# Enhancing nutritional content, phytochemical levels, growth and yield of okra (*Abelmoschus esculentus* L.) using the Organic Medium Enclosed Trough system

By

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## Declaration

I, Tyson Tebogo Mokgalabone, declare that neither I nor anyone else has ever submitted a dessertation for a degree to the University of Limpopo or any other institution titled "Enhancing nutritional content, phytochemical levels, growth and yield of okra (*Abelmoschus esculentus L*.) using the Organic Medium Enclosed Trough" for a Master of Agricultural Management (Plant Production). Also, this is my work in design and in execution, while related materials contained herein had been duly acknowledged.

_____ _____

**Mokgalabone T.T** **Date**

## Dedication

I dedicate this full dissertation to my late family members, my mother Frengelina Mokgadi Mokgalabone, my grandparents Khuluma Mokgalabone, Dorah Mokgalabone and Mahlane “Lahla” Thete. May their soul rest in eternal peace. Along with my lovely family at large who have always desired the best for me and helped me through all walks of life.

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## Publications

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**Mokgalabone, T.T.**, Mpai, S., Ndhlala, A.R. 2022. Enhancing primary and secondary metabolites in okra grown using Organic Medium Enclosed Trough technology. 12th Faculty of Science and Agriculture Postgraduate Research Day. 21-23 September 2022. Bolivia Lodge, Polokwane.

**Mokgalabone, T.T.**, Mpai, S. and Ndhlala, A.R. 2022. Targeted metabolite profile approach to elucidate response of stem thickness in okra grown under OMET system. IPUF. 04 to 07 July 2022 Virtual platform.

**Mokgalabone, T.T.**, Mutshekwa, N., Mpai, S. and Ndhlala, A.R. 2022. Growing Okra on an organic medium enclosed trough system improves growth, yield and water-use efficiency. South African Association of Botanist 47th annual conference. Virtual platform. 17-20 January 2022.

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## List of abbreviations

%= percentage

°C= Degrees Celsius

µg= microgram

µL= Microliter

µm= Micrometre

AAS= Amino Acid Score

Ala= Alanine

$AlCl_3$= Aluminium chloride

AOAC= Association of Official Analytical Chemists

Arg= Arginine

Asp= Asparagine

Asp= Aspartate

C= Carbon

Ca= Calcium

CAF=Central Analytical Facilities

Cm- Centimeter

CRD= Completely Randomised Design

Cu= Copper

DAFF= Department of Agriculture, Fishery and Forestry

DPPH= 2, 2-Diphenyl-1-picrylhydrazyl

DSI= Department of Science and Innovation

$EC_{50}$=Half maximal effective concentration

FAO= Food and Agricultural Organization

Fe= Iron

GBRCE= Green Biotechnologies Research Centre of Excellence

Glu= Glutamine

Gly= Glycine

$H_2SO_{4=}$ Sulphuric acid

His= Histidine

$HNO_{3=}$ Nitric acid

ICPE= inductively coupled plasma optical emission spectrometry

K= Potassium

LATS= Limpopo Agro-Food Technology Station

Leu= Leucine

Lys= Lysine

Met= Methionine

Mg- Milligrams

mg CE/g = milligram of catechin equivalence/ gram

Mg= Magnesium

mg= Milligram

mmol/L =millimole

Min= Minute

mL= Millilitre

Mn=Manganese

MS= Mass-spectrometer

N= Nitrogen

Na= Potassium

$NaNO_2$= Sodium nitrite

NaOH= Sodium hydroxide

Non-OMET= Non-Organic Medium Enclosed Trough

NRF= National Research Fund

OMET- Organic Medium Enclosed Trough

P= Phosphorus

PCA= Principal Component Analysis

PDA= Photodiode array

Phe= Phenylanine

Pro= Proline

QTOF=Quadrupole time-of-flight (

RC= Ratio Coefficient

RCBD= Randomized Complete Block Design

Se= Selenium

SE= Standard Error

Ser= Serine

SSA- Sub-Saharan Africa

Thr= Threonine

Tyr= Tyrosine

UPLC= Ultra-Performance Liquid Chromatography

UPLC-MS- Ultra-Performance Liquid Chromatograph Mass Spectrometer

UV/VIS=Ultraviolet–visible spectroscopy

WHO= World Health Organization

Zn=Zinc

## Abstract

Okra is an indigenous vegetable consumed in Southern Africa. Its growth and yield are negatively affected by water and nutrient deficit. There is insufficient scientific information on the growth and yield attributes of underutilised indigenous vegetables such as okra. The information on enhancement and evaluation of growth, yield, nutrients, and phytochemical compositions of okra using the climate-smart OMET growing technique has not been documented. The Organic Medium Enclosed Trough (OMET) system was developed as a non-drainable growing technique which improves crop yield by reducing water and nutrient seepage. The aim of this study was to develop scientific information on the effects of the OMET growing technique and growing environment on growth and yield, nutritional composition and phytochemical composition in okra. The objectives of this study were to (1) investigate the effect of the OMET growing technique and growing environment on growth and yield attributes, (2) nutritional composition and (3) the phytochemical composition of okra grown under greenhouse and micro-plots conditions. To achieve the objectives, four-week-old okra seedlings were transplanted on the established OMET and non-OMET growing technique concurrently in both the growing environment (greenhouse and micro-plot) following a randomised complete block design (RCBD) for a period of 110 days, with three replications and twelve plants per replicate. The amount of irrigation water used in both experiments was recorded until harvest and computed as cumulative irrigation water. The mean separation was done using a parametric T-test at the significance level of 5% using the Genstat version 18.0 statistical package. The growth attributes which included plant height and stem diameter (n=9) were taken on a weekly basis. At harvest, yield components including the number of branches per plant, plant biomass, fresh pod weight, number of pods per plant, fresh pod length and fresh pod diameter width were recorded. The harvested leaves and pods were then used for nutritional and phytochemical composition analysis.

The OMET growing technique significantly ($p \leq 0.05$) affected the growth and yield attributes of okra regardless of the growing condition. At termination (110 days after transplanting), the OMET growing technique had significantly increased the stem

diameter by 40 and 37%, while the plant height was increased by 68 and 48% under greenhouse and micro-plot experiments respectively. When evaluating the yield attributes, a similar trend was observed where the OMET system significantly increased the yield attributes of okra as follows: biomass by 64 and 50%, number of branches by 67 and 50%, number of pods per plant by 60 and 49%, fresh pod weight by 75 and 53%, pod length by 64 and 51% in both the growing environment, while the pod diameter width was increased by 68% in the greenhouse environment and there was no significant difference on the micro-plot trial.

The OMET growing technique significantly affected the nutritional composition of okra leaves and pods ($p \leq 0.05$). Both the essential and non-essential amino acids were determined and quantified in the leaves and pods of okra grown under OMET and Non-OMET growing techniques. The OMET growing technique significantly improved the essential amino acid composition of okra leaves with Thr (0.57 mg/kg), Val (0.70 mg/kg) Leu (0.90 mg/kg) and Phe (1.03 mg/kg) being higher than the non-OMET grown okra leaves. The pods showed that the OMET growing technique also significantly improved the accumulation of all the tested non-essential amino acids with Lys being the highest ($p \leq 0.05$). It was observed that the micro-plot experiment resulted in the OMET growing technique significantly enhancing the accumulation of all the tested essential amino acids in both the leaves and pods with Phe and Lys (1.53 and 0.70 mg/kg) being the highest in the leaves and pods respectively. The non-essential amino acid composition was also significantly improved using the OMET growing technique in both the leaves and pod with Glu.. The non-essential amino acid composition was also significantly improved using the OMET growing technique in both the leaves and pod of okra grown under micro-plot with Glu (2.73 mg/kg and 4.05 mg/kg) being the highest respectively. The OMET growing technique showed the ability to maintain a daily recommended amino acids ratio coefficient equal to 1 by consuming 100g of the tested okra. The nutritional composition which includes proteins, mineral elements and amino acids of the tested okra leaves and pods grown using the OMET system regardless of the growing condition has resulted in an increase in the % protein content compared to the non-OMET grown okra. Nutritional elements Ca, K, P, Mg and Na were predominant in okra leaves and pods irrespective of

the growing environment and growing technique (OMET). The results generated showed that the OMET system significantly ($p \leq 0.05$) improved the nutritional composition in okra leaves and pods regardless of the growing condition, though the micro-plot experiment resulted in higher nutritional composition as compared to the greenhouse experiment.

Untargeted metabolites, phenolic acids and antioxidant activity were also determined and compared for both the okra leaves and pods extract grown using the OMET and non-OMET growing techniques in both the growing conditions. For untargeted metabolites, methanol extracts were analysed using UPLC-ESI-QTOF-MS. The UPLC-MS untargeted metabolites profile detected 161 polar analytes classified within the glucuronic acid, tricarboxylic acids, O-glycosyl derivatives, flavonoid-O-glycosides, iridoid o-glycosides and terpene glycosides. Explorative principle component analysis demonstrated three main clusters according to metabolites heterogeneity in plant tissue (pods and leaves) and growing conditions (greenhouse or mirco-plot). There was major heterogeneity in the metabolome profile of leaves tissue along the vertical PC1 suggesting their metabolic moiety. Okra leaves grown under micro-plot were highly predominated by the 2-O-caffeoylglucaric acid (286.13 mg/kg) and 2-(E)-O-feruloyl-D-galactaric acid (111.69 mg/kg). Leaves samples grown in non-OMET were predominated by citroside A (412.04 mg/kg). Okra pods grown under OMET micro-plot enhanced the accumulation of quercetin 3-galactoside (87.83 mg/kg) and quercetin 3-galactoside-7-glucoside (150.00 mg/kg). The OMET under greenhouse conditions encouraged the accumulation of icariside F2 and benzyl beta-D-apiofuranosy (49.21 mg/kg). The results generated showed that flavonoids were the major contributors to the total antioxidant activity and OMET enhanced the accumulation of the majority of the metabolites. Phytochemical analysis showed that the OMET growing technique significantly increased the concentration of total phenolics and flavonoids in both the growing environment ($p \leq 0.05$). The OMET growing technique significantly affected the antioxidant activity in both growing conditions.

# Chapter 1: General Introduction

## 1.1 Introduction

*Abelmoschus esculentus* (L.) (Moench) also known as okra, belongs to the Malvacea family. Okra is an underutilised vegetable consumed in the world. Okra production accounts for an estimated area of 2 million ha globally with a total annual production of 9 million tons (FAO, 2018). The common okra is praised for its thickened, fleshy, engorged leaves and tender immature fresh pods which are consumed as vegetables in the preparation of daily meals (Gemede *et al.,* 2015; Purseglove, 1987; DAFF, 2012). In some countries, they are usually boiled in water to make slimy soups and sauces (Gemede *et al.,* 2015). In fact, the young leaves are commonly used as a relish like spinach. Whilst roasted okra seeds are used in some areas as a substitute for coffee (Siemonsma and Kouame, 2004). On the other hand, matured and dry long ridged seed pods are a rich source of edible oils (Reddy *et al.,* 2013). The consumption of 100 g of okra provides vitamins (31 mg), carbohydrates (7.6 mg), proteins (2.0 mg), dietary fibre (3.2 mg), calcium (75 mg) and magnesium (57 mg) (Liu *et al*., 2005; Kumar *et al*., 2009). In Africa, the production of okra is predominated in the northern and eastern areas including Nigeria, Morroco, Cameroon and Ghana (Kumar *et al*., 2009) whilst, there is lower productivity in Sub-Saharan African (SSA) areas (Kumar *et al*., 2009). Okra is widely grown in tropical and subtropical regions under irrigation (Siemonsma and Kouame, 2004). The crop can be planted in different soil types but performs best in a well-drained sandy loam with pH 6-7, and a high content of organic matter. Okra pods are sometimes marketed as canned or dehydrated (Liu *et al*., 2018, Kumar *et al*., 2009).

OMET farming is a climate-smart production system that is more water efficient, simpler and produces a higher yield. In addition, this method produces a significant quantity of crops in a relatively small area. All plant waste goes into the compost that feeds the soil again. The sheet mulch used in the OMET system virtually eliminates evaporation, dramatically reducing the water requirements of the crop. Heavy rainfall or drought has minimal impact on the system as the enclosed trough keeps the soil at a constant, optimal temperature that will result in increased growth and ultimately improved yield (Ferreira,

2013). Its smooth surface deters crawling insects and prevents weed growth, minimizing the need for weeding or herbicides. Pests and diseases are controlled when there is a need, since OMET is a closed trough system, the micro-organisms in the soil suppress soil-borne diseases such as root rot. Earthworms thrive in the growth medium, a clear sign of healthy living soil (Ferreira, 2013). Although the system is totally enclosed and there is no drainage, it conserves all the nutrients healthy plants need. The plants grow in optimal growing conditions and are easily cared for. This method results in record growing times (Ferreira, 2013), with exceptional high-quality produce, environmentally friendly and organic (Ferreira, 2013).

The use of metabolite (untargeted and targeted) and chemometric analyses has gained popularity as an important tool in crop sciences. High-performance liquid chromatography (HPLC) is one of the most popular, modern, powerful, and versatile chromatographic separation techniques that have been routinely used to separate the components in a mixture, to identify each component (or at least as many components as possible), to quantify separated components, and to obtain the chemical profile. HPLC is the most widely used analytical separation technique for the qualitative and quantitative determination of compounds in extracts (Santos *et al*., 2017). Chemometrics is not a single tool but a range of methods including basic statistics, signal processing, factorial design, calibration, curve fitting, factor analysis, detection, pattern recognition, and neural network. Chemometrics is the tool for extracting information from multivariate chemical data using tools of statistics and mathematics (Saxena *et al*., 2013). With the advance of computational techniques, chemometrics has become a leading tool for faster analysis of results/data and shorter product development time. It is generally applied for one or more of three primary purposes to explore patterns of association in data, track properties of materials on a continuous basis and prepare and use multivariate classification models. This tool has the capacity for analysing and modeling a wide variety of data types for an even more diverse set of applications. Chemometrics are basically classified into two main categories: pattern recognition methods (unsupervised and supervised) when a qualitative evaluation is considered and multivariate calibration for quantitative purposes. The design of the experiment, data preprocessing, classification, and calibration are the

main practical steps involved in any chemometrics analysis. Experimental design primarily screens factors that are important for the success of a process (Saxena *et al*., 2013). Principal component analysis (PCA) is an unsupervised pattern recognition technique used for handling multivariate data without prior knowledge about the samples under investigation (Santos *et al*., 2017). The central idea of PCA is to reduce the dimensionality of a data set consisting of large amounts of interrelated variables, while keeping maximum variation in the data set. PCA is generally used to evaluate the discrimination ability of common components using relative peak areas of common peaks as input data instead of the full fingerprint (Santos *et al*., 2017).

### 1.1.1 Description of the research problem

The consumption of indigenised vegetables in Sub-Saharan Africa was encouraged by the World Health Organisation with the aim to improve food security. Currently, a total of 21 leafy vegetables have been added to the South African food database owing to their nutritional composition that enhances balanced diets. Yet, the consumption of okra in Sub-Saharan Africa is lower compared to northern and eastern Africa. Indigenous vegetables such as okra are rich in vitamins, minerals, proteins and antioxidants (van den Heever, 1995), while on the other hand, they are drought tolerant. Inland South Africa predictions suggested that temperatures would increase by 2% in 2030, whereas rainfall would decline by 10% (IPCC, 2007). Generally, the scarcity of water is due to the greater frequency and severity of droughts in most semi-arid areas, and excessive heat conditions, all of which could limit plant growth and yields (Parry *et al*., 2007). Smallholder farmers are mostly affected by water deficits (Altieri and Koohafkan, 2008).. The reduction of yields due to water scarcity and use of sustainable cropping systems have since become an economic problem since the presidential outcomes for agriculture which directly affect food security, job creation and wealth generation.

### 1.1.2 Impact of the research problem

Due to increasing unfavorable conditions for exotic crops, climate-smart agriculture has since dictated the increased focus on adapted underutilised plant species. However, production information for most of the plant species with the potential for improving food security, job creation and wealth generation is not empirically supported. Information on increasing the growth, yield, phytochemicals and accumulation of essential nutrient elements in okra using a climate-smart agriculture has not been documented. The study was intended to investigate the possibility of enhancing growth, yield, phytochemicals and nutrients profiling of okra using the OMET growing technique in different growing environments (greenhouse and field) in order to enhance the development of best agricultural production practices under the disposal of lower irrigation water as water is becoming a scarce resource worldwide.

Sub-Saharan Africa is characterized by water scarcity and the challenges of rapid population growth which results in food and nutrition insecurity. The majority of smallholder communities are located in marginal areas where crops struggle to survive due to poor growing conditions. Ultimately, residents and farmers face food insecurity and malnutrition challenges as a result of poor growing conditions and a scarcity of production factors. Furthermore, commercial or irrigated crop production occurs in areas where water is scarce.

There is insufficient scientific information on the growth and yield attributes of underutilised indigenous vegetables such as okra. Furthermore, the information on the enhancement and evaluation of growth, yield, nutrients, and phytochemical compositions of okra using the climate-smart OMET growing technique has not been documented. The OMET growing technique is a production system that utilises less water and restricts water and nutrients seepage while increasing the yield in a relatively small area.

Strategies to improve growth and yield in crop production without compromising nutritional composition and its phytochemical profiling are a necessity to improve socio and economic factors of small-scale farmers. Such investigations will aid in proper water management and understanding adaptation mechanisms. Studies on metabolites

profiling have been adopted in the crop sciences to elucidate potential bio-makers involved during crop adaptation to different growing conditions. Such studies in concomitant to chemometric analysis have been adopted to underpin metabolites induced during water deficit irrigation, fertilizer application, and saline water in different crops. These advances in biological research have also resulted in exciting discoveries that when applied in food and agriculture, will help to mitigate the many challenges that exist today.

Metabolomics is one such innovation that has great potential to advance agricultural research. The science for the profiling and characterization of metabolites is called Metabolomics. Plant metabolites are small organic molecules that are produced by protein catalysis. Studies of plant metabolites can provide valuable information regarding the plant biosynthetic and catabolic pathways and are therefore very important for understanding the physiological and biochemical regulations of plant cells. Further, as plant metabolites are direct indicators of plant performance, they can potentially guide the selection and development of biochemical markers, providing valuable tools for the improvement of plant cultivation practices (Seger and Sturm, 2007). The application of chemometrics in crop production can provide the incentive to address a number of biologically significant issues, including climate change, biotic and abiotic stresses, crop breeding, nutritional properties, genetic engineering and adaptation mechanisms.

### 1.1.3 Possible causes of the research problem

Water remains a limited resource worldwide because of the ever-changing climate coupled with the nature of the extremely high temperatures experienced in arid regions like SSA. The observed heat waves, intense droughts, shifts in rainfall patterns, and an increase in average temperatures have an impact on every aspect of crop production (Zhongming *et al*., 2021). Climate change is already having an impact on the amount of water available for irrigation. The availability of water and nutrients has a direct impact on crop growth, yield and accumulation of metabolites.

Increase in population is also a possible cause of the research problem which results in food insecurity which is the basic cause of malnutrition. The underutilization of indigenous vegetable crops such as okra is increasing dramatically, most likely due to a lack of knowledge about the benefits of eating these highly nutritious indigenous vegetables. In fact, the lack of food diversification, whereby only one type of staple food is consumed adds to nutritional food insecurity. There is a tremendous need to grow indigenous vegetables to nutrify our generation to improve our well-being. This however requires a more efficient method of crop production that are environmentally friendly to ensure sustainability and counteracts malnutrition (FAO, 2018). New climate-smart growing methods have to be developed to outsmart unfavorable climatic conditions to improve crop yields (Ferreira, 2013).

