. <https://doi.org/10.11648/j.ijnfs.20160504.16>.
- Ravelombola, W., Shi, A., Chen, S., Xiong, H., Yang, Y., Cui, Q., Olaoye, D., Mou, B. (2020a).** Evaluation of cowpea for drought tolerance at seedling stage. *Euphytica* 216, 123. <https://doi.org/10.1007/s10681-020-02660-4>
- Richardson, A. E and Simpson, R. J. (2011).** Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology* 156 (3):989–996.
- Rochange, S., Goormachtig, S., Lopez-Raez, J. A., and Gutjahr, C. (2019).** “The Role of Strigolactones in Plant–Microbe Interactions.” In *Strigolactones-Biology and Applications*. Cham: Springer. pp 121–142.
- Rodrigues, G., Reis, H. B., Rotili, E. A., Carlos Mourão, D., Andrade de Farias, D. I. O.,**

- Pereira¹, T. A., Ferreira, T. P., Chagas Junior, A. F. (2020).** Inoculation of Rhizobium associated with *Trichoderma asperellum* on the development and yield of cowpea. *Agronomy*, 1–8. <https://doi.org/10.5039/agraria.v15i2a8082>
- Rusike, J., van den Brand, G., Boahen, S., Dashiell, K., Kantengwa, S., Ongoma, J., Mongane, D. M., Kasongo, G., & Jamagani, Z. B., Aidoo, R., and Abaidoo, R. (2013).** Value chain analyses of grain legumes in N2Africa: *Kenya, Rwanda, eastern DRC, Ghana, Nigeria, Mozambique, Malawi and Zimbabwe*. 1–96.
- Samuels, G. J. (2006).** *Trichoderma*: Systematics, the Sexual State, and Ecology. *Phytopathology*, 96(2), 195–206.
- Sharma, S., Kour, D., Rana, K. L., Dhiman, A., Thakur, S., Thakur, P., Thakur, S. et al. (2019).** *Trichoderma*: Biodiversity, Ecological Significances, and Industrial Applications. In A. N. et al Yadav (Ed.), *Recent Advancement in White Biotechnology Through Fungi, Fungal Biology* (pp. 85–120). <https://doi.org/10.1007/978-3-030-10480-1>
- Shukla, N., Awasthi, R. P., Rawat, L., & Kumar, J. (2014).** Seed biopriming with drought tolerant isolates of *Trichoderma harzianum* promote growth and drought tolerance in *Triticum aestivum*. *Annals of Applied Biology*. <https://doi.org/10.1111/aab.12160>.
- Sibhatu, B., Belete, K., & Tessema, T. (2015).** Effect of Cowpea density and nitrogen fertilizer on a Sorghum-Cowpea Intercropping System in Kobo, Northern Ethiopia. *International Journal of Agriculture and Forestry*, 5(6), 305–317. <https://doi.org/10.5923/j.ijaf.20150506.02>.
- Singh, B. (2020).** Cowpea: the food legume of the 21st century. John Wiley & Sons
- Singh, B. B., Ajeigbe, H. A., Tarawali, S., Fernandez-Rivera, S. and Abubakar, M. (2003).** Improving the production and utilization of cowpea as food and fodder. *Field Crops Research*, 84:169-177
- Singh, B. B. (2014),** "Cowpea: The Food Legume of the 21st Century", In. Cowpea: The Food Legume of the 21st Century, (ACSESS Publications) [<https://dl.sciencesocieties.org/publications/books/pdfs/acsesspublicati/cowpeathefoodle/17>]
- Siwach, P., and Gill, A. R. (2014).** Micropropagation of *Ficus religiosa* L. via leaf explants and comparative evaluation of acetylcholinesterase inhibitory activity in the micropropagated and conventionally grown plants. *Biotechnology*, 4, 477–491.

- Soriano-Porras, A., Soriano-Martín, M. L., Porras-Piedra, A., and Azco'n, R. (2009).** Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *Journal of Plant Physiology*, 166, 1350–1359. <https://doi.org/10.1016/j.jplph.2009.02.010>.
- Souleymane, A., Aken'Ova, M. E., Fatokun, C. A., and Alabi, O. Y. (2013).** Screening for resistance to cowpea aphid (*Aphis craccivora* Koch) in wild and cultivated cowpea [*Vigna unguiculata* (L.) Walp.] accessions. *International Journal of Science, Environment and Technology*, 2, 611–621.
- Souza, P. J., Farias, V. D., Lima, M. J., Ramos, T. F., and Sousa, A. M. (2017).** Cowpea leaf area, biomass production and productivity under different water regimes in Castanhal, Pará, Brazil. *Revista Caatinga*, 30(3), 748–759. <https://doi.org/10.1590/1983-21252017v30n323rc>.
- Srivastava, R., Khalid, A., Singh, U. S., and Sharma, A. K. (2010).** Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. lycopersici for the management of tomato wilt. *Biological Control*, 53, 24–31. <https://doi.org/10.1016/j.biocontrol.2009.11.012>.
- Ste-Marie, C., and Houle, D. (2006).** Forest floor gross and net nitrogen mineralization in three forest types in Quebec, Canada. *Soil Biology and Biochemistry*, 38 (8), 2135–2143
- Stewart, A., and Hill, R. (2014).** Applications of *Trichoderma* in Plant Growth Promotion. In *Biotechnology and Biology of Trichoderma* (pp. 415–428). <https://doi.org/10.1016/B978-0-444-59576-8.00031-X>
- Stoddard, F. L., Nicholas, A. H., Rubiales, D., Thomas, J., and Villegas-Fernández, A. M. (2010).** Integrated pest management in faba bean. *Field Crops Research*, 115(3), 308–318. <https://doi.org/10.1016/j.fcr.2009.07.002>
- Sudharani, Y., Mohapatra, P. P., Pattanaik, M., Hans, H., and Maitra, S. (2020).** Effect of Phosphorus on Cowpea (*Vigna unguiculata* (L.) Walp): A review. *Journal of Pharmacognosy and Phytochemistry*, 9(4), 425–427. [doi:10.22271/phyto.2020.v9.14e.11721](https://doi.org/10.22271/phyto.2020.v9.14e.11721)
- Tamasloukht, M., Sejalon-Delmas, N., Kluever, A., Kluever, A., Jauneau, A., Roux, C., Becard, G., and Franken, P. (2003).** Root Factors Induce Mitochondrial-Related Gene Expression and Fungal Respiration during the Developmental Switch from Asymbiosis to Presymbiosis in the Arbuscular Mycorrhizal Fungus *Gigaspora rosea* 1. *Plant*