### 1.1.4 Proposed solutions

This study is designed to establish new information in the form of documentation on the use of the OMET growing technique to enhance the growth, yield, nutritional composition and phytochemical levels of okra. The OMET growing technique is a highly sustainable method of crop production that can be practiced by farmers to increase crop production using less inputs to meet the needs of present and future generations. It is aimed at reducing input production systems such as labour cost, and water to suit resource-poor farmers to enable them to commercialize crop production. The issue of water scarcity will be effectively addressed through the execution of climate-smart OMET growing technique.

The use of the climate-smart OMET growing technique is intended to produce high-quality nutritious food to fight malnutrition which is the basic cause of food insecurity in Southern Africa. Most modern farming system such as hydroponic farming is said to be labor intensive and requires a lot of investment to install and maintain. They also require more knowledge and skills to adopt or put into practice. The study is aimed to improve the scientific way of farming to address the challenges faced by farmers. The findings of this study will address sustainable agriculture and the efficient use of irrigation water to improve food security. The knowledge gained will push farmers to adopt this system to

improve yield under the disposal of lower irrigation water to produce food to feed the growing population (Ferreira, 2013).

### 1.1.5 General focus of the study

Quantitative, qualitative and horticultural attributes of a crop form an integral part of the health of any society. This has however been a very big challenge sitting at the heart of many developing countries such as South Africa which has been threatening human development and prosperity. Inorganic farming possesses many environmental and health hazards that affects soil quality, the quality of food we eat and our well-being. Therefore, there is a significant need to grow good quality produts to nourish our generation. This however requires a more efficient method of crop production that is environmentally friendly to ensure sustainability and counteract malnutrition.

Issues surrounding water scarcity is one the major problems faced by farmers today as water remains limited for agricultural purpose due to climate change and global warming. The problem however is worsened by varied extreme weather events normally experienced in SSA. The rapid population growth and the nature of characteristics of smallholder farmers in South Africa. The majority of smallholder farmers in South Africa are illiterate and do not have adequate resources to commercialize crop production. This as a result tends to cause farmers to focus on less demanding crops such as maize which reduces the cultivation of other crops. The rapid population growth, malnutrition and water scarcity put pressure on farmers to produce more food with limited resources. Moreover, most modern farming system such as hydroponics are too expensive to install and maintain and requires a high level of expertise. This, therefore, hinder most smallholder farmers to adopt this kind of system. This study is focused on the introduction of climate-smart agriculture with new innovative strategies that use less water for crop production to maximise yields, nutritional and phytochemical composition, which is necessitated by recent changes in the climate which result in water scarcity.

## 1.2 Problem statement

Okra production accounts for an estimated area of 2 million ha globally with a total annual production of 9 million tons (FAO, 2018). The African continent accounts for 32.8% of the world's okra production. West and Central African countries contribute to over 75% of total okra production in SSA (Kumar and Reddy, 2016). Despite the significant contribution by SSA towards global okra production, average yields are low and variable in the region. For example, pod yields of 2.5, 6.2 and 8.8 tons/ha were reported in West, East and North Africa, respectively (FAO, 2018). Low and variable yields in SSA are attributed to poor cultivation techniques (Alake, 2020) associated with poor management practices. Further, biotic (i.e., cucumber mosaic virus) and abiotic stress (i.e., drought and heat) are the major constraints to okra production and declining yields (Mkhabela *et al*., 2021). Freshwater is becoming the scarcest and most critical commodity nationally and internationally, which necessitates a need to find a more sustainable way of farming to reduce irresponsible irrigation methods, leaking infrastructure, high evaporation, changes in climatic conditions, industrial and domestic water wastage while increasing yield (Ferreira, 2013). There is a need to introduce new innovative strategies that use less water for okra production to maximise yields and metabolites which is necessitated by recent changes in the climate which result in water scarcity.

Organic Medium Enclosed Trough (OMET) farming is a climate-smart production system that is more water-efficient, simpler and produces a higher yield, in a relatively small area. All plant waste goes into the compost that feeds the soil again. The sheet mulch used in the OMET system eliminates evaporation, reducing the water requirements of the crop (Ferreira, 2013). Changes in the climatic condition have minimal impact on the system as the enclosed trough keeps the soil at a constant, optimal temperature that will result in an increased yield (Ferreira, 2013). There is a need to develop and introduce okra cultivation practices to improve yield, quality, food and nutrition security faced by the growing population (DAFF, 2012). Growing okra on an OMET system is a promising tool for improved yield. However, since this is a newly developed technique, there is limited information on the actual rates of the yield of okra grown under this OMET system.

## 1.3 Rationale of the study

Okra is a source of medicinal properties for the prevention and treatment of cancer, sugar diabetes, reduced serum cholesterol, relieving constipation, heart problems and improved brain health (Gemede *et al*., 2015; Dubey and Mishra, 2017; Liu *et al.*, 2018), the balanced nutritional composition of okra makes it a vital source of nutrition to reduce malnutrition in Asian and SSA countries. The seeds are a rich source of protein (22.14%), amino acids (lysine and tryptophan), fat, fibre, vitamins (A, C and K), and mineral elements (calcium, potassium, sodium, and magnesium) (Sanjeet *et al*., 2010; Petropoulos *et al.,* 2018; Gerrano, 2018). Furthermore, the crop is cultivated under various agricultural cropping systems due to its ease of cultivation and wide adaptation to diverse environmental conditions (Kumar and Reddy, 2016).

The information on enhancement and evaluation of growth, yield, nutrients, and phytochemical compositions of okra using climate-smart OMET growing technique has not been investigated and documented. Considering the above mentioned, this facilitates a need for research to commercialize the crop, maximize yield and ensure that okra is conserved and adequate information is available to farmers. This will assist in conserving water and reduce production costs without compromising the horticultural attributes, nutritional and biochemical quality of the crop. Mulching is a similar technology and has been suggested to improve the growing conditions thus increases the yield. Plastic mulching affects the physical, chemical, and biological properties of soil, as well as promotes the decomposition of soil organic matter and the transformation and release of soil nutrients (Acharya *et al.,* 2005; Ferrini *et al*., 2008). Plastic mulches directly affect the microclimate around the plant, modifying the surface radiation budget and reducing soil water loss (Ferrini *et al*., 2008), and their use contributes substantially to an improved biological value of yield (Ferrini *et al*., 2008). For determining the stress response to different cropping systems in crops, untargeted metabolites have become a powerful tool to investigate unpredictable metabolism to elucidate how plant growth and yield response to various cropping systems (Akhatou *et al*., 2016; An *et al*., 2016). There is a necessity for research on innovative strategies that conserve water while maintaining nutritional,

phytochemical and biochemical quality in crop production thus the development of the OMET growing technique.

## 1.4 Purpose of the study

### 1.4.1 Aim

The purpose of the study was to develop scientific information on the effects of thy OMET growing technique and growing environment on growth and yield, nutritional composition and phytochemical composition in okra.

### 1.4.2 Objectives

i. To determine the effect of the OMET growing technique and growing environment (greenhouse and micro-plot) on growth and yield attributes of okra.
ii. To determine the effect of the OMET growing technique and growing environment (greenhouse and micro-plot) on nutritional composition in the leaves and pods of okra.
iii. To assess the effect of OMET growing technique and growing environment (greenhouse and micro-plot) on phytochemical composition in the leaves and pods extracts of okra.

## 1.5 Reliability, validity and objectivity

For this study, reliability was based on using appropriate statistical analysis of data using GenStat 18th version (VSN International, Hempstead, UK) at a significance level ($p \leq 0.05$). Validity is achieved through replicating the treatment to increase the range of validity as well as controlling and repeating the experiments in time. Objectivity was achieved by discussing the findings based on empirical evidence as shown by statistical

analyses, with findings checked for similarities and differences with findings in other studies, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

### 1.6 Bias

In this study, bias was minimized by ensuring that the experimental error in each experiment and trial was reduced through increased replications and randomization (Leedy and Ormrod, 2005).

### 1.7 Scientific contribution

The documented knowledge regarding the use of the OMET growing technique will contribute to the farmers of okra about the strategy to improve growth, yield and nutrition. This study will provide useful information on the absorption of the OMET growing technique if it increases the nutritional content, phytochemical levels, growth and yield of okra, then finally we will be having a solution to our dwindling food resources, which is influenced by the growing population. The information generated will be distributed between small-scale farmers and growers of traditional crops to enlighten them on a more sustainable way of farming. This system has no rain influence, no high evaporation and no water or nutrient seepage, because it is practiced under an enclosed trough. Resulting in more easily and profitable farming and production of quality produce thus poverty being a thing of the past to the vulnerable communities because they can be able to produce under the disposal of lower irrigation.

### 1.8 Structure of the dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was outlined as a literature review (Chapter 2). Then (Chapter 3) looked at the effect of the OMET growing technique as compared to the non-OMET system and growing environment on the growth and yield

attributes of okra. Chapter 4 outlined the effect of the OMET growing technique and growing environment on the nutritional composition of okra in the leaves and pods and Chapter 5 addressed the final objective which was to investigate the effect of the OMET growing technique and growing environment on the phytochemical composition of both the leaves and pods extracts. Chapters 3, 4 and 5 are structured in a research publication format. In the final chapter (Chapter 6), the significance of the findings was summarized and integrated to provide their significance with recommendations for future research. In the study, citations in text and references followed the Harvard style of author-alphabet as approved by the Senate of the University of Limpopo.

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# Chapter 2: Literature review

## 2.1. Introduction

Under climate-smart agriculture or water-constrained conditions, the cultivation of underutilised indigenous crops is becoming more important. Abiotic stress factors, particularly drought stress, are responsible for small-scale farmers' yield losses of between 30 -100 percent (Wiersum *et al.,* 2006). The development of sustainable and environment-friendly methods as a best practice strategy in Sub-Saharan Africa can improve production and agricultural productivity of semi-arid originated plants as underutilised crops under the impeding climate change, where predictions suggest that extremes related to drought and high temperatures would be common (Zuluaga *et al*., 2020). The ensuring review intended to assess what has already been done and not done on indigenous alternative medicinal crops with reference to okra.

## 2.2 Work done on okra

### 2.2.1 Morphology of okra

Okra is dicotyledonous plant which is is robust, erect and an annual herb, ranging between 1 to 2 m in height. The leaves are alternate, heart shaped and three to five-lobbed. The flowers are regular, solitary and yellow in colour with a crimson centre, with superior ovaries and numerous stamens. The fruit which is the pod is variable in colour when fresh and is hairy at the base, is a tapering 10-angled capsule 10-25 cm (4-10 inches) in length that contains numerous oval dark-coloured seeds (McGregor, 1976; Kumar *et al.,* 2010). Figure 2.1 shows okra plant with the leaves, flowers and the pods.

Figure 2.1: Okra plant showing the flowers, immature pods, petals and the leaves

2.2.2 Origin, distribution and production of okra

Okra is one of the oldest cultivated crops, and it is now grown in many nations, particularly in tropical and subtropical areas, as well as in Asia, Africa, Europe, and America. Okra is thought to have originated in Ethiopia and was first cultivated in Egypt in the 12th century BC. After that, the cultivation spread throughout the Middle East and North Africa. Even though it is documented that it originates from Ethiopia the center of origin remains unclear (Arapitsas, 2008). It was previously classified as *Hibiscus*, section *Abelmoschus*, of the Malvaceae family (Qhureshi, 2007). *Abelmoschus* was later proposed to be elevated to the level of a separate genus. The broader use of Abelmoschus was later recognized in taxonomy and contemporary literature (Kumar *et al*., 2009).

The annual global production of okra is 9,953,537 tonnes. With an annual production of 6,176,000 tonnes, India is the world's largest okra grower. India generates more than 60% of the okra consumed worldwide. With an annual production of 1,819,018 tonnes, Nigeria ranks second. Mali is the third-largest producer of okra, with 512,855 tonnes produced annually. United States of America is ranked 24th with 10,192 tonnes of production annually (Table 2.1) (Sorapong, 2012; MEF, 2013). Eight of the top ten okra-producing countries are from the African continent.

Table 2.1: Production of okra worldwide (www.atlasbig.com)

| | Country | Production/tons | Production per Person (Kg) | Acreage/ha | Yield (kg/ ha) |
|---|---|---|---|---|---|
|  | India | 6,176,000 | 4.621 | 513,000 | 12,039 |
|  | Nigeria | 1,819,018 | 9.215 | 1,802,463 | 1,009.2 |
|  | Mali | 512,855 | 26.84 | 46,741 | 10,972.3 |
|  | Sudan | 309,413 | 7.584 | 29,365 | 10,536.8 |
|  | Côte d'Ivoire | 152,325 | 6.116 | 54,061 | 2,817.7 |
|  | Pakistan | 124,779 | 0.618 | 16,239 | 7,683.9 |
|  | Cameroon | 104,216 | 4.38 | 36,975 | 2,818.6 |
|  | Niger | 103,854 | 4.838 | 148,431 | 699.7 |
|  | Egypt | 88,819 | 0.911 | 5,719 | 15,530.5 |

| Country | Production/tons | Production per Person (Kg) | Acreage/ha | Yield (kg/ ha) | |
|---|---|---|---|---|---|
| | Ghana | 68,954 | 2.328 | 3,270 | 21,086.9 |
| | Benin | 60,341 | 5.311 | 16,932 | 3,563.7 |
| | Iraq | 59,353 | 1.509 | 10,990 | 5,400.6 |
| | Malaysia | 58,204 | 1.782 | 3,781 | 15,393.8 |
| | Mexico | 42,207 | 0.338 | 4,387 | 9,620.9 |
| | Senegal | 35,200 | 2.238 | 968 | 36,363.6 |
| | Philippines | 31,708 | 0.298 | 4,011 | 7,905.3 |
| | Turkey | 31,428 | 0.389 | 6,143 | 5,116.1 |
| | Saudi Arabia | 24,210 | 0.725 | 1,561 | 15,509.3 |
| | Burkina Faso | 23,100 | 1.141 | 3,744 | 6,169.9 |
| | Yemen | 22,814 | 0.789 | 4,539 | 5,026.2 |
| | Syria | 17,121 | 0.936 | 4,625 | 3,701.8 |
| | Oman | 16,843 | 3.361 | 813 | 20,717.1 |
| | Guyana | 14,977 | 19.147 | 281 | 53,298.9 |
| | United States of America | 10,192 | 0.031 | 1,272 | 8,012.6 |
| | Albania | 8,023 | 2.795 | 690 | 11,627.5 |
| | Jamaica | 6,838 | 2.506 | 679 | 10,070.7 |
| | Guatemala | 6,581 | 0.38 | 1,080 | 6,093.5 |
| | Jordan | 5,212 | 0.509 | 739 | 7,052.8 |
| | Malawi | 4,157 | 0.232 | 2,138 | 1,944.3 |
| | Kenya | 4,107 | 0.081 | 1,445 | 2,842.2 |
| | Kuwait | 3,474 | 0.822 | 191 | 18,188.5 |
| | Palestinian Territories | 3,114 | 0.684 | 443 | 7,029.3 |
| | Fiji | 2,761 | 3.12 | 309 | 8,935.3 |
| | Trinidad and Tobago | 1,802 | 1.328 | 536 | 3,361.9 |
| | Congo-Brazzaville | 1,748 | 0.324 | 581 | 3,008.6 |
| | United Arab Emirates | 1,695 | 0.178 | 77 | 22,013 |
| | Mauritius | 1,258 | 0.995 | 177 | 7,107.3 |
| | Lebanon | 973 | 0.16 | 427 | 2,278.7 |
| | Cyprus | 558 | 0.653 | 31 | 18,000 |
| | Barbados | 509 | 1.777 | 44 | 11,568.2 |
| | The Bahamas | 502 | 1.316 | 19 | 26,421.1 |
| | Brunei | 432 | 1.022 | 525 | 822.9 |
| | Qatar | 188 | 0.077 | 45 | 4,177.8 |
| | Bahrain | 56 | 0.037 | 2 | 28,000 |
| | Belize | 32 | 0.081 | 26 | 1,230.8 |

### 2.2.3 Environmental requirements of okra

Temperatures over 20°C are necessary for the normal growth and development of okra (Kumar *et al.*, 2009). Germination percentage and speed of emergence are optimal at 30-35°C. With rising temperatures, flowering of okra is delayed (there is a positive correlation between temperature and the number of vegetative nodes) (NRC, 2009). *Abelmoschus* spp. is a short-day plant, but its wide geographical distribution (up to latitudes of 35-40° N) indicates that cultivars differ markedly in sensitivity. Flower initiation and flowering are hardly affected by day length in popular subtropical cultivars. Most tropical cultivars show quantitative short-day responses, but qualitative responses also occur (Ndangui *et al.,* 2010). The fruits also serve as soup thickeners. Okra seeds can be dried, the dried seeds are a nutritious material that can be used to prepare vegetable curds or roasted and ground to be used as a coffee additive or substitute (Kumar *et al*., 2010).

### 2.2.4 Culinary uses and preparation of okra.

Okra is suitable for medical and industrial use. Roasted okra seeds are used in some areas as a substitute for coffee (FAO, 2018). Okra is not only used as a vegetable but also in the rope-making and paper industry. Okra seed oil is also used for food and biodiesel production. Young immature okra fruits are consumed cooked or fried (Mihretu *et al*., 2014). Okra is usually boiled in water resulting in slimy soups and sauces, which are relished. Okra leaves are considered good cattle feed, but this is seldom compatible with the primary use of the plant. The leaf buds and flowers are also edible (Mihretu *et al*., 2014).

### 2.2.5 Nutritional status of okra

Nutrition assessment is the best way to determine whether people's nutritional needs are effectively being met. Nutrients are the components found in our food such as carbohydrates, vitamins, minerals, fats, etc. These components are necessary for survival. Nutrition analysis of medicinal plants provides quality, quantity, and evidence-

based information for future research, planning, commercialization and utilization, all together aiming at eradicating hunger, poverty and reducing the burden of malnutrition in southern Africa (Messing *et al.,* 2014).

Deficiencies of iron, zinc, calcium and vitamins are widespread, with over 300 million people affected every year, and a much greater number being at risk of malnutrition deficiencies (Habwe *et al.*, 2009). Malnutrition deficiencies increase the vulnerability to infectious diseases, causing numerous human deaths (Davis, 1996). Micronutrient deficiencies affect mainly pregnant women and children and contribute significantly to the global disease burden of children by limiting proper cognitive development, impairing physical development, and increasing susceptibility to infectious diseases (Joseph *et al.,* 2017). Most countries in Sub-Saharan Africa are still struggling to address the problems of under-nutrition and micronutrient deficiencies (Nesamvuni *et al.,* 2014). Indigenous leafy vegetables such as okra are increasingly being recognised as possible contributors of both micronutrients and bioactive compounds to human diets of populations worldwide (Smith and Eyzaguirre, 2007). Indigenous vegetables such as okra play an important role in the survival of the growing populations at risk and help to reduce malnutrition. These indigenous vegetables are significant contributors to food security and nutrition for smallholder farmers (Njeme *et al.,* 2014). Though there is a need to introduce cultivation practices with good ecological interaction to be able to increase productivity.

As presented in Table 2.2 below, the majority of vital nutrients are available in okra. These nutrients are vital in supplying several health advantages which include but are not restricted to the risk of the development of chronic non-communicable diseases for example obesity, hypertension, coronary heart diseases, hypercholesterolemia and gastrointestinal disorder. Okra plays a vital role in the human diet by supplying fats, proteins, carbohydrates, minerals and vitamins (Gopalan *et al*., 2007; Varmudy, 2011). Carbohydrates are mainly present in the form of mucilage (Liu *et al*., 2005; Kumar *et al*., 2009). The main components of the young fruits long-chain molecules are galactose (25%), rhamnose (22%), galacturonic acid (27%) and amino acids (11%). The mucilage is highly soluble in water. Its solution in water has an intrinsic viscosity value of about

30%. The okra seeds incorporate about 20% protein and 20% oil (Tindall, 1983). The okra pods are among the very low-calorie vegetables. They provide just 30 calories per 100 g besides containing no saturated fats or cholesterol. Nonetheless, they are rich sources of dietary fiber, minerals, and vitamins, often recommended by nutritionists in cholesterol-controlling and weight-reduction programs. The pods are one of the rich sources of mucilage substance that helps in smooth peristalsis of digested food through the gut and ease constipation condition. Vitamin-A is essential for maintaining healthy mucosa and skin (Gopalan *et al*., 2007). Fresh pods are a good source of folates, providing about 22% of RDA per 100 g. Consumption of foods rich in folates, especially during the pre-conception period helps decrease the incidence of neural tube defects in the newborn. They are rich in the B-complex group of vitamins like niacin, vitamin B-6 (pyridoxine), thiamin, and pantothenic acid. The pods also contain good amounts of vitamin-K which is a co-factor for blood clotting enzymes and is required for bone health. The pods are also a good source of many essential minerals such as iron, calcium, manganese, and magnesium (Gopalan *et al*., 2007).

Table 2.2: Nutritional profile of okra pods per 100 g.

| Principle nutrient | Nutrient value | %RDA |
|---|---|---|
| Energy | 33 kcal | 1.5 |
| Carbohydrates | 7.03 g | 5.4 |
| Protein | 2.0 g | 4 |
| Total fat | 0.1 g | 0.5 |
| Cholesterol | 0 mg | 0 |
| Dietary fiber | 3.2 g | 9 |
| Vitamins | | |
| Folates | 88 µg | 22 |
| Niacin | 1.000 mg | 6 |
| Pantothenic acid | 0.245 mg | 5 |
| Pyridoxine | 0.215 mg | 16.5 |
| Riboflavin | 0.060 mg | 4.5 |

| Principle nutrient | Nutrient value | %RDA |
|---|---|---|
| Thiamin | 0.200 mg | 17 |
| Vitamin C | 21.1 mg | 36 |
| Vitamin A | 375 IU | 12.5 |
| Vitamin E | 0.36 mg | 2.5 |
| Vitamin K | 53 µg | 44 |
| Electrolytes | | |
| Sodium | 8 mg | 0.5 |
| Iron | 0.80 mg | 10 |
| Magnesium | 57 mg | 14 |
| Manganese | 0.990 mg | 43 |
| Phosphorus | 63 mg | 9 |
| Selenium | 0.7 µg | 1 |
| Zinc | 0.60 mh | 5.5 |

The table is retrieved from: USDA SR-21 Nutrient Database, 2015.

2.2.6. Secondary metabolites reported in okra

Okra is known for being high in antioxidant activity in both the leaves and the pods. With reference to the work done by Arapitsas (2008), whereby they reported that okra seed is rich in phenolic compounds, mainly composed of flavanol derivatives and oligomeric catechins. According to Khomsug *et al*., (2011), the total phenolic content of pod pulp and seed extracts of okra was found to be 10.75±0.02 mg GAE/100 g extract and 142.48±0.02 mg GAE/100 g extract which corresponds with scavenging activities. Besides they have also found procycanidin B2 as the predominant phenolic compound followed by procycanidin B1 and rutin in seeds. In pulped seed catechin, procycanidin B2, epicatechin and rutin are reported to be present, Liao *et al*., (2005) have done a comparative analysis of total phenolics and total flavonoids and antioxidant ability of different organs (flower, fruit, leaf, and seed) and different enrichment fractions of water extracts of the okra plant. They confirmed the fruitful presence of total phenolics, and total flavonoids related to antioxidant ability in all the extracts of the plant organs although the percentage varied. In the flower of okra, the highest amount of total phenolics and total flavonoids were found (Liao *et al*., 2005; Ngoc *et al.,* 2008). Table 2.3 indicate phyto-nutrients profile of fresh, raw pods per 100 g. This data suggests okra as a good contributor to the antioxidant status and a promising chemo-preventive agent as described in several traditional medicines for the human race.

Table 2.3: Phyto-nutrients profile of fresh, raw pods, value per 100 g

| Principle | Phytochemical Value µg |
|---|---|
| Carotene-ß | 225 |
| Crypto-xanthin-ß | 0 |
| Lutein-zeaxanthin | 516 |

The pods compose of healthy amounts of flavonoid antioxidants such as beta-carotene, xanthin, and lutein. It is one of the vegetables with the highest levels of these antioxidants.

Consumption of natural vegetables and fruits rich in flavonoids helps to protect from lung and oral cavity cancers (Middleton, 2000).

### 2.2.7 Medicinal uses and human health benefits of okra

Okra is a multipurpose crop due to its various uses of fresh leaves, buds, flowers, pods, stems and seeds (Mihretu *et al*., 2014). Okra may be considered a medicinal plant due to its nutritional enhancement in dipping the blood glucose level in hyperglycemia induced by diabetes (Kumar *et al*., 2010). It is also an important component of preventive therapy in the management of diabetes and its related complications. Moreover, the pods are also used to reduce diarrhea and acute inflammation dysentery (Middleton, 2000). The plant is also used to reduce kidney catarrhal infections, ardor urine, dysuria irritation of the stomach and gonorrhea. Okra has found medical applications as a plasma replacement or blood volume expander (Arapitsas, 2008; Madison, 2008; Kumar *et al*., 2010). Tests conducted in China suggest that an alcohol extract of okra leaves can eliminate oxygen-free radicals, alleviate renal tubular-interstitial diseases, reduce proteinuria, and improve renal function (Liu *et al*., 2018; Kumar, 2014).

Okra improves cardiovascular disease, reduces serum cholesterol and therefore decreases the chance of heart disease. The use of okra is an efficient method to manage the body's cholesterol level. Okra is additionally loaded with pectin that can help in reducing high blood cholesterol simply by modifying the creation of bile within the intestines (Madison, 2008; Dubey and Mishra, 2017; Liu *et al*., 2018). Table 2.4 indicate different bioactive components derived from okra showing their therapeutic benefits on human health, along with their mechanisms of action.

Table 2.4. Different bioactive components derived from okra showing their therapeutic benefits on human health, along with their mechanisms of action.

| Bioactive Components | Therapeutic Benefits | Mechanisms of Action | References |
|---|---|---|---|
| Polysacharide | Antidiabetic | In C57BL/6 mice fed a high-fat diet, it aids in lowering body weight, blood glucose levels, improving glucose tolerance, and lowering total serum cholesterol levels. | Fan *et al*., 2014 |
| Rhamnogalacturonan | Antidiabetic | Effect on blood sugar. | Liu *et al*., 2018 |
| Lectins | Anticancer | Stop the cell cycle and start the caspase cascades. | Damodaran et al., 2007 |
| | | In vitro inhibition of cellular proliferation in human breast cancer. | Sharma and Sabyasachi, 2019 |
| Pectin | Anticancer | Involved in cell adhesion, growth, and Survival as well as tumor development and cancer prevention therapy. | Lengfeld *et al*., 2004 |
| | Antiproliferative | Induce apoptosis and inhibit cellular proliferation. | Liu *et al.,* 2018 |
| Pectin | Lower bad cholesterol | Promotes cholesterol degradation while inhibiting fat production in the body. This aids in the removal of clots and deposited cholesterol. | Soma das and Ghosh, 2019 |

| Bioactive Components | Therapeutic Benefits | Mechanisms of Action | References |
|---|---|---|---|
| Quercetin3-O-glucosyl (1!6) glucoside (QDG) and quercetin3-O-glucoside (QG) | Antioxidant | Excellent reducing power and free radical scavenging capabilities, including DPPH, superoxide anions, and hydroxyl radicals. | Hu *et al*., 2014 |
| Vitamin C, calcium, iron, manganese, and magnesium | Antioxidant | Eliminating free radicals. | Soma das and Ghosh, 2019 |
| Quercetin derivatives and Epigallocatechin | Antioxidant | Inhibitory effects on the generation of reactive oxygen species (ROS). | Khomsug *et al*., 2010 |
| Polysaccharide | Metabolic disorders | Inhibition of LXR and PPAR signaling. | Fan *et al*., 2014 |
| Vitamin A; B vitamins (B1, B2, B6); and vitamin C and traces of zinc, calcium, folic acid, and fiber | Pregnancy benefits | Folates help to prevent miscarriages. They are also beneficial in the formation of the foetal neural tube and in protecting these tubes, preventing defects. This aids in the prevention of birth defects such as spinal bifida and can even alleviate constipation during pregnancy. | Hurrell, 2003 |

| Bioactive Components | Therapeutic Benefits | Mechanisms of Action | References |
|---|---|---|---|
| Polyphenols like catechin and flavonoids like quercetin possess | Antifatigue effects | Lowers blood lactic acid levels, which may help with physical fatigue and recovery. | Fan *et al*., 2014 |
| Probiotics | Gut bacteria-friendly | Biosynthesis of the vitamin B complex. | Soma das and Ghosh, 2019 |
| Mucilaginous | Ulcer treatment | Okra's slimy stuff is alkaline. This aids in acid neutralization. Furthermore, it forms a protective coating within the digestive tract, hastening the healing of peptic ulcers. | Soma das and Ghosh, 2019 |
| Mucilaginous with fiber | Relieves Constipation | Toxins are bound and the large intestines are lubricated. Because of its natural laxative properties, this ensures easy and normal bowel movement. | Soma das and Ghosh, 2019 |
| Vitamin K and C | Bone health and prevent blood-clotting process. | Several mechanisms have been proposed to explain how vitamin K can influence bone metabolism. Apart from the gamma-carboxylation of osteocalcin, a protein thought to be involved in bone mineralization, there is growing evidence that vitamin K also has a positive effect on calcium balance, a key mineral in bone metabolism. | Faruqu *et al*., 2013 |

| Bioactive Components | Therapeutic Benefits | Mechanisms of Action | References |
|---|---|---|---|
| Glycosylated compounds | Antibacterial activity | Helicobacter pylori adhesion to the human gastric mucosa is inhibited. | Lengsfeld *et al*., 2019 |
| Rhamnogalacturonan Polysaccharides | Antiadhesive properties | Interfere with the outer membrane proteins of H. pylori to prevent it from adhering to human stomach tissues. | Lengsfeld *et al*., 2019 |
| Polyphenols and flavonoids | Antifatigue | Enhances antioxidant capacity. . | Xia *et al*., 2015 |

## 2.3. Secondary plants metabolites

Plant secondary metabolites are a unique source of food additives, pharmaceuticals, commercials and agro-chemicals and are also exploited in many other industrial values (Saxena *et al*., 2013). They can be classified based on their chemical structure, composition, and solubility in various solutes and or pathways (Kennedy and Wightman, 2011). Due to their diverse biological activities, secondary metabolites have been utilized as medicinal components for centuries. According to the nomenclature adopted by the British Nutrition Foundation, plant secondary metabolites can be divided into 4 major groups: phenolic and polyphenolic compounds (about 8000 compounds), terpenoids (about 25000 compounds), alkaloids (about 12000 compounds), and sulfur-containing compounds (Goldblatt and Manning, 2000; Kennedy and Wightman, 2011). They are viewed as potential sources of new natural drugs, antibiotics, insecticides, and herbicides (Crozier *et al*., 2006).

As a result of the commercial importance of secondary metabolites, there has been a great interest in their production and the potential of increasing their biosynthesis by means of biotechnology while the ecological relationship is in harmony (Zhao *et al.,* 2005; Saxena *et al*., 2013). The practice of using sustainable agriculture in the improvement of secondary metabolites is beneficial in smart climate agriculture as a reliable source to produce high-quality bio-actives under controlled and uncontrolled conditions taking into consideration the climatic and geographical limitations.

The rise in molecular biology and the improvement of biochemical techniques have undoubtably verified the major role these secondary metabolites play in the way that plants adapt to their environment (Makkar *et al., 2007*). These compounds can protect plants from toxins from other plants and pathogens due to their antifungal, antiviral, and antibiotic properties. Secondary metabolites protect leaves from light damage which is very detrimental in primary smart agriculture (Li *et al*., 1993). There are more than 2,140,000 known secondary metabolites and they are generally categorized according to

their function, structural diversity, and biosynthetic pathways (Saxena *et al*., 2013). Terpenoids and steroids, alkaloids, enzyme co-factors and non-ribosomal polypeptides, polyketides and fatty acids derive substances that define the five main classes of secondary metabolites, however, secondary metabolites can be divided into three chemically distinct groups: terpenes, phenolics and nitrogen-containing compounds (Kennedy and Wightman, 2011; Lengsfeld *et al*., 2019).

One of the largest groups of plant metabolites is phenols. They generally have a common characteristic, the presence of one or more phenol groups (Marinova *et al.*, 2005). Some of these compounds are also pharmacologically valued for their anti-hepatotoxic properties like silybin and anti-inflammatory activities such as quercetin. A great number of phenolic molecules are free radical scavengers, possessing effective antioxidant activities, particularly flavonoids (Lengsfeld *et al*., 2019). Phenolics are classified into eight different groups namely: simple phenolics, flavonoids, tannins, coumarins, xanthones, chromones, lignans and stilbenes. Overall, secondary metabolites have shown several biological effects, providing the scientific basis for their use as phytopharmaceuticals and phytomedicines.

### 2.3.1 Phenolic compounds

Phenolic compounds constitute probably the largest group of plant secondary metabolites, varying in size from a simple structure with an aromatic ring to complex ones such as lignin. Natural phenolic compounds are plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl group (Kennedy and Wightman, 2011). More than 8000 phenolic compounds as naturally occurring substances from plants have been reported (Kennedy and Wightman, 2011; Kumar and Pandey, 2013). They are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants (Tomas-Barberan *et al.*, 2001; Lapornik *et al.,* 2005). Simple phenolics have at least one hydroxyl group attached to an aromatic ring while polyphenolics have two or more hydroxyl groups attached to a matrix of aromatic rings. They possess a wide range of biochemical activities such as antioxidant, antimutagenic

and antimicrobial activity (Marinova *et al.*, 2005). Phenolic compounds are reported to contribute to quality in food production in terms of modifying colour, taste, aroma and flavour, besides health effects (Ndhlala *et al*., 2007). Structures of some of the phenolic compounds which included phenol, pyrocatechol, pyrogallol and phloroglucinol are shown in figure 2.2.

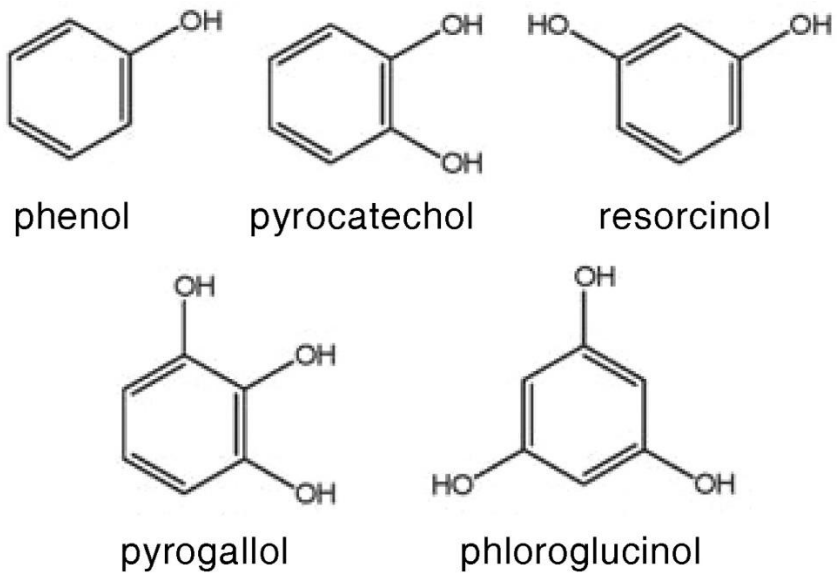


Figure 2.2: The structure of some of the phenolic compounds.

2.3.2 Flavonoids

They are the largest group of phenolic compounds. They can be divided into sub-groups namely: flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and anthocyanins (Kennedy and Wightman, 2011). Flavones and flavonols (Figure 2.3) are the most widely distributed of all the flavonoids. Flavonoids occur in different plant parts both in a free state and as glycosides. Studies have reported flavonoids to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer and antiarthritic (Ndhlala *et al*., 2007; Sulaiman and Balachandran, 2012). Flavonoids are categorically beneficial, acting as antioxidants and providing protection against

cardiovascular diseases, certain forms of cancer and age-cognate degeneration of cell components (John *et al*., 2014).

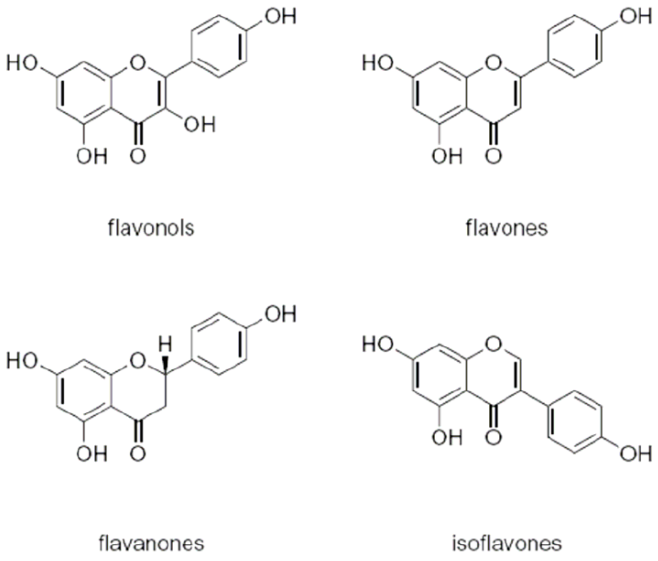


Figure 2.3 Chemical structures of the different groups of flavonoid compounds.

## 2.4 Application of metabolomics in crop sciences

Metabolomics is a comprehensive metabolic profiling approach that enables the analysis of a wide range of metabolite classes simultaneously in a non-biased manner (Cevallos-Cevallos *et al.,* 2009). It is a concept for describing the analysis of a comprehensive metabolite characterization on plant tissue. Its fundamental goal is based on the analysis of multiple analytes concurrently (Cubero-Leon *et al*., 2014). It is an important comparative tool for studying global metabolite levels of plant materials grown under different conditions (Li *et al.*, 2017). Metabolomics has increasingly been used to optimize the selection of disadvantageous accessions and improve breeding materials. Metabolomics offers a holistic characterization of the analyte, including the quantitative and qualitative. Metabolomics is an essential tool mainly applied to demonstrate a potential relationship between changes in parameters, or the response to environmental stress through the use of modern technologies (Cevallos-Cevallos *et al.,* 2009). The untargeted analysis offers a holistic profile of the sample without the identification of the

peaks (Cevallos-Cevallos *et al.,* 2009). Untargeted metabolomics strategies, are based on comparing metabolite profiles from different samples using chemo-metric approaches, which is critical in identifying the markers which play the most relevant role in various conditions (Perez *et al.*, 2010; Arapitsas *et al.,* 2014;). The approach could generate highly sensitive results and facilitate high-throughput data acquisition (Cevallos-Cevallos *et al.,* 2009).