Physiology, 131, 1468–1478. <https://doi.org/10.1104/pp.012898.ment>.

- Tijjani, A. R., Nabinta, R. T. and Muntaka, M. (2015).** Adoption of innovative cowpea production practices in a rural area of Katsina State, Nigeria. *Journal of Agricultural and Crop Research*. 3(4), 53-58.
- Tchameni, S. N., Ngonkeu, M. E. L., Begoude, B. A. D., Nana, L. W., Fokom, R., Owona, A. D., Mbarga, J. B., Tchana, T., Tondje, P. R., Etoa, F, X., and Kuaté, J. (2011).** Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease. *Crop Protection*, 30(10), 1321–1327. <https://doi.org/10.1016/j.cropro.2011.05.003>.
- Tharanathan, R. N., and Mahadevamma, S. (2003).** Grain legumes—A boon to human nutrition. *Trends in Food Science and Technology*, 14, 507–518. <https://dx.doi.org/10.1016/j.tifs.2003.07.002>
- Torres-olivar, V., Villegas-torres, O. G., Domínguez-patiño, M. L., Sotelo-nava, H., Rodríguez-Martínez, A., Melgoza-Alemán, R. M., Valdez-Aguilar, L. A., and Alia-Tejagal, I. (2014).** Role of Nitrogen and Nutrients in Crop Nutrition. *Journal of Agricultural Science and Technology*, 4, 29–37.
- Van Reeuwijk, L. P. (2002).** Compiled and edited FAO, Sixth edition vegetable crops. *Karnataka Journal Agriculture science*. 24(1), 55-59.
- Walinga, I., Vark W., van Houba, V. J. G., and Lee, J. J. van der. (eds.) 1989.** Soil and plant analysis. Part 7. Plant analysis procedures. Syllabus. Wageningen Agricultural University, the Netherlands.
- Wang, Z., Li, T., Wen, X., Liu, Y., Han, J., Liao, Y., and DeBruyn, J, M. (2017).** Fungal Communities in Rhizosphere Soil under Conservation Tillage Shift in Response to Plant Growth. *Frontiers in Microbiology*, 8(1301), 1–11. <https://doi.org/10.3389/fmicb.2017.01301>
- Willis, A., Rodrigues, B. F., and Harris, P. J. C. (2013).** The Ecology of Arbuscular Mycorrhizal Fungi. *Critical Reviews in Plant Sciences*, 32, 1–20. <https://doi.org/10.1080/07352689.2012.683375>
- Woomer, P. L., and Mulei, W. (2015).** Better farming in Western Kenya; Finding solutions to common farm problems. International Institute of Tropical Agriculture-Kenya, Nairobi and WeRATE R4D Platform, Mbale, Kenya.

- 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*, 10(43), 8593–8598. <https://doi.org/10.5897/AJB10.1494>.
- Zhang, M., Duan, L., Zhai, Z., Li, J., Tian, X., Wang, B., He, Z., and Li, Z. (2004).** Effects of plant growth regulators on water deficit-induced yield loss in soybean. Proceedings of the 4th International Crop Science Congress, Brisbane, Australia.
- Zhang, Z., Zhang, J and Huang, Y. (2014).** Effects of arbuscular mycorrhizal fungi on the drought tolerance of *Cyclobalanopsis glauca* seedlings under greenhouse conditions. *New Forests*, 45, 545–556. <https://doi.org/10.1007/s11056-014-9417-9>.
- Zhang, S., Gan, Y., and Xu, B. (2019).** Mechanisms of the IAA and ACC deaminase producing strain of *Trichoderma longibrachiatum* T6 in enhancing wheat seedling tolerance to NaCl stress. *BMC Plant Biology*. 19(22). <https://doi.org.101186/s12870-018-1618-59>
- Zhou, H., Zhou, G., He, Q., Zhou, L., Ji, Y., and Zhou, M. (2020).** Environmental explanation of maize specific leaf area under varying water stress regimes. *Environmental and Experimental Botany*, 171, 1–10. <https://doi.org/10.1016/j.envexpbot.2019.103932>.

APPENDIX

Appendix: Research license


REPUBLIC OF KENYA


NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 571413 Date of Issue: 12/June/2023

RESEARCH LICENSE



This is to Certify that Mr. Daniel Wanjala of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kiambu on the topic: Arbuscular Mycorrhizal fungi and Trichoderma spp Influence on the Nutrient uptake and Water Stress Tolerance in Vigna unguiculata L. (Walp) (Cowpea) for the period ending : 12/June/2024.

License No: NACOSTI/P/23/26115

571413

Applicant Identification Number


Director General
NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY &
INNOVATION

Verification QR Code



NOTE: This is a computer generated License. To verify the authenticity of this document,
Scan the QR Code using QR scanner application.

See overleaf for conditions