The interpretation of the dataset from the untargeted analysis can be done by using different chemo-metric models, such as principle component analysis (Cubero-Leon *et al*., 2014). However, the drawback of untargeted analysis is that there is no direct assessment of metabolic profiles due to unidentifiable compounds in the sample. Targeted analysis on the other hand detects a limited number of compounds that are at target. Pure standards are injected for quantification of the compound's content within a sample (Perez *et al.*, 2010). Using this approach, we can explore different metabolites using a more sustainable growing technique.

### 2.5 The effect of mulching on yield and phenotypic attributes

Producers of horticultural crops often use plastic mulches to warm the soil, inhibit weeds or conserve moisture near the roots of the crop which is called mulching. Plastic mulches affect not only the soil environment but also the above-ground environment. How the mulch changes the plant's environment depends on the properties of the plastic (Erenstein, 2002.) and the degree of physical contact between the plastic and the underlying soil (Salmeron *et al*., 2006). Over the last several decades, vegetable production has shown significant yield increases in many areas of the world as a result of water scarcity and nutrients deficiency. The utilisation of plastic mulch in combination has played a major role in the increases in the production of tomato, pepper, eggplant, watermelon, muskmelon, cucumber, and squash, among other vegetables. There are, however, few reports on the utilisation of plastic mulches in underutilised medicinal crops. The growth, yield and quality attributes of okra are hampered by a lack of knowledge about the best management and cultivation practices, low awareness of nutritional and

health benefits (Brown *et al.,* 1987; Csizinszky and Martin 1988; White 1988). Mulching conserves soil moisture, reduces infiltration rate, reduces fertilizer leaching, prevents from extremes of temperature, reduces weed growth and ultimately increases the yield of crops (Salmeron *et al*., 2006).

## 2.6 Work done on the research problem

In realization of the limited water resource coupled with extremely hot weather conditions the OMET system was invented. OMET system is a more efficient and sustainable method of crop production than hydroponics. It does not require a high level of expertise which makes it easy to be used by smallholder farmers who are often uneducated (Ferreira, 2013). The OMET system was developed by the industrial and agricultural researcher, Helmuth Rohrer. It is used in many countries to produce great quantities of vegetables under different climatic conditions, and it is a better solution to conserving water. This system is better suited to different regions including arid and windy conditions. It has less rain and drought influence.

The system is very cheap to install because of the once-low setup cost. The drips are placed directly in the root zone of the crop which further conserves water. The top and bottom of plastic act as a mulch to cover and protect the medium to prevent water and nutrient loss respectively (Ferreira, 2013). Its smooth surface can deter crawling insects and prevents weed growth, eliminating the need for weeding or herbicides. This system is not affected by less rainfall and drought, and the enclosed trough helps to keep the soil at a constant, optimal temperature. Earthworms can thrive in the growth medium which is a good indication of healthy living soil (Ferreira, 2013). The crop has all the nutrients that a healthy plant needs. The enclosed trough can also help to control soil-borne diseases. Many different types of crops can be grown in the OMET system. For deep-rooted plants, one can use grow bags, but the principles remain the same. It is even suitable for fruit trees such as strawberries (Ferreira, 2013). Irrigation can be done through the dripper lines laid on top of the growth medium in the sheet mulch. OMET growing technique is highly efficient with virtually no water loss. Moreover, less labour is required to run the

system cutting production costs. It is said the system can help to keep the soil above 12°C (Ferreira, 2013).

## 2.7 Work not yet done on the research problem

Research conducted on how to lower crop water requirements has mainly focused on reducing the evaporation of water from the soil. However, research targeting the management of drainage below the root zone still needs to be investigated further. The OMET system addresses the evaporation of water from the soil and the drainage of water below the plant's effective root zone. According to Ferreira (2013), the OMET system is highly efficient with virtually no water loss. Questions have been raised about the growth of plants in a system that has no drainage. In the OMET system, irrigation is conducted with less water compared with conventional agriculture to avoid the organic medium becoming waterlogged.

## 2.8 Work done on other crops regarding the research problem

The work done on other crops using the OMET system has been scanty. However, Ferreira (2013), reported improved production of three tons of Swiss chard in a month on an 1110 $m^2$ OMET system. This gave an insight on the effectiveness and efficiency of the system in crop production. To alleviate the overexploitation of groundwater and achieve sustainable agriculture, it is important to develop and implement climate-smart growing techniques for protected crop production.

## 2.9 Addressing the identified gaps

Due to the increase in the importance of medicinal plants and changes in the climatic conditions that affect the production of agricultural crops, there are several factors that can be investigated to increase growth, yield attributes and the levels of active ingredients. There is no data documented on the effect of the OMET system on the

growth, yield and phytochemical properties of okra. The lack of scientific documentation on growth, yield and phytochemical properties of okra grown using a climate-smart production system has resulted in their neglect and under-utilisation. Providing scientific knowledge with the aim of enhancing yield and phytochemical composition can play a significant role to mitigate food insecurity and alleviate malnutrition in the country. However, okra has been considered a minor crop and scant attention was paid to its improvement in the international research program in the past (Sanjeet *et al.*, 2010). It is envisaged that the results of this study will initiate the exploitation of their potential. This study will examine the possibility of enhancing the growth, yield, nutritional and phytochemical properties of okra as part of measures to further appraise its role in health promotion and the fight against diseases and food insecurity. Okra is a nutritional source of power used throughout history for both medicinal and culinary purposes. Okra is a good source of minerals, vitamins and nutrients that are responsible for health benefits. The crop has been reported to possess pharmacological properties like antidiabetic and antioxidant. The current research is an effort towards providing sustainable production of okra using climate-smart agriculture.

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## Chapter 3: The effect of OMET growing technique and growing environment on growth and yield attributes of okra.

Abstract

Okra is an underutilized indigenous vegetable crop consumed in Sub-Saharan Africa. Its growth and yield are negatively affected by water and nutrient deficit. The Organic Medium Enclosed Trough (OMET) system was developed as a non-drainable growing technique which improves crop yield by reducing water and nutrient seepage. The objective of this study was to investigate the effects of the OMET system on the growth and yield of okra grown under greenhouse and micro-plots conditions. Four weeks old okra seedlings were transplanted on the established OMET and non-OMET systems concurrently under greenhouse and micro-plot conditions following a randomised complete block design (RCBD) for a period of 110 days, with three replications and twelve plants per replicate. The amount of irrigation water used was recorded until harvest and computed as cumulative irrigation water. Data were subjected to statistical analysis using GenStat 18th Edition. The mean separation for significant treatments was achieved using a parametric T-test at the significance level of 5%. The growth attributes which included plant height and stem diameter were taken on a weekly basis (n=9). At harvest, yield components including the number of branches per plant, plant biomass, fresh pod weight, number of pods per plant, fresh pod length and fresh pod diameter were recorded. OMET growing technique resulted in enhancement of growth and yield of okra regardless of the growing environment. The OMET growing technique significantly affected the growth and yield of okra either grown under greenhouse or micro-plot conditions. ($p \leq 0.05$). At harvest, the OMET system significantly increased the stem diameter by 40 and 37% while the plant height was increased by 68% and 48% under greenhouse and micro-plot experiments respectively. When evaluating the yield parameters, a similar trend was observed for the yield attributes where the OMET system significantly increased them as follows: biomass by 64 and 50%, no of branches by 67 and 50%, no of pods per plant by 60 and 49%, fresh pod weight by 75 and 53%, pod length by 64 and 51% under greenhouse and micro-plot conditions respectively, while the pod diameter was increased

by 68% in the greenhouse environment and there was no significant difference in the micro-plot trial. The use of OMET growing technique under micro-plot conditions can be used to improve growth and yield attributes in okra production.

### 3.1 Introduction

The growth and yield of okra are hampered by a lack of knowledge about the best crop production practices (Adejumo *et al.,* 2018). Although there is still a lack of literature based on OMET and its efficacy to improve growth and yield, plastic mulching possesses similar principles and has been reported as a great sustainable technology for vegetable production (Ferreira, 2013). Climate-smart agriculture is an approach for transforming and reorienting agricultural systems to support food security under the new realities of climate change. Widespread changes in rainfall and temperature patterns threaten agricultural production and increase the vulnerability of people dependent on agriculture for their livelihoods, which includes most of the world's poor. Climate change disrupts food markets, posing population-wide risks to the food supply. Threats can be reduced by increasing the adaptive capacity of farmers as well as the development of a new growing technique to outsmart changes in climate and to us be able to use the available resources sustainably. Climate change alters agricultural production and food systems, and thus the approach to transforming agricultural systems to support global food security and poverty reduction. Climate change introduces greater uncertainty and risk among farmers (Zhongming, 2021).

Okra is a crop of tropical and subtropical climates, its growth is vigorous during the rainy season compared to spring and summer. Water is an essential requirement in agricultural production (Alake, 2020). However, water availability is becoming a major challenge in farm production. Water resources in South Africa at present face many challenges, including increasing demands in many sectors. Maximum stress created directly or indirectly is due to the agricultural sector. So, it is important to judiciously use the already existing water resources by using suitable cultivation practices that not only increase

production per unit area but also per unit of water used. Thus, scientific and efficient management of water is needed to enhance water use efficiency and yield of crops.
Mulching conserves soil moisture, reduces infiltration rate, reduces fertilizer leaching, prevents extremes temperature, reduces weed growth, and ultimately increases the yield of crops (Birbal *et al*., 2013). The mechanism involved with the use of plastic mulch is that the plastic promotes nutrient and water accumulation thus promoting the growth of the crop grown resulting in higher yields. The growers and researchers use different mulches in vegetable production (Salmeron *et al*., 2006). The popularity of using plastic mulching in crop production has resulted in the development and introduction of the OMET growing technique, a non-drainable vegetable growing technique that is similar to plastic mulching beside that the OMET growing technique is practiced under an enclosed trough.

The OMET growing technique is a more sustainable system that prevents water and nutrient seepage while improving crop growth and yield. The OMET growing technique is similar to plastic mulch in many ways the main difference is that OMET is practiced under an enclosed trough. The advantages include the ability to conserve nutrients and water, raise temperatures, and eliminate erosion and weed growth (Ferreira, 2013). The sheet mulch also promotes soil health and pest control. The OMET system, like mulching, manipulates the crop's growing conditions to make them more favorable, resulting in increased growth and yield (Ferreira, 2013). This system raises and optimises the temperature of the growing medium, promoting greater root development. It promotes the accumulation and concentration of nutrients and water around the plant's root system by eliminating nutrients and water seepage (Ferreira, 2013). In addition to impacting crop development and yield, the availability of nutrients and water to the root system of the crop helps to resolve the nutritional and wilting crises. The use of plastic mulch has been reported to improve the growth and yield of crops (Shi *et al.* 2020).

In SSA, the crop is often grown under marginal conditions characterised by low and erratic rainfall, lack of agricultural inputs and modern production technologies. Abiotic stress factors notably drought stress account for yield losses in okra ranging between 30-100% (Ayub *et al.,* 2020). Drought stress occurring during the flowering and pod-filling stages

causes severe yield losses (Mbagwu and Adesipe, 1987). As a result, yield losses in okra are dependent on the cultivation practice and the phenological stage at which drought stress occurs (Barzegar *et al.,* 2016; Munir *et al*., 2016; Adejumo *et al.*, 2018; Shi *et al.* 2020). Limited studies are available that determined the climate-smart cultivation practice to improve the yield of okra. Moreover, there are fewer cultivation efforts to develop drought-tolerant okra production practice for cultivation in arid and semi-arid regions of SSA hampering efforts to improve yield, quality, food, and nutrition security faced in the region. The number of pods per plant, pod weight, pod length and width, number of seeds per plant, hundred seed weight and number of branches, fresh pod weight, stem diameter and plant height are key yield-contributing traits which can be targeted for selection in water restricted environments (Ariyo *et al*., 1987; Akinyele and Osekita, 2006; El-Fattah *et al*., 2020). Further, there is scant information on the production of okra using a more sustainable production system that requires less application of water to eradicate the negative effects caused by drought stress conditions. Considering the above background, the objective of this chapter was to determine the response of okra to climate-smart OMET growing technique and growing environment to improve the growth and yield attributes.

## 3.2 Materials and methods

### 3.2.1 Description of the study site

The experiments were conducted concurrently in a greenhouse (Figure 3.1) and under open field in micro-plot (3.2) conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53''10'S, 29°44''15'E) from September to December 2021. The greenhouse structure had ambient day/night temperatures averaging 20 to 25°C, with maximum temperatures controlled using thermostatically activated fans and a wet wall on the other end to regulate relative humidity around 45%. Open field micro-plots were characterised by hot-dry weather with day/night temperatures ranging between 28 to 38°C and precipitation mean average of less than 500 mm.

### 3.2.2 Treatments and experimental design

In each growing condition, a growing technique of OMET versus non-OMET was established following a randomised complete block design (RCBD) to accommodate twelve test plants replicated three times, which accounted for a population size of 36 (n=36). The non-OMET system was used as the control to check if the system is viable and efficient. In the greenhouse, blocking was performed for the wind currents that are blown by thermostatically controlled fans and in the field, blocking was done for the changes in environmental conditions.

### 3.2.3 Procedures

Seeds of okra (Starke Ayres Pty Ltd, South Africa), cultivar Clemson spineless, of moderately ribbed and medium green straight spineless pods, were sown in a disinfected 200 polystyrene seedling trays filled with Hygromix® growing medium up to 4 weeks of post-emergence. In a greenhouse condition, OMET system was prepared in such a way that a polystyrene black plastic sandwich a prepared mixture of growth medium in a raised seedbed of approximately 40 cm deep (Figure 3.1). Similarly, to the greenhouse, the open field micro plot OMET trial was prepared by demarcating an area of 200 m by 200 m space, whereby an underlying plastic was set underneath at 30 cm height down the surface to cover 20 cm growing plastic pots filled with the growth medium. Prior to transplanting, the plastic was carefully closed and holes were created on top of the plastic with row spacing of 40 cm × 40 cm between plants. The growth medium enclosed in this trough consisted Hygromix® growing medium, pasteurized (300°C for 45 minutes) loam soil and fine sand at a 2:1:1 ratio. The non-OMET system was prepared by planting the plants in 30 cm pots with the same spacing but without the use of any plastic as can be observed in Figure 3.2. The transplanted seedlings were irrigated as per need and the irrigation water was recorded


Figure 3.1: Three weeks old okra seedlings grown under greenhouse conditions. (A) OMET and (B) non-OMET


Figure 3.2: Three weeks old okra seedlings grown under micro-plot conditions. (A) OMET and (B) non-OMET

### 3.2.4 Data collection

Growth and yield parameters were collected from nine randomly selected and tagged plants from each replicate under micro-plot and greenhouse experiments. After initiating the experiment, growth parameters (stem diameter and plant height) were measured once every week. The plant height was measured with a meter ruler and the measurements were taken from the soil level to the highest point of the stem apex and the mean was calculated and expressed in cm. Stem diameter was measured at 30 cm from the soil, using a digital Vernier Calliper (KTV150-major Tech) and expressed in mm. Eight weeks after initiating the experiment, it was terminated and yield parameters including number of branches per plant, biomass in grams, number of pods per plant, fresh pod weight per plant in grams, pod length and width in mm were recorded from the data plants per replicate.

### 3.2.5 Statistical analysis

Data were subjected to statistical analysis using GenStat 18$^{th}$ version statistical package (VSN International, Hempstead, UK). The mean separation for significant treatments was achieved using a T-test at the significance level of 5%.

## 3.3 Results and discussion

### 3.3.1 The effect of OMET growing technique and growing environment on growth attributes of okra.

In okra production, various growth, and yield components, including plant height, stem diameter, number of branches per plant, fresh pod length and width, number of pods per plant, biomass, root length and pod weight influence pod productivity (Ariyo *et al*.,1987). The influence of both treatments on plant growth was observed throughout the experiment on a weekly basis, while yield parameters were observed during termination.

The results obtained for both the greenhouse and micro-plot experiment on plant growth and yield parameters are presented in Figure 3.3 up to 3.8 and Table 3.1.

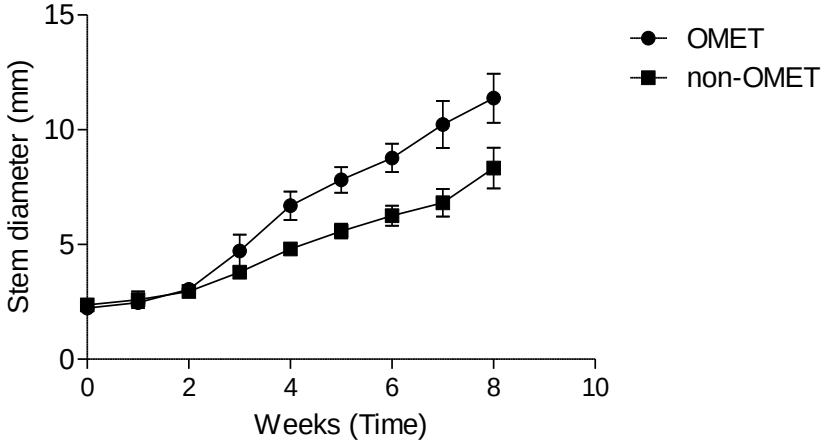


Figure 3.3: The effect of the OMET growing technique on stem diameter (n=9) of okra grown under greenhouse conditions

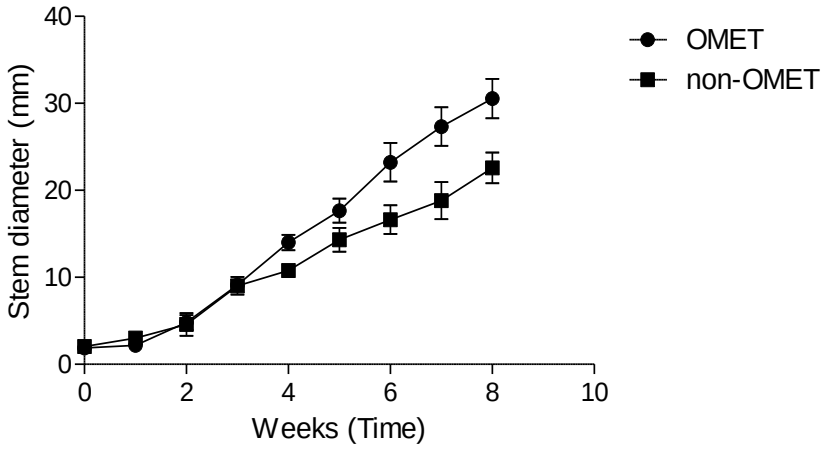


Figure 3. 4: The effect of the OMET growing technique on the stem diameter (n=9) of okra grown under micro-plots conditions.

The OMET growing technique significantly ($p \leq 0.05$) affected the stem thickness of okra in all the two sets of the experiment over time (n=9). The OMET system promoted an increase in stem diameter when compared to the non-OMET system; the OMET-grown

okra resulted in a gradual increase in stem diameter from week three until the end of the experiment, as shown in Figures 3.3 and 3.4. The results demonstrated that the OMET system can increase okra stem diameter over time. At termination, the OMET system in both sets of the experiment had a stem diameter that was two-fold higher than the non-OMET. Stem diameter is an important agronomic trait for increasing okra yield potential (Yadav *et al*., 2010; Kumar *et al*., 2011; Asare *et al*., 2016). According to Eshiet and Brisibe, (2015) thin stems are not desirable because they are prone to lodging and subsequently lead to a decrease in fresh pod yield. The variation in stem diameter among the treatments might be due to what was reported by Altaf Romaisa *et al*. (2015) that stems are attributed to the maximum plant height which captures maximum sunlight for photosynthesis which results in the formation of maximum photosynthates that accumulate in the stem. They were thicker stems in the micro-plot experiments as compared to the greenhouse conditions.

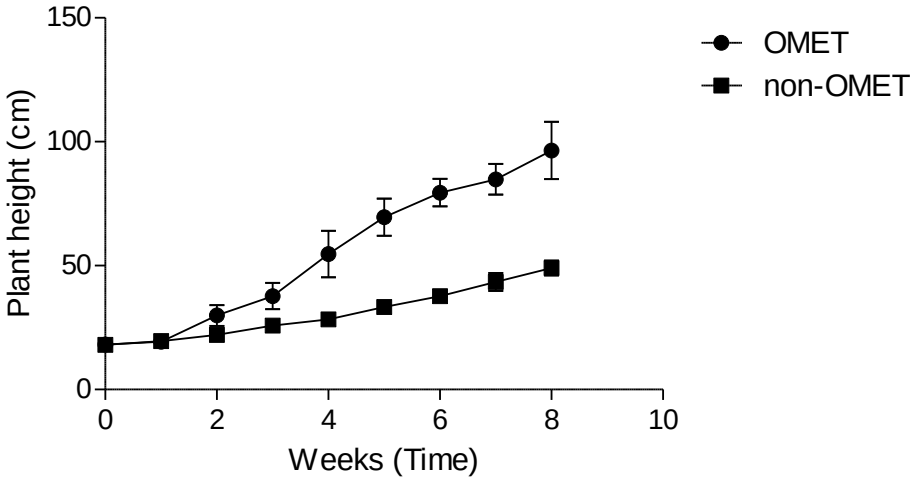


Figure 3.5: The effect of the OMET growing technique on plant height of okra grown under greenhouse conditions (n=9).

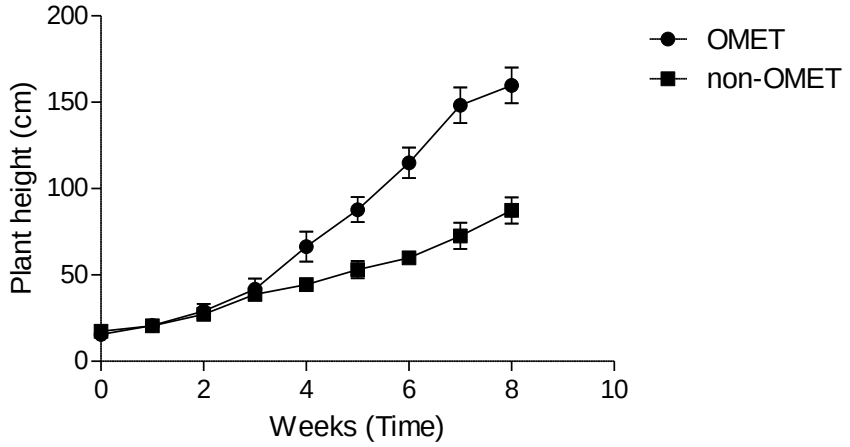


Figure 3.6: The effect of the OMET growing technique on plant height (n=9) of okra grown under micro-plot conditions.

The OMET system in both the field and greenhouse was significant ($p \leq 0.05$) on plant height which meant that the use of the OMET system improved the plant height as compared to the non-OMET systems. There was a gradual increase in plant height (Figures 3.5 and 3.6) in all two sets of the experiment over time. The OMET growing technique produced plants which were two-fold taller than the non-OMET (Figures 3.7 and 3.8). As reported by Reddy *et al*. (2012) taller plants are crucial to accommodate a greater number of pods on the main stem, and this has a direct effect on pod yield in okra. The ability of the OMET system to preserve water and nutrients, which results in a moderate availability of water and good nutrient solubility and absorption by the plants, was likely what caused the growing technique's effectiveness in increasing stem diameter and plant height (Ferreira, 2018). The OMET system restricts water and nutrient seepage because it is practiced under an enclosed trough, therefore we assume that the OMET system provided better conditions for the crop, resulting in an improved plant height. Growth parameters play a key role in the absorption and transportation of water and nutrients from the soil to various parts of the plant. The micro-plot experiment yielded the largest stem diameter and tallest plant height, which may be due to the process of cell division and elongation. Hasegawa *et al.* (2000) also discovered nutrition, hormones, and environmental factors such as light and temperature all have an impact on the rate and extent of elongation.

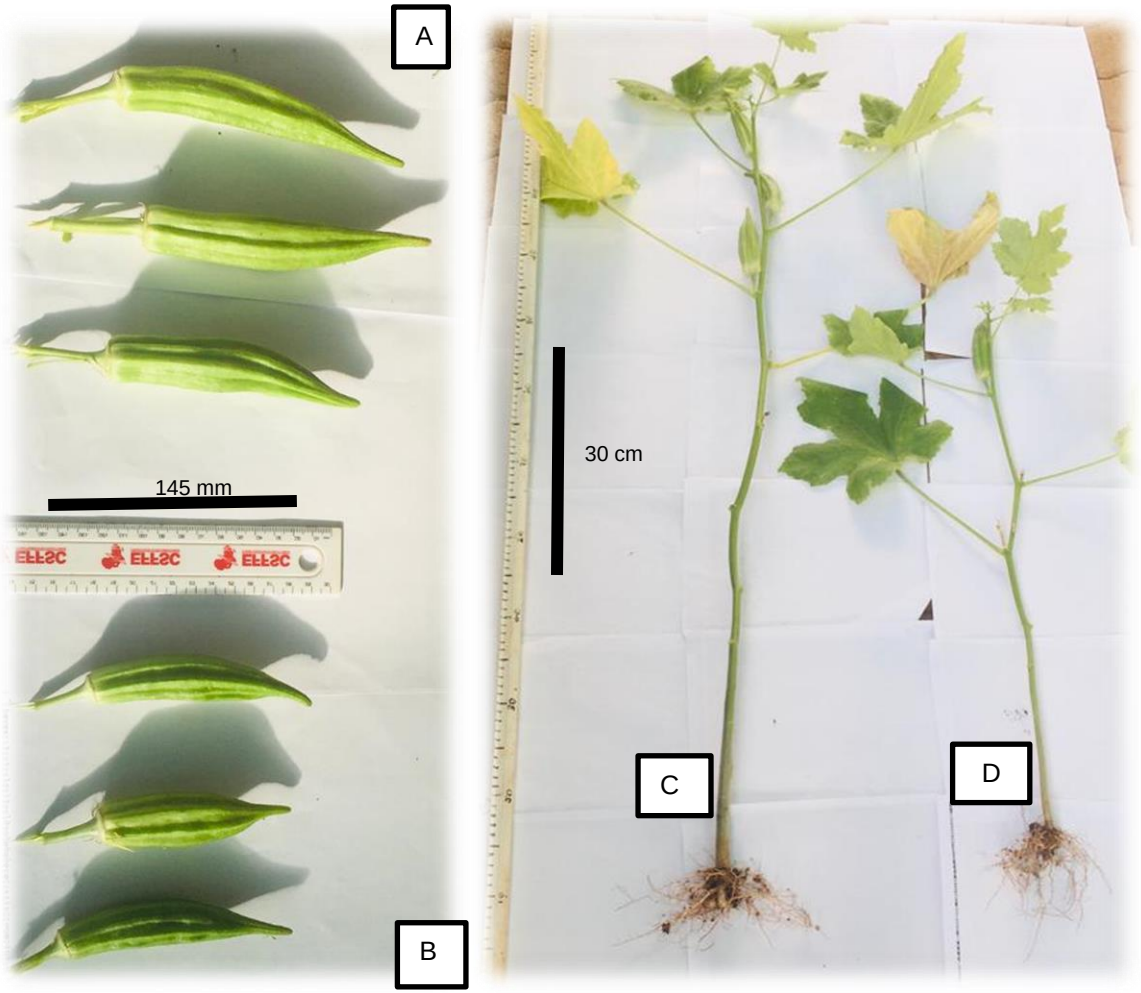


Figure 3.7. Freshly harvested okra pods (A) OMET and (B) non-OMET and plants (C) OMET and (D) non-OMET grown under greenhouse conditions at day 110 after transplant.

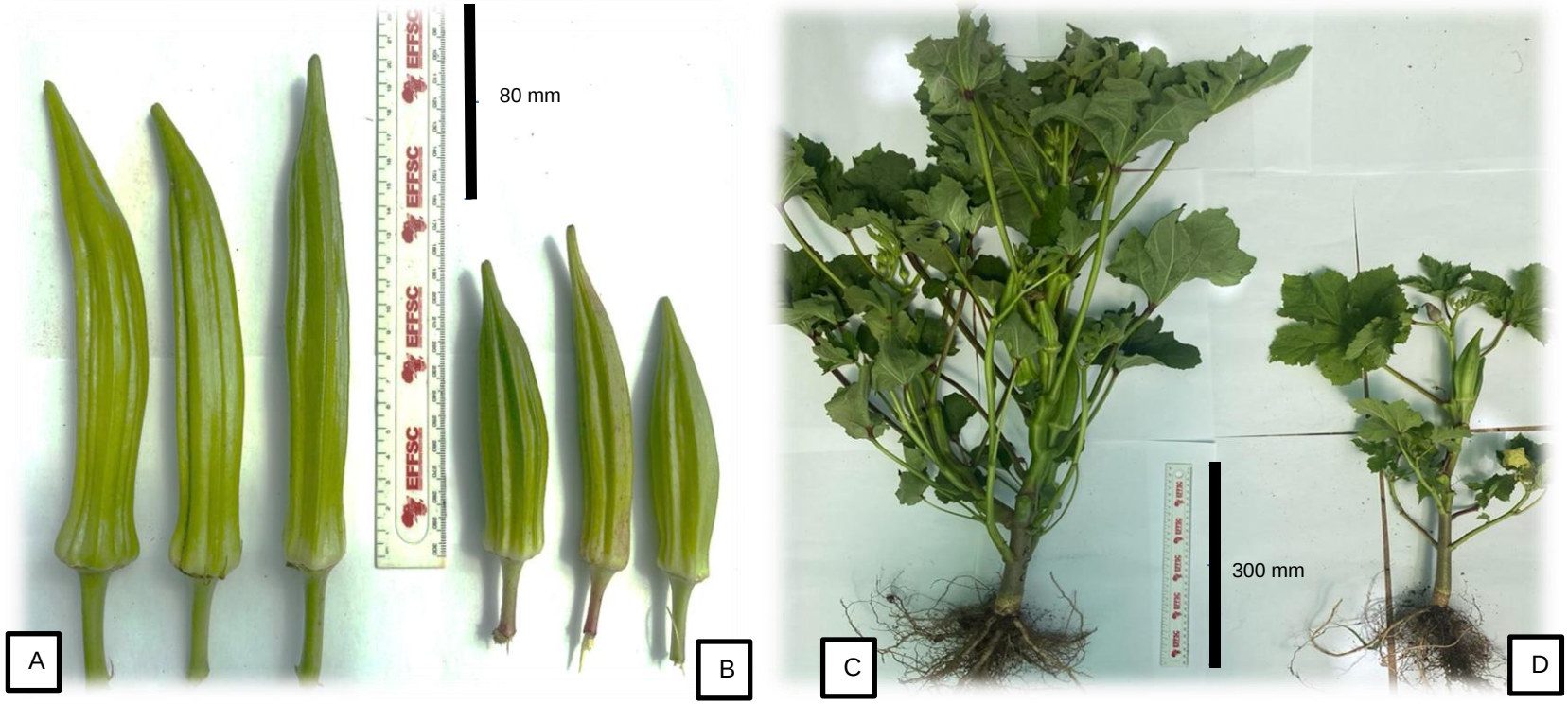


Figure 3.8: Freshly harvested okra pods (A) OMET and (B) non-OMET and plants (C) OMET and (D) non-OMET grown under micro-plot conditions at day 110 after transplant.

3.3.2 Effect of OMET growing technique and growing environment on yield attributes of okra.

Table 3.1 Yield parameters in okra grown on OMET and non-OMET under greenhouse and micro-plot conditions.

| Treatments | Biomass (g) | No. of branches | No. of pods per plant | Fresh pod weight (g) | Pod length (mm) | Pod diameter (mm) |
|---|---|---|---|---|---|---|
| Greenhouse | | | | | | |
| OMET | 15.86±1.23[a] | 3.00±0.17[a] | 5.00±0.29[a] | 7.73±0.88[a] | 139.45±6.3[a] | 24.27±0.62[a] |
| Non-OMET | 3.87±0.45[b] | 1.00±0.24[b] | 2.00±0.18[b] | 2.69±0.39[b] | 47.19±5.46[b] | 8.85±0.25[b] |
| p-value | 0.025 | 0.038 | 0.018 | 0.04 | 0.02 | 0.00 |
| | | | | | | |
| Micro-plot | | | | | | |
| OMET | 51.76±2.28[a] | 8.00±0.24[a] | 16.00±0.4[a] | 43.64±2.02[a] | 172.43±6.2[a] | 27.26±0.8[a] |
| Non-OMET | 26.06±1.32[b] | 4.00±0.18[b] | 8.00±0.29[b] | 20.3±0.87[b] | 130.18±2.8[b] | 28.65±0.8[a] |
| p-value | 0.041 | 0.021 | 0.001 | 0.000 | 0.003 | 0.083 |

Values are expressed as means ± standard error (n=9). For all the values within a column, different letters superscripts mean significant differences ($p \leq 0.05$)

The okra pod is the most important and economical part of okra production and is utilized as a vegetable (Reddy *et al*., 2013; Eshiet and Brisibe, 2015). Sustainable cultivation practices selection of okra is mostly focused on developing practices which will result in desirable pod characteristics including length, width, and size. The OMET system significantly ($p \leq 0.05$) improved the biomass of okra in both the growing environment. The present study revealed that the use of the OMET system can result in an increase in yield (Figure 3.7, 3.8 and Table 3.1). At harvest, the OMET system significantly increased the yield attributes as follows: biomass by 64 and 50%, no of branches by 67 and 50%, no of pods per plant by 60 and 49%, fresh pod weight by 75 and 53%, pod length by 64 and 51% under greenhouse and micro-plot conditions respectively, while the pod diameter was increased by 68% in the greenhouse environment and there was no significant difference in the micro-plot environment.

The increase in the pods recorded in the OMET compared to the non-OMET may be attributed to the greater number of branches that were observed in the OMET system which implied that the plant can be able to accommodate a greater number of pods as compared to the non-OMET which had a smaller number of branches. These results supported those of dos Santos-Fariasa *et al*. (2019) and Shi *et al*. (2019) that fresh pod yield in okra is a complex character that depends on many yield components traits such as number of branches, stem diameter, plant height, root length, fresh pod length and width, number of seeds per plant and number of pods per plant.

The study also supported the findings of Kumar and Reddy (2016) that showed that branching capacity in okra has a direct effect on pod yield and phenotypic traits associated with yield response are useful to improve the yield potential of okra in climate-smart agriculture. The results obtained in this study were also supported by those of Jha *et al.* (2018) who reported that the use of plastic mulch significantly influenced the plant height whereby okra yield was highest (8104 kg/ha) under silver plastic mulch. Plastic mulch enhanced the growth parameters like canopy length, plant height, leaf number, stem diameter, leaf length and yield attributes of okra. The black plastic mulches increased total yield by 84.9%, 46.0% and 46.9%, respectively, compared to the control.

This may be attributed to the fact that plastic mulches can suppress annual weeds and offer other important benefits, such as organic matter, nutrients, moisture conservation, soil protection, and moderation of soil temperature. Studies have shown that drought stress induces a series of physiological and morphological changes in plants, for example, growth inhibition and reduction in crop yields (Eshiet and Brisibe, 2015), enhancement of root systems and root-shoot ratios (Lu *et al*., 2014), regulation of the closure of stomata (Wan *et al*., 2009), disruption of photosynthesis (Zivcak *et al*., 2013), activation of respiration (Sanjeet *et al*., 2010), accumulating compatible solutes and protective proteins (Shi *et al*., 2019), and the strengthening of anti-oxygenic enzyme activity (Hu *et al*., 2015). These changes are controlled by gene expression, which is particularly affected by biotic and abiotic factors in the environment. The growing environment also significantly affected the yield attributes as the okra grown under micro-plot conditions resulted in higher yields as compared to the greenhouse-grown okra regardless of the growing technique. The variation in yield attributes between the environments supported the results obtained by Hu *et al.* (2015) who reported that changes in the environmental conditions affect the growth and yield of okra.

### 3.3.3 Effects of OMET technique on irrigation water quantity.

The OMET growing technique significantly positively affected the amount of irrigation water as more water was used to irrigate the non-OMET grown okra in both the growing condition ($p \leq 0.05$). The cumulative irrigation water increased in weeks (Figure 3.9). The OMET system proved that it can enhance growth and yield in the disposal of lower irrigation water. The results may be attributed to the fact that the OMET growing technique is practiced under an enclosed trough which eliminates water seepage thus advocating for climate-smart agriculture. The results were similar to those reported by Ferreira (2018) whereby the OMET growing technique improved the growth and yield of Swiss chard under the disposal of lower irrigation. The top plastic of the OMET system reduces the evaporation rate.

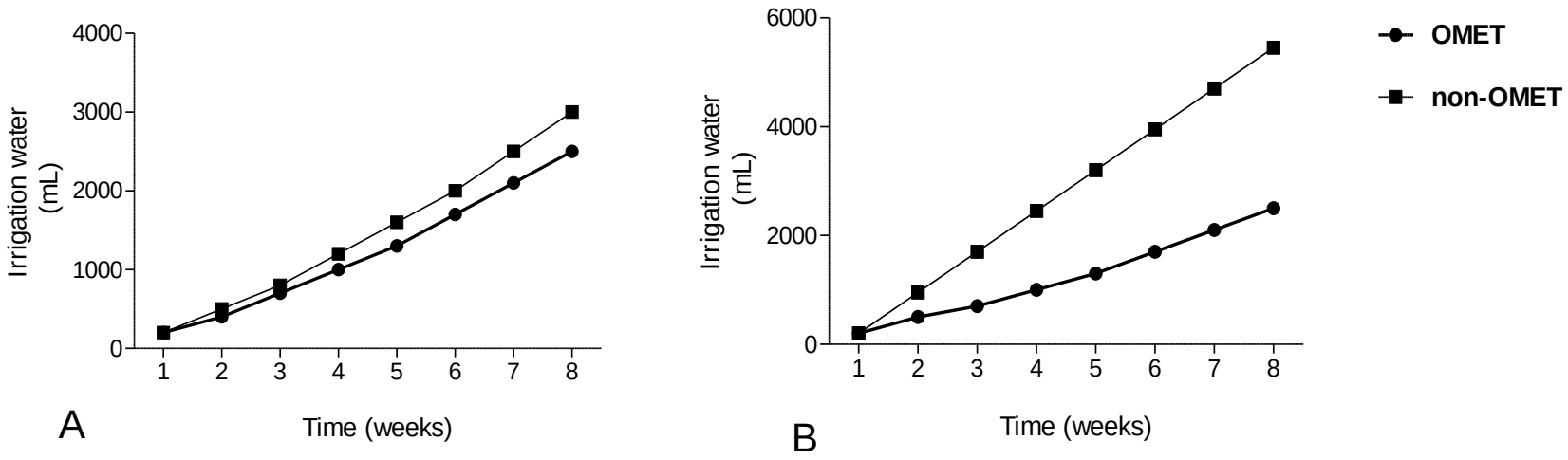


Figure 3.9: Effect of the OMET growing technique on cumulative irrigation water under greenhouse (A) and micro-plot (B) conditions.

## 3.4 Conclusion

This study proved that the growth and yield parameters of okra are affected by the growing technique. The use of the OMET system significantly increased growth and yield components which included stem diameter (10.31 and 28.29 mm), plant height (84.29 and 149.44 mm), biomass (15.86 and 51.76 g), number of branches per plant (3 and 8) number of pods per plant (5 and 16), fresh pod weight (7.73 and 43.64 g) and pod length (139.45 and 172.43 mm) of okra grown under greenhouse and micro-plot conditions respectively. The present study presented evidence that the OMET growing techniques offer improved yield and crop quality as compared to the non-OMET under the disposal of lower irrigation water. The OMET growing technique is a climate-smart production system that does not require high technological skills. It prevents water and nutrient seepage. Therefore; this technique is recommended for okra small-scale and organic farmers under cultivation in water-restricted environments. For improved growth and yield of okra, it is recommended to plant it under field conditions using the OMET growing technique as the results of the micro-plot experiments in terms of yield were higher than those grown under greenhouse conditions. Both biomass and number of pods per plant were higher by 70 and 69% respectively compared to the greenhouse OMET gown okra. For future research, the OMET system growing technique can be used to grow other crops in the disposal of different water regimes. The results obtained can assist small-scale farmers to improve the production of medicinal crops with less input cost thus improving their socio and economic factors.

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## Chapter 4: The effect of OMET growing technique and growing environment on the nutritional composition of okra leaves and pods.

Abstract

Okra contains many vital nutrients, these nutrients confer a number of health advantages, including a decreased risk for the development of chronic non-communicable diseases, such as obesity, hypertension, hypercholesterolemia, coronary heart diseases, and gastrointestinal disorders. The OMET growing technique significantly improved the essential amino acid composition of okra leaves with Thr (0.57 mg/kg), Val (0.70 mg/kg) Leu (0.90 mg/kg) and Phe (1.03 mg/kg) being higher than the non-OMET grown okra leaves. The pods showed that the OMET growing technique also significantly improved the accumulation of all the tested non-essential amino acids with Lys being the highest ($p \leq 0.05$). It was observed that the OMET growing technique used in the micro-plot experiment significantly enhanced the accumulation of all the tested essential amino acids in both the leaves and pods with Phe and Lys (1.53 and 0.70 mg/kg) being the highest in the leaves and pods respectively. The non-essential amino acids composition was also significantly improved using the OMET growing technique in both the leaves and pod with Glu (2.73 mg/kg and 4.05 mg/kg) being the highest respectively of OMET growth technique to maintain the daily recommended amino acids ratio coefficient equal to 1. Whereby Lys, Met, Thr in the pods, and Thr, Lys, Tyr+Phe, Leu, Ile, and Met were maintained in the leaves. The nutritional composition which includes proteins, mineral elements and amino acids of the tested okra leaves and pods grown using the OMET system regardless of the growing condition has resulted in an increase in the % protein content compared to the non-OMET grown okra. Nutritional elements Ca, K, P, Mg and Na were predominant in okra leaves and pods irrespective of the growing environment and growing technique (OMET). The results generated showed that the OMET system significantly ($p \leq 0.05$) improved the nutritional composition in okra leaves and pods regardless of the growing condition, though the micro-plot experiment resulted in higher nutritional composition as compared to the greenhouse experiment. OMET can be used to manipulate and improve nutritional composition towards the daily recommended intake.

## 4.1 Introduction

Food and nutrition security are two challenges that are faced by an increasing global population estimated to reach 9.7 billion by 2050. These challenges have been worsened by the Covid-19 pandemic which started in 2020. The number of people facing hunger in the world was between 720 and 811 million (The World Bank, 2022). Food insecurity refers to the inability to access adequate healthy and nutritious food (Wekeza *et al*., 2022). It often leads to hidden hunger or micro-nutrient deficiency which further contributes to malnutrition and undernourishment (Wekeza *et al*., 2022). Nevertheless, good health can only be achieved by eating a well-balanced diet, with adequate amounts of minerals and nutrients. Minerals are nutritional chemical elements required for the functioning of the body, in order to sustain life (Heber *et al.*, 2016). Most minerals in the human diet are obtained by ingestion of plants and animals, or in the form of consuming water (Soetan *et al*., 2010). Minerals are broadly classified as macro (major) or micro (trace) elements. Potassium, magnesium, calcium, zinc, sodium, and phosphorus, alongside chlorine and sulphur, are the main quantity elements or macro-minerals, as they are required in amounts greater than 100mg/dl, to maintain the human body's physiochemical processes (Rosborg, 2016). The trace elements or micro-minerals are required in smaller quantities of less than 100 mg/dl (Eruvbetine, 2003; Harris, 2014). Furthermore, macro-nutrients such as carbohydrates, amino acids, fatty acids, and organic acids are involved in growth and development, respiration and photosynthesis, hormone and protein synthesis in plants (Khan *et al.*, 2020).

Research has demonstrated the impact of consuming indigenous fruits and vegetables as a potential weapon against malnutrition or diet-related diseases. Okra has been indigenized in African countries where it is consumed as a relish in daily meals. The consumption of 1 cup of okra pods contains dietary fiber (3.2 g), carbohydrate 91.2 g), protein (2 g) and vitamin C (21.1 mg) (Sanchez *et al.*, 2022). In addition, Okra contains noticeable amounts of amino-acid, and mineral elements (iron, potassium, and calcium) (Salameh, 2014). Therefore, it is a good source of nutrients to alleviate hidden hunger and malnutrition. Okra is a tropical to subtropical crop which is negatively affected by

droughts (Dhankhar *et al*., 2005). In response to this challenge, extensive research has focused on cropping systems such as mulching with different materials to reduce irrigation water evaporation (Tiwari *et al.*, 1998; Adekiya *et al.*, 2017).

However, different cropping systems affected the nutritional composition. According to Fawibe *et al*. (2022), plastic mulch under *Elaeis guineensis* (Jacq.) canopy reduced the ash, crude protein, and carbohydrates as compared to the canopy without mulching. On the other hand, different mulching materials including *Pueraria phaseoloides, Mucuna pruriens, Pennisetum pur-pureum* and *Panicum maximum* impacted the mineral element composition such that nitrogen, phosphorus, potassium, calcium and magnesium were increased in comparison to no-mulch treatment (Adekiya *et al*., 2017). Yet, reports on the enhancement of the nutritional composition of okra using a sustainable cultivation practice in the disposal of lower irrigations are relatively few. In this study, okra was grown on the OMET technique to improve water and nutrient retention. This is a new cropping technique which should be evaluated for its efficacy in improving the nutritional composition before being recommended to the farm industry. Its advantages are based on reducing water and nutrients evaporation and seepage using plastic which encloses a growth medium. However, there is also no comprehensive literature regarding characteristics of the nutritional composition produced from the leaves and pods of okra using the OMET system. Therefore, this chapter was aimed to assess scientific information regarding the composition of amino acids and minerals in okra leaves and pods grown using the OMET system under greenhouse and micro-plot conditions.

## 4.2 Materials and methods

### 4.2.1 Description of the study location

Mineral composition experiment took place at the University of Limpopo, Limpopo Agro-Food Technology Station (LATS), while amino acids composition was carried out at Stellenbosch University in the Central Analytical Facilities (CAF).

### 4.2.2 Treatments and design

Harvested leaves and pods of okra grown under OMET and non-OMET growing techniques under greenhouse and micro-plot conditions (Chapter 3) were oven-dried at 40ºC until they reached a constant dried weight. Then both leaves and pods were ground into a powder.

### 4.2.3 Determination of the amino acid content

Amino acid analysis was performed according to Grobbelaar (2013) as described by Mpai *et al.* (2018), using dried leaves and pods of okra seperately. A volume of 100 g was vortexed with 6 N HCl 0.5 mL with the resulting mixture held in an oven at 110°C for 18 h and after cooling, centrifuged and filtered. The resulting filtrate was dried using a speed vacuum and reconstituted in a borate buffer (70 µL) for derivatisation. Samples were derivatised using an accQ-Tag ultra-amino acid kit and the sample was analysed twice. The derivatisation kit contains five vials of each of the following: AccQ-Tag derivatising agent (6-aminoquinolyl-N- hydroxysuccinimidyl carbamate (AQC), dry acetonitrile for preparing the AQC, and sodium borate buffer (0.2 M, pH 8.8) to be used in the derivatisation reaction. Initially, the samples were undiluted and then diluted 10 times in order to quantify the amino acids that are present in higher concentrations. The derivatisation process was performed by adding 10 µL aliquot of the prepared undiluted sample (which contained 20 µL/L norvaline in 80 µL of the sample) to the 20 µL of AQC, vortexed and held in the oven at 55 °C for 10 min. Thereafter, the vials were cooled and the samples were ready for the Ultra Performance Liquid Chromatograph (UPLC) analysis. Amino acid separation and detection were performed using a Waters Aquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector. An aliquot of 1 µL of the sample was injected into the mobile phase which conveys the derivatised amino acids onto a Waters Ultra Tag C18 column (2.1 x 50 mm x 1.7 µm) held at 60 °C.

The gradient was set up and commenced with 99.95 eluent A (water) and 1 % eluent B (acetonitrile). The total run time was 9.5 min and the run flow rate was 0.7 mL/ mi. The amino acid peak area of standards and samples was calculated to determine amino acid concentration.

The quantified amino acid values were used to generate the amino acids score (AAS). The AAS refers to the ratio between the amino acid of the test protein and the amino acid of the reference protein as tabulated in a publication by Graciela et al. (2013). The reference protein contains various essential amino acids, and the content of each essential amino acid can completely satisfy the needs of the human body. The amino acid in the test protein that showed the lowest proportion was termed as the first limiting amino acid, and the ratio obtained was the score. The AAS equation is as follows:

$$\text{AAS} = \frac{\text{Amino acid o f test protein (mg/g)}}{\text{Amino acid of protein in re f erence protein (mg/g)}} \times 100\% \qquad \text{(Graciela } et\ al\text{., 2013)}$$

The Ratio Coefficient of Amino Acids (RC) is the ratio of amino acids in food equivalent to the reference amino acid, which was designed based on the theory of amino acid balance to evaluate the nutritional value of proteins was calculated as follows:

$$\text{RC} = \frac{\text{AAS}}{\text{Ave AAS}}$$

Where Ave AAS is the average of individual AAS from different species of tested samples for each amino acid.

Once RC is equal to 1 (R=1) means that the test amino acid is to the reference amino acid. More than 1 (>1) defines a surplus of the test amino acids against the reference amino acid and lastly, less the 1 (<1) indicates insufficient amino acid in relation to its reference standard (Ma *et al.*, 2022).

4.2.4 Determination of mineral composition

Approximately 10 g of dried materials were digested in 40 mL of 4% nitric acid ($HNO_3$), followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, thereafter incubated in a 95°C water bath for 90 minutes, allowed to cool down at room temperature, filtered, and decanted into 50 mL tubes which were covered with a foil and then selected nutrient elements were analysed using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

4.2.5. Determination of protein

The micro Kjeldahl method described by the Association of Official Analytical Chemists (AOAC) (1990), was used. Two grams (2 g) of each sample was mixed with 10 mL of concentrated sulphuric acid ($H_2SO_4$), in a heating tube. One tablet of selenium catalyst was added to the tube and the mixture was heated inside a fume cupboard. The digest was transferred into a 100 mL volumetric flask made up with distilled water. Ten millilitre portion of the digest was mixed with an equal volume of 45% NaOH solution and poured into a Kjeldahl distillation apparatus. The mixture was distilled and the distillate was collected into a 4% boric acid solution, containing 3 drops of indicator. A total of 50 mL of distillate was collected and titrated as well. The sample was duplicated and the average value was taken. The nitrogen content was calculated and converted to percentage protein by using a protein conversion factor of 6.25.

**4.3 Statistical analysis**

Both the OMET and non-OMET analysed leaves and pods of okra grown under greenhouse and micro-plot conditions were subjected to statistical analysis using GenStat 18th version (VSN International, Hempstead, UK). The mean separation for significance treatments was achieved using a T-test at the significant level of 5%.

## 4.4 Results and discussion

### 4.4.1. Effect of OMET growing technique and growing environment on the amino acid composition of Okra leaves and pods.

The effect of the OMET growth technique has significantly ($p \leq 0.05$) affected the nutritional status of okra. In the greenhouse environment, a total of 16 amino acid compounds were quantified in the leaves and pods of okra grown under OMET and non-OMET growing techniques. The non-essential amino acids compounds included: Arginine (Arg), Serine (Ser), Glycine (Gly), Aspartate (Asp), Glutamate (Glu), Alanine (Ala), and Proline (Pro). Whilst essential amino acids such as Histidine (His), Threonine (Thr), Methionine (Met), Lysine (Lys), Tyrosine (Tyr), Leucine (Leu), Phenylanine (Phe), Asparagine (Asp) and Glutamine (Gln) were quantified (Figure 4.1). Quantitatively, all essential amino acid compounds were 79%, 74%, 35% and 31% higher than the non-essential amino acids in Okra pods and leave grown under OMET and non-OMET conditions, respectively.

Glutamic acid (1.66-4.18 mg/kg) and aspartate acids (1.10-1.92 mg/kg) were the major components of amino acids in okra, followed by Arg (0.59-1.32 mg/kg), Phe (0.57-1.02 mg/kg), Leu (0.26- 0.90 mg/kg), Lys (0.35- .77 mg/kg), Gly (0.28- 0.65 mg/kg), Val (0.26-0.70 mg/kg), and Ser (0.28-0.60 mg/kg) regardless of the growth technique. Methionine was the least amino acid component found in the studied okra samples, followed by Tyr (0.12-0.31 mg/kg), His (0.16-0.30 mg/kg), Thr (0.23-0.56 mg/kg), (0.38-0.62 mg/kg) and Ile (0.19-0.52 mg/kg).

For non-essential amino acids, samples of okra pods grown under OMET conditions exhibited the highest contents of Asp and Glu (1.32 mg/kg and 4.18 mg/kg respectively) in comparison to non-OMET pods (0.94 mg/kg and 3.66 mg/kg respectively) and the leaves grown under OMET (0.56 mg/kg and 1.69 mg/kg respectively) and non-OMET (0.59 mg/kg and 1.69 mg/kg respectively) samples. Overall, the samples grown under OMET exhibited higher contents of all non-essential amino acids including Ser, Gly, Asp, Ala and Pro more than the non-OMET pods' sample.

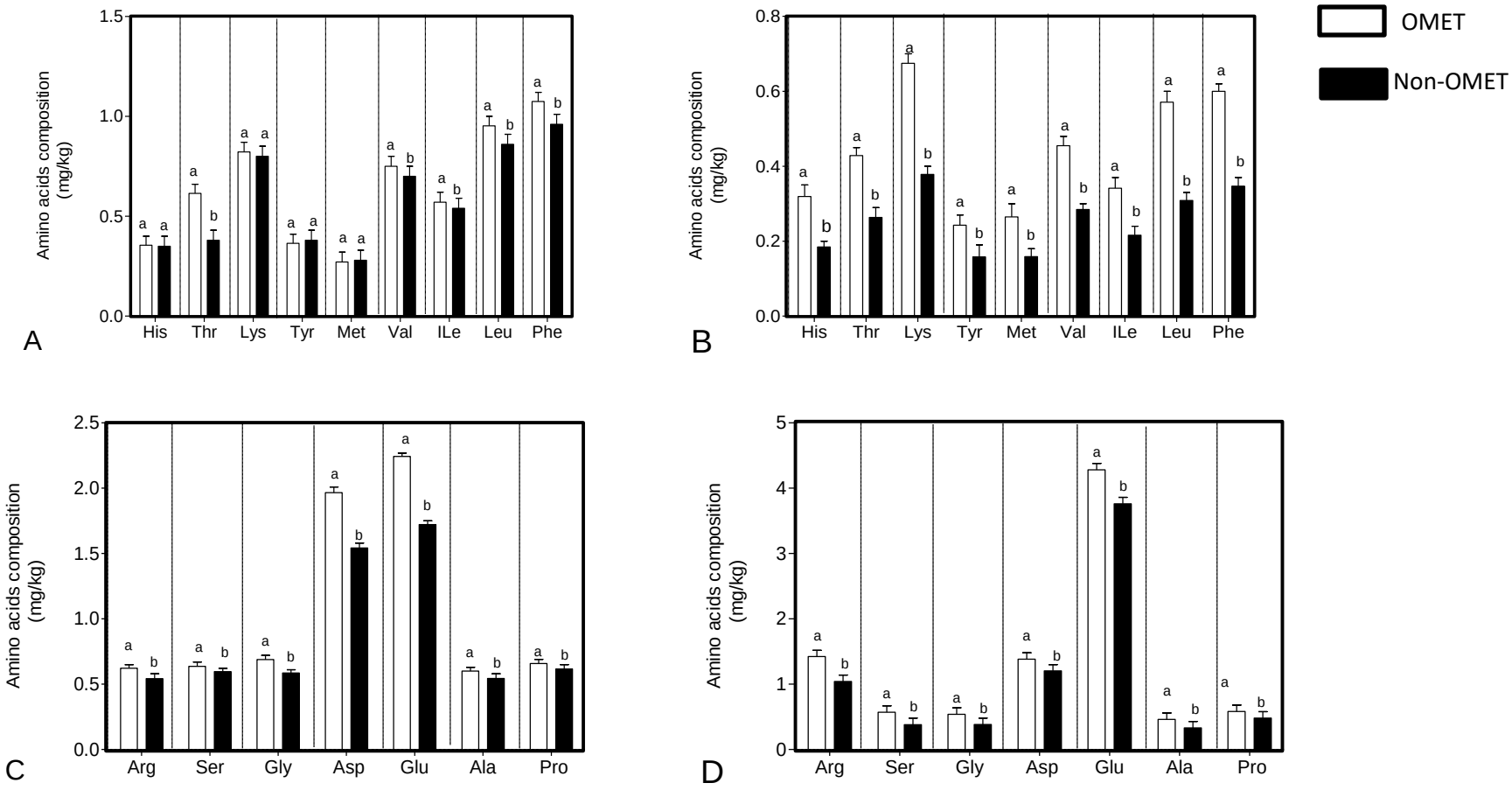


Figure 4.1 Total content of essentia essential (A=leaves and B=pods) and non-essential (C=leaves and D=pods) amino acids of okra leaves and pods grown under greenhouse condition respectively. Bars (± SE) with different letters are significantly different ($p \leq 0.05$). Mean separation was done using a T-test. His= Histidine, Thr= Threonine, Lys=Lysine, Tyr=Tyrptophan, Met= Methionine, Val=Valine, Leu=Leucine, Phe= Phenylalanine, Arg= Arginine, Ser= Serine, Gly= Glycine, Asp= Asparagine, Glu=, Ala= Alanine and pro= Proline

Under micro-plot growing conditions, the amino acid composition of okra pods and leaves was significantly ($p \leq 0.05$) affected by OMET growing conditions. Glutamine and Aspartate amino acids were the most predominant compounds in both leaves and pods of okra grown under OMET and non-OMET. The Glu contents ranged between (4.04 and 3.64 mg/kg) and (2.73 and 2.38 mg/kg), while Asp range was between (1.11 and 1.00 mg/kg) and (2.59 and 2.06 mg/kg) for okra leaves and pods grown using the OMET and non-OMET growing technique respectively.

Overall, the trend of the non-essential amino acids in okra pods grown under OMET growing technique showed that the contents of Arg (1.06 mg/kg), Ser (0.51 mg/kg), Gly (0.52 mg/kg), Asp (1.11 mg/kg) and Glu (4.04 mg/kg), were higher than those grown under non-OMET growing technique (Fig 4.2 D). However, a non-significant effect between OMET and non-OMET growing techniques were observed for Ala and Pro amino acids compounds in okra pods.

All the quantified non-essential amino acids which included Arg, Ser, Gly, Glu, Ala, Pro and Asp were higher in OMET-grown leaves samples (Figure 4.2 C). On the other hand, essential amino acids including His, Lys, Met, Leu, Tyr and Phe were higher in pods of okra grown under OMET (Figure 4.2 B). Yet, Thr (0.38 mg/kg), Val (0.49 mg/kg), and ILe (0.36 mg/kg) were unaffected by the OMET growing technique. Okra leaves grown under the OMET growing technique exhibited higher contents of His, Tyr, Lys, Thr, Met, Val, ILe, Leu and Phe in comparison to non-OMET (Figure 4.2 A).

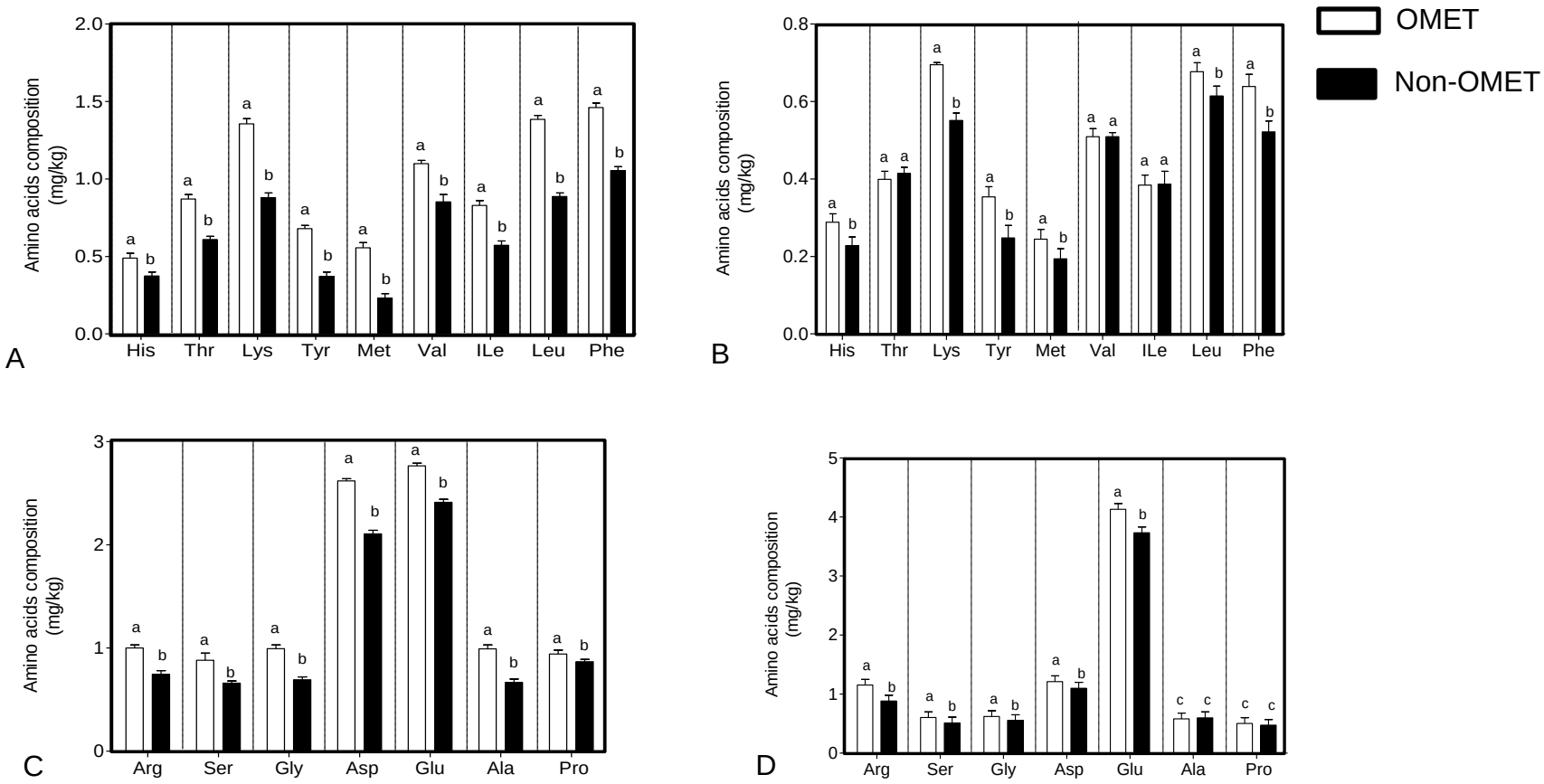


Figure 4.2 Total content of essentia essential (A=leaves and B=pods) and non-essential (C=leaves and D=pods) amino acids of okra leaves and pods grown under micro-plot condition respectively. Bars (± SE) with different letters are significantly different ($p \leq 0.05$). Mean separation was done using a T-test. His= Histidine, Thr= Threonine, Lys=Lysine, Tyr=Tyrptophan, Met= Methionine, Val=Valine, Leu=Leucine, Phe= Phenylalanine, Arg= Arginine, Ser= Serine, Gly= Glycine, Asp= Asparagine, Glu=, Ala= Alanine and Pro= Proline.

4.4.2 Effect of the OMET growing technique and growing environment on the mino cid Score (AAS) of okra leaves and pods.

In this study, the OMET system samples had higher AAS values for each of the essential amino acids investigated under greenhouse conditions. The OMET system under greenhouse conditions showed a significant impact on AAS for Thr, His and Tyr + Phe on the leaves of okra while for the pods there was a significant difference on His, Thr, Lys, Tyr + Phe, Val and Leu. Among the essential amino acid compositions, the highest AAS was found for Phe + Tyr for both the leaves and pods which means that the content of Phe + Tyr was higher than the standard protein amino acid composition. Both the leaves and pods recorded the lowest AAS for Met, indicating that Met was the first limiting amino. The second limiting amino acid was found to be Ile for both the leaves and pods.

Evaluating the micro-plot results, the highest AAS of each essential amino acid was found under OMET growing technique for both the leaves and pods. The growing technique and growing condition showed significant difference in AAS for the leaves while for the pods the significant difference was only found for His, Lys and Tyr + Phe. For both the leaves and pods the highest AAS was found at Tyr + Phe which were two-fold higher in OMET than the non-OMET respectively. The first limiting amino acid was Meth for both the leaves and pods as it recorded the lowest AAS while the second limiting amino acid was Ile (Table 4.1).

Table 4.1: The effect of the OMET growing technique and growing environment on the amino acid score

| Treatments | His | Thr | Lys | Tyr + Phe | Val | Leu | Ile | Met |
|---|---|---|---|---|---|---|---|---|
| Greenhouse OMET leaves | 0.88 | 0.07 | 0.09 | 33.08 | 0.08 | 0.07 | 0.07 | 0.002 |
| Greenhouse non-OMET leaves | 0.91 | 0.11 | 0.1 | 31.93 | 0.09 | 0.08 | 0.07 | 0.002 |
| Greenhouse OMET pods | 0.5 | 0.04 | 0.04 | 12.60 | 0.03 | 0.03 | 0.02 | 0.01 |
| Greenhouse non-OMET pods | 0.85 | 0.08 | 0.08 | 21.61 | 0.05 | 0.05 | 0.04 | 0.02 |
| Micro-plot OMET leaves | 1.35 | 0.17 | 0.17 | 66.09 | 0.13 | 0.12 | 0.11 | 0.05 |
| Micro-plot non-OMET leaves | 1.02 | 0.12 | 0.11 | 34.22 | 0.10 | 0.09 | 0.08 | 0.02 |
| Micro-plot OMET pods | 1.61 | 0.08 | 0.07 | 32.86 | 0.06 | 0.06 | 0.05 | 0.02 |
| Micro-plot non-OMET pods | 0.79 | 0.08 | 0.09 | 21.64 | 0.06 | 0.06 | 0.05 | 0.02 |

AAS refers to the ratio between the amino acid of the test protein and the amino acid of the reference protein (Graciela et al., 2013). The amino acid in the test protein that showed the lowest AAS was termed as the first limiting amino acid. A protein with an amino acid score close to 100 is considered high quality (Joint *et al.,* 1973).

### 4.4.3 Effect of the OMET growing technique and growing environment on the Ratio Coefficient of Amino Acids (RC)

Under greenhouse conditions, the OMET growing technique significantly affected the ratio coefficient (RC) of amino acids. According to Graciela *et al*. (2013), a lack of essential amino acids affects the nutritional value of proteins, while an excess of certain amino acid compositions also limits the nutritional value of proteins (Bjorck *et al.,* 1984). The highest RC of each essential amino acid composition was found under non-OMET. The RC values for the leaves were all >1 either grown under OMET or non-OMET, indicating that the contents of the essential amino acids were excessive in the leaves. The pods also followed the same trend with the highest RC value being at the non-OMET. Though the RC value for Lys, Tyr+ Phe, Val, Leu and Ile were less than 1 indicating that the contents of these amino acids were scarce in the pods and limited the nutritional value of proteins. For the other essential amino acids of Met, His and Thr the RC values were closer to the standard amino acid (~1).

When evaluating the micro-plot experiment, the highest RC of each essential amino acid composition was found under the non-OMET except for Tyr + Phe for the leaves. Either grown under the OMET or non-OMET the essential amino acids RC values were greater than 1 except for Tyr + Phe and met grown under the non-OMET indicating that the contents of the essential amino acids were excessive in the leaves. For the pods, all the essential amino acids were found to be higher in the OMET as compared to the non-OMET. The RC values for the pods grown under the OMET system were all greater than 1 indicating that the pods' essential amino acids were excessive. The results indicated that the OMET system did not limit the nutritional value of protein.

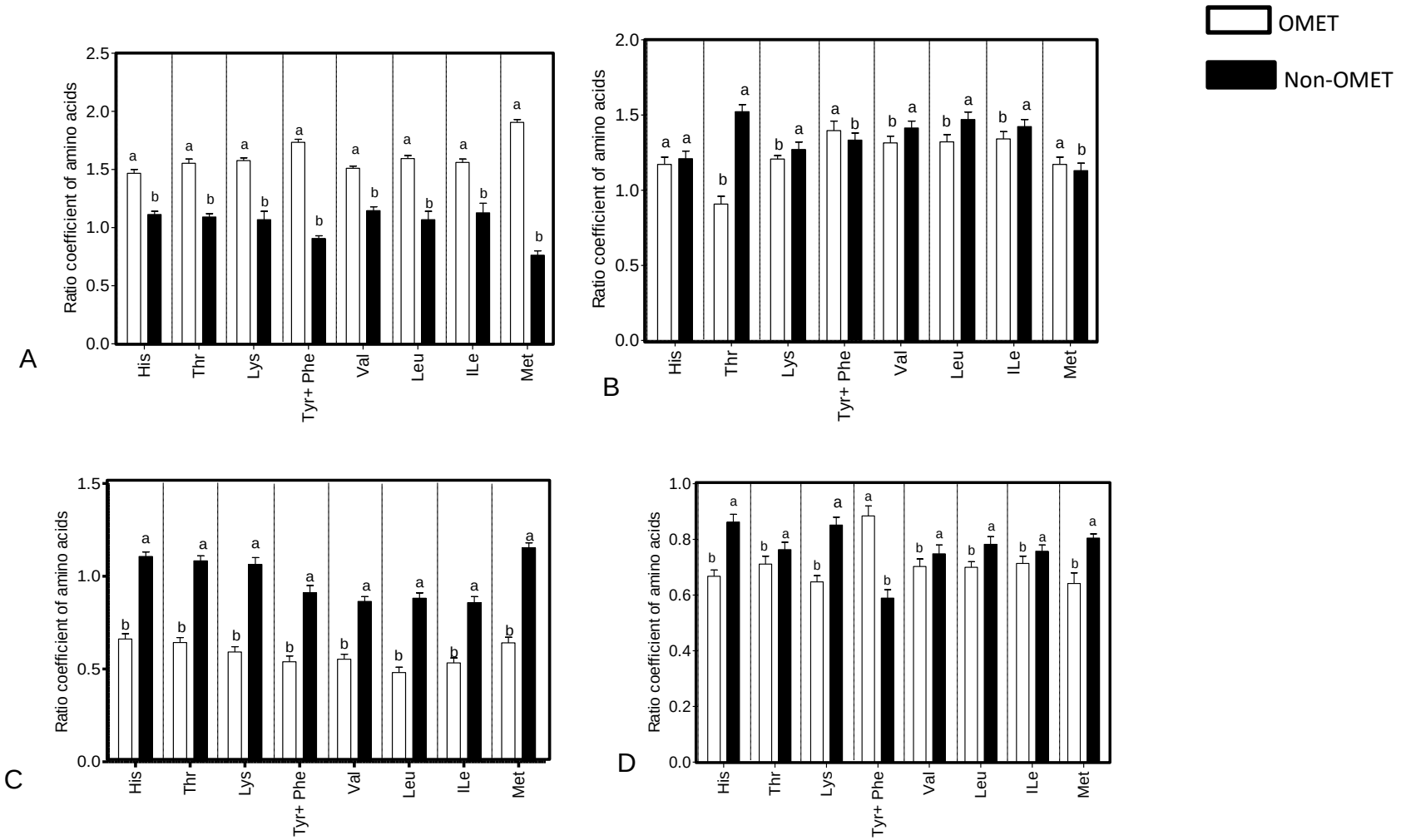


Figure 4.3:The effect of the OMET growing technique on ratio coefficient of amino acids of okra leaves and pods grown under greenhouse (A=leaves and B=pods) and micro-plot (C=leaves and D=pods) conditions . Bars (± SE) with different letters are significantly different ($p < 0.05$). Mean separation was done using a T-test. His= Histidine, Thr= Threonine, Lys=Lysine, Tyr=Tyrptophan, Met= Methionine, Val=Valine, Leu=Leucine and Phe= Phenylalanine

4.4.4 Effect of OMET growing technique and growing environment on elemental nutrient content in okra leaves and pods.

Evaluating the effect of OMET growing technique and growing condition showed a significant impact on the mineral composition of okra ($p \leq 0.05$). Results showed that elemental nutrients including calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) were predominant attributes for the nutritional status of okra leaves and pods (Figure 4.4). A clear trend demonstrated higher contents of the nutrient element in micro-plot and OMET-grown samples than their counterpart grown under greenhouse and non-OMET conditions except for K content which demonstrated higher content in the greenhouse environment. Furthermore, the leaves of okra contained higher nutrients than the pods.

In a greenhouse growing condition, Ca content ranged between 50.7 mg/kg and 90.6 mg/kg for okra pods grown under non-OMET and OMET, respectively (Figure 4.4 B). This range was two and three-fold lower in okra leaves which extended between 213.8 mg/kg and 220 mg/kg in OMET and non-OMET samples respectively. In addition, the content of K (100 mg/kg) and Mg (51.3 mg/kg) in non-OMET pods was two and three-fold lower than the highest mean value recorded in OMET leaves. The concentration of P ranged between 5.68 mg/kg and 7.26 mg/kg and maintained the lowest nutrient value. Results for the micro-plot condition demonstrated the highest content of Ca (237 mg/kg), K (170 mg/kg), Mg (110 mg/kg), and P (6.62 mg/kg) recorded in OMET leaves, whilst the lowest contents for Ca (49 mg/kg), Mg (37.6 mg/kg) and P (4.75 mg/kg) were recorded in okra non-OMET pods, except for K (110 mg/kg) which recorded the lowest content in the leaves of non-OMET.

Calcium, potassium, and magnesium are found in okra, providing about 8%, 9% and 14%, respectively, of the daily value (Sacks *et al*., 2001). Calcium is well known for its function in maintaining bone and teeth health but is also critical to cell signaling, blood clotting, muscle contraction, and nerve function. Dietary potassium intake has been demonstrated to significantly lower blood pressure in a dose-responsive manner in both hypertensive

and normotensive individuals in observational studies (Appel *et al.*, 1997; Sacks *et al.,* 2001; Appel, 2010; Sacks and Campos, 2010).

Elemental nutrients including (in descending order) selenium (Se), sodium (Na), zinc (Zn), iron (Fe) and manganese (Mn) were quantified in both environmental conditions and growing technique. These nutrients were downregulated in a greenhouse growing condition and non-OMET pod such that the lowest content of 1.79 mg/kg, 3.26 mg/kg, 3.55 mg/kg, 1.54 mg/kg and 1.07 mg/kg were archived for Se, Na, Zn, Fe and Mo, respectively. Leaves OMET-grown samples exhibited the highest contents of these elements. The results under the micro-plot pointed OMET grown pods and leaves to exhibit similar and highest contents of Se (12.26 and 12.8 mg/kg) and Na (6.19 and 6.77 mg/kg), which were slightly higher than Se (11.8 mg/kg) and two-fold higher than Na (3.73 mg/kg) content found in non-OMET leaves. OMET-grown leaves maintained the higher contents of Zn, Fe and Mn which were recorded to be up to three-fold, six-fold and two-fold higher than the mean values presented in non-OMET pods.

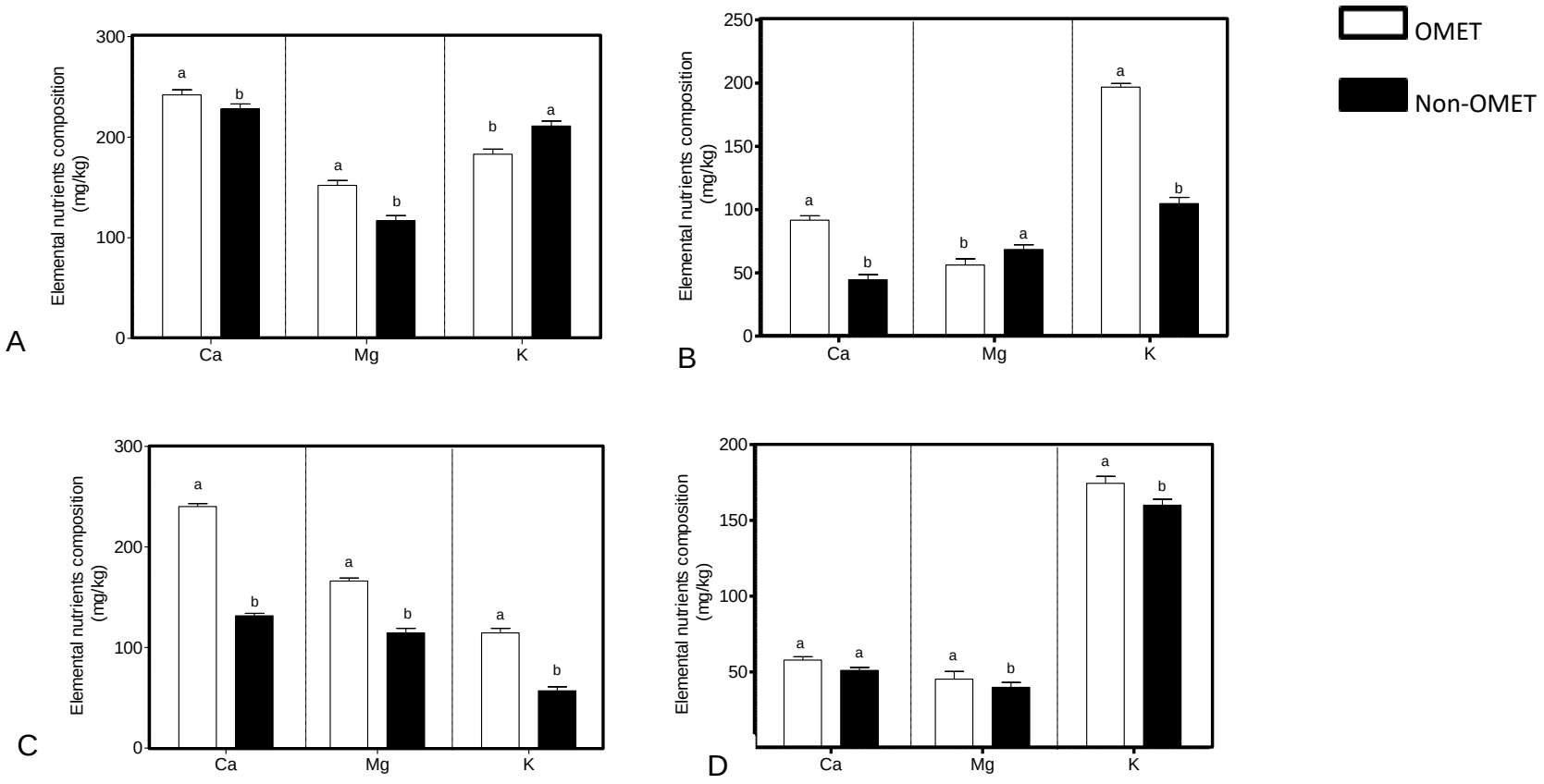


Figure 4.4 Elemental nutrients composition of okra leaves and pods grown under greenhouse (A=leaves and B=pods) and micro-plot (C=leaves and D=pods) conditions respectively. Bars (± SE) with different letters are significantly different ($p \leq 0.05$). The separation of the means was done using a T-test. Fe= iron, Mn= manganese, Na= sodium, Se= sellenium and Zn= zinc.

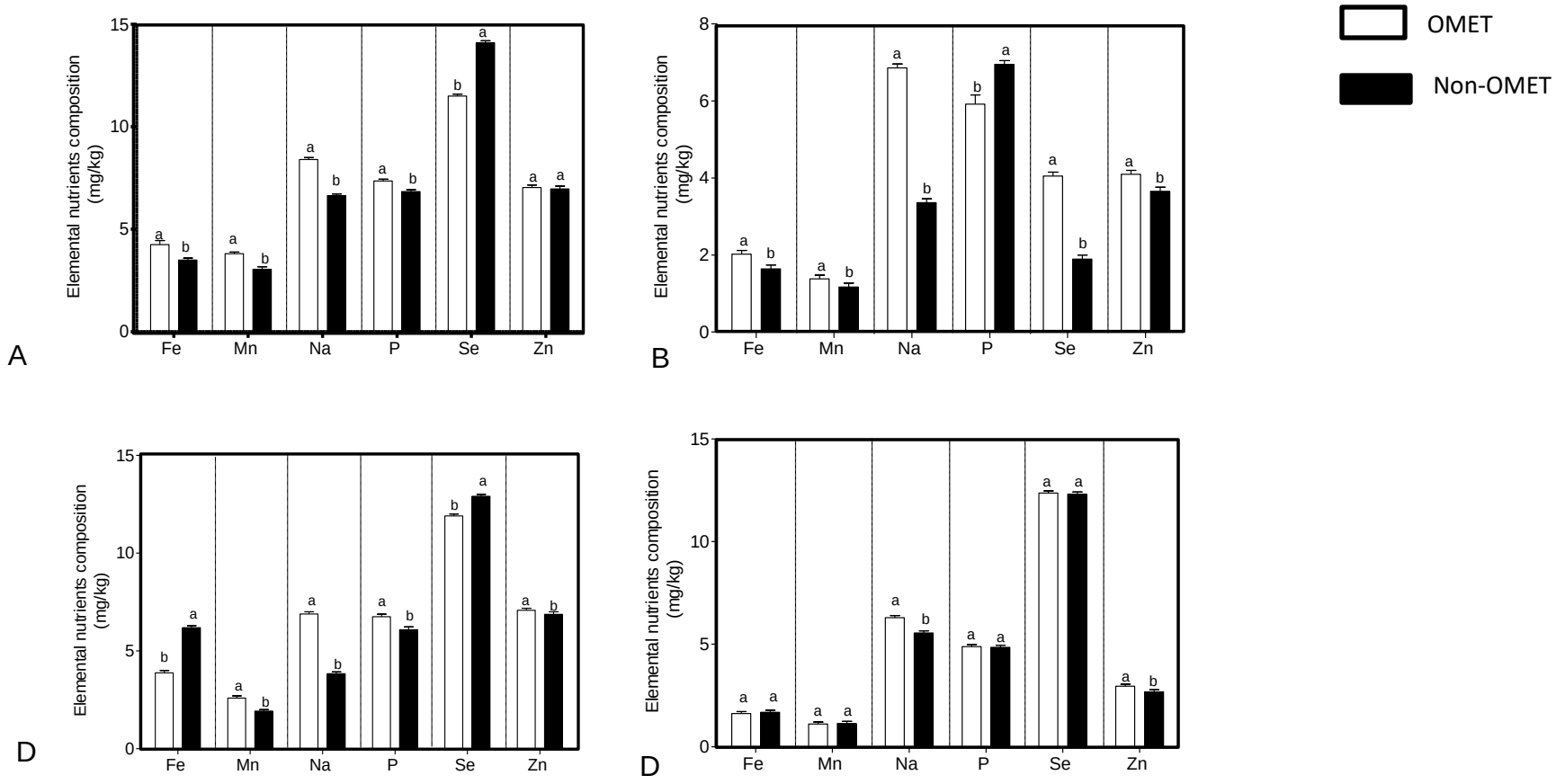


Figure 4.5:. Elemental nutrients composition of okra leaves and pods grown under greenhouse (A=leaves and B=pods) and micro-plot (C=leaves and D=pods) conditions respectively. Bars (± SE) with different letters are significantly different ($p \leq 0.05$). The separation of the means was done using a T-test. Fe= iron, Mn= manganese, Na= sodium, Se= sellenium and Zn= zinc.

### 4.4.5 The effect of OMET growing technique and environmental condition on protein.

Evaluating the effect of the OMET growing technique and growing condition on protein percentage statistically showed a significant difference ($p \leq 0.05$) on both the leaves and pods. Under greenhouse conditions, the OMET growing technique resulted in a higher protein percentage as compared to the non-OMET (2.27 and 2.02%) in the leaves respectively. The pods followed the same trend with protein percentage of 2.56% being recorded in the OMET growing technique as compared to 1.86% that was recorded in the non-OMET growing technique. Under the greenhouse condition, the highest protein percentage was recorded in the pods.

The results under micro-plot conditions, resulted in the leaves of the OMET-grown okra recording the protein percentage of 3.13% as compared to 2.7% of the non-OMET. While the pods protein percentage was 2.18% and 2.03% for both the OMET and non-OMET grown okra respectively. Under the micro-plot conditions the highest protein percentage was recorded in the leaves.

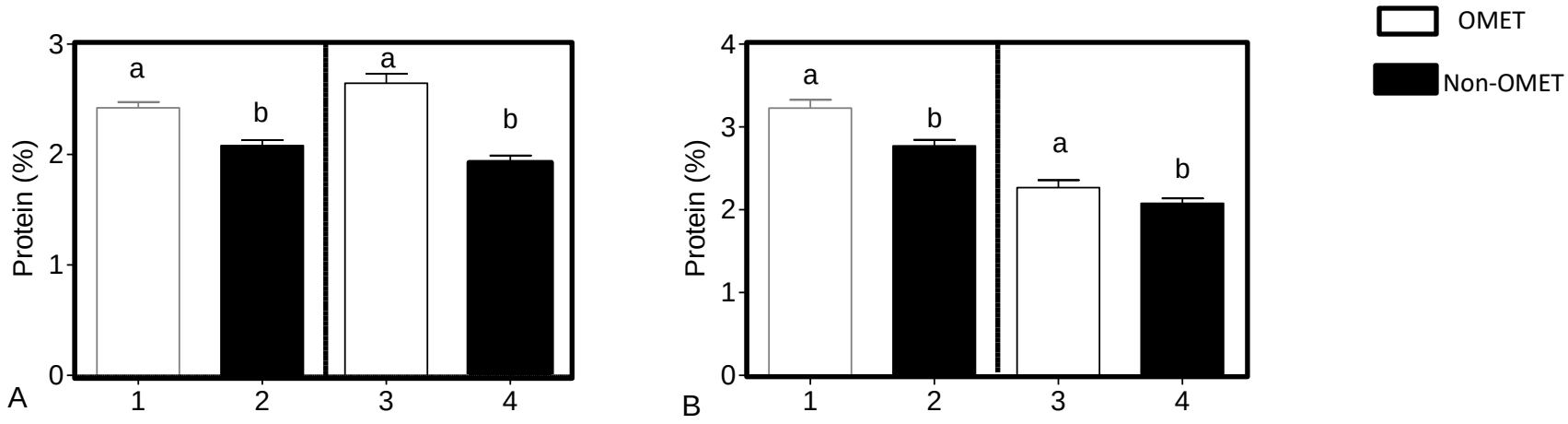


Figure 4.6: Protein percentage of okra leaves and pods grown under Greenhouse (A) and micro-plot (B) conditions respectively. Bars (± SE) with different letters are significantly different ($p \leq 0.05$). The separation of the means was done using a T-test. With 1= Leaves (OMET), Leaves (non-OMET), 3=Pods (OMET) and 4=Pods (non-OMET).

South Africa is a developing country which intends to maximise food security by up to 50% by 2050 (WWF, 2019). To archive this, sustainable cultivation practices aiming at water-use efficiency to produce high yields and uncompromised nutritional contents are required. In this study, the growth of okra under different environmental and in OMET growth techniques affected the nutritional composition on basis of protein basic units: amino acids and elemental nutrients. From the perspective of human nutrition, protein can promote the growth and development of the human body and provide energy for human health. As the basic unit of protein, amino acids and elemental nutrients play an important role in the processes of protein synthesis. It was interesting to observe that Glu and Asp were predominant amino acids in okra which was confirmed by a study reported by Sami *et al*. (2013). Furthermore, the same study showed that Tyr falls among the most limiting amino acid compounds (Sami *et al*., 2013). The Glu and Asp were found to predominate the soybean genotypes which had the highest preferred sensory score in relation to good taste (Carrera *et al*., 2011; Kumar *et al.*, 2011). The results obtained showed that the OMET growing technique has the potentialto maintain daily recommended amino acids ratio coefficient equal to 1.. Whereby, Lys, Met, Thr in the pods, and Thr, Lys, Tyr+Phe, Leu, Ile, and Met were maintained in the leaves.

In addition, both growing conditions and OMET growth technique directly brought change to primary factors such as soil temperature, light, water availability and evapotranspiration which affect the nutritional composition (amino acids and mineral nutrients). Amino acid accumulation is directly proportional to growing environment temperature (Climate *et al*., 2021; Ma *et al.*, 2022). A study reported by (Ma *et al*., 2022) revealed that Asp increased linearly with temperature, which was a fact that can be proven by my results. Amino acids including Ile, Met, Phe, Ala, Glu, and Pro have a tendency to increase with temperature up to a certain threshold which if it is surpassed will lead to a drop in their contents (El-kereamy *et al*., 2012).

For example, Pro showed to decrease at 22˚C, followed by Phe at 21˚C, Glu at 20˚C, Met at 19˚C and Ile at 16˚C (El-kereamy *et al*., 2012). Such evidence had now accounted for results obtained in this study which demonstrated OMET growing technique and micro-plot condition to maintain higher amino acids contents than their counterparts. Although the greenhouse condition maintained an average temperature

of 25˚C throughout the growing period, the OMET could have modified the root surface temperature to be more favourable than in non-OMET. A similar disclaimer may have been the case under micro-plot conditions, whereby, the OMET growing technique reduced trans-evaporation, irrigation intervals, water seepage and acts as a shade for the rooting zone. On the other hand, amino acids are known defense mechanisms against any abiotic conditions. The Pro, is well known for its potential to regulate osmotic adjustment substances in response to cold resistance (Khan *et al*., 2020). However, the content of Pro was moderately upregulated in both the growing environment and OMET growth technique and thus suggesting optimum growth temperature for okra production. This was further authenticated by the maintained higher contents of Glu: a precussor for Pro, which gets depleted in cold environments. In a limited water availability, reports by Khan *et al*. (2020), demonstrated the translocation and exudation of amino acids compounds such as Pro, Ser, and Ala, from the shoot down to the roots which likely affects soil water presence.

In this study, nutritional elements (in descending order): Ca, K, P, Mg and NA were predominant in okra leaves and pods irrespective of the growing environment and growing technique (OMET). The results were similar to those reported by Manuel *et al*. (2015). Reports stated that severe drought conditions of up to 30% water application increased Ca and Mg in *Amaranthus cruentus* species. The same study reported an increase in the contents of Zn, Na, P and K in 60% of water availability. According to Kanda *et al*. (2020), moderate deficit irrigation on cowpea increased the carbohydrate and fibre content due to water availability around the root surface. These reports account for the impacts of water availability on okra nutrients availability. On the other hand, mulching using plant materials such as increased N, P, K, Ca and Mg nutrient contents in okra leaves (Adekiya *et al.*, 2017). In this, study, OMET growing technique maintained higher contents of minerals than in non-OMET. In a non-OMET condition, samples were often under dry conditions caused by aeration, water seepage and trans-evaporation, which often lead to moderate drought conditions. These drought conditions were reported to reduce element nutrients such as protein, Zn, Fe, P and N (Sehgal *et al*., 2018). Furthermore, it is possible to suggest that consumption of approximately 50 g of okra pods and 25 g of leaves grown under OMET and cultivated in either greenhouse or micro-plot conditions can ensure the 1000 mg for adults' adequate intake of Ca according to the Institute of Medicine (2011).

## 4.5 Conclusion

This study validates the use of the OMET growing technique in both greenhouse and micro-plot conditions to improve growth and yield attributes while improving the nutritional composition of okra vegetables. Under the greenhouse growing condition, the use of the OMET growing technique significantly improved the essential amino acid composition of okra leaves with Thr (0.57 mg/kg), Val (0.70 mg/kg) Leu (0.90 mg/kg) and Phe (1.03 mg/kg) being higher than the non-OMET grown okra leaves. The pods showed that the OMET growing technique significantly improved the accumulation of all the tested non-essential amino acids with Lys being the highest. It was observed that the micro-plot experiment resulted in the OMET growing technique significantly enhancing the accumulation of all the tested essential amino acids in both the leaves and pods with Phe and Lys (1.53 and 0.70 mg/kg) being the highest in the leaves and pods respectively. The non-essential amino acid composition was also significantly improved using the OMET growing technique in both the leaves and pod with Glu (2.73 and 4.05 mg/kg) being the highest respectively. Regardless of the growing environment the OMET growing technique significantly improved the accumulation of the amino acids, nutritional elements and proteins though the highest accumulation was recorded under micro-plot conditions. The results generated showed that to obtain a higher accumulation of nutritional composition in okra, it is recommended to grow it under micro-plot conditions. The OMET growing technique can be used as a sustainable tool to improve the productivity of okra under organic farming regulations and for the improvement of food security to fight malnutrition.

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## Chapter 5: The effect of OMET growing technique and environmental condition on the phytochemical composition of okra leaves and pods.

Abstract

Okra is an underutilised indigenous crop belonging to the family Malvacea. It is a good source of medicinal components for the prevention and treatment of cancer, sugar diabetes, relieving constipation, heart problems and improving brain health. Organic Medium Enclosed Trough (OMET) farming is a climate-smart production system that is more water efficient, simpler and produces higher biomass yields in a relatively small area. The study objective was to investigate the effects of the OMET system on untargeted and targeted metabolites profile in okra leaves and pods grown under micro-plot and greenhouse conditions. Methanol extracts were analysed using UPLC-ESI-QTOF-MS. The UPLC-MS untargeted metabolites profile detected 161 polar analytes classified within the organic acid, sugars, amino acid, phenolic acid and flavonoids chemical classes. Explorative principle component analysis demonstrated three main clusters according to metabolites heterogeneity in plant tissue (pods and leaves) and growing conditions (greenhouse or mirco-plot). There was major heterogeneity in the metabolome profile of leaves tissue along the vertical PC1 suggesting their metabolic moiety. Okra leaves grown under micro-plot were highly predominated by the 2-O-caffeoylglucaric acid (286,13 mg/kg) and 2-(E)-O-feruloyl-D-galactaric acid (111.69 mg/kg). L-Tryptophan (amino acid) was enhanced in samples grown under the OMET system and greenhouse. Leaves samples grown in non-OMET were predominated by Citroside A (412. 04 mg/kg). Okra pods grown under OMET micro-plot enhanced the accumulation of Quercetin 3-galactoside (87. 83 mg/kg) and Quercetin 3-galactoside-7-glucoside (150 mg/kg). The OMET under greenhouse conditions encouraged the accumulation of Icariside F2; Benzyl beta-D-Apiofuranosy (49.21mg/kg) and L-Tryptophan (107.04 mg/kg). The results generated showed that flavonoids were the major contributors to the total antioxidant activity and OMET enhanced the accumulation of the majority of the metabolites.

Keywords: UPLC, OMET, Phytochemicals, Amino acids, Metabolites, Okra, PCA.

## 5.1 Introduction

Plants produce a diverse assortment of organic compounds that do not participate directly in growth and development. These substances, traditionally called secondary metabolites, are often differentially distributed among taxonomic groups within the plant kingdom. Secondary metabolites are plant products with no known nutritional and photosynthetic functions (Hartmann, 1991). They can be classified based on their chemical structure, composition, solubility in various solutes and or pathways (Kennedy and Wightman, 2011). Their functions, many of which remain unknown, are being elucidated with increasing frequency. Development of strategies to improve the nutritional quality of plant foods requires an understanding of the biochemical and molecular mechanisms of metabolite synthesis in plants as well development of analytical approaches for metabolite identification. Over the past decade, significant efforts have been made to enhance the production of plant metabolites through traditional breeding and the application of genomics technologies (Akhatou *et al*., 2016; Chen *et al*., 2019)

The introduction of production systems that will result in the enhancement of phytochemical-in plant production requires extensive metabolite control because background levels of other metabolites are largely unknown. Plant metabolomics is a new research discipline, which aims to develop a comprehensive approach to metabolite detection, identification and quantification. Metabolite profiling and metabolite fingerprinting are fast-growing technologies for phenotyping and diagnostic analyses of plants (Krishnan *et al.,* 2004; Mishra *et al.*, 2017). They allow the identification of the most important compounds (or groups of compounds) underlying differences between genotypes or phenotypes (Mishra *et al.*; 2017).

We are now approaching a comprehensive high-throughput analysis of plant metabolites due to recent advances in analytical and computational methods. Plant metabolomics is the large-scale study of metabolites within plant tissues, which helps to understand plant physiology and biology because metabolites represent the endpoint of biological activities. (Barbehenn and Constabel, 2011). Methodologies and instrumentations of plant metabolomics have been developing rapidly. There are two

types of metabolomics strategies: untargeted and targeted. Targeted metabolomics is the measurement of targeted groups of chemically characterized and biochemically annotated metabolites, whereas untargeted metabolomics is a global analysis of all measurable compounds in a sample. Untargeted analysis can detect a new metabolite and cover all measurable analytes in a sample, whereas targeted metabolomics provides more precise characteristics and quantitative analyses (Coutinho *et al*., 2018). Untargeted metabolomics allows for the investigation of phenotypes in metabolomics involving thousands of metabolites in a single run. It allows for the unbiased screening of all metabolites, but it results in more complex data steps. Plant-targeted metabolomics, on the other hand, provides a high-resolution tool for identifying and quantifying targeted metabolites in plants, as well as measuring their responses to environmental cues (An *et al*., 2018).

The consumption of indigenous vegetables does not only resolve the issue of food insecurity, but they also offer chemopreventative and cardio-preventative measures as well as protection against oxidation of the free radicals in the human body (Gorshkova *et al*., 2000). These preventative measures are due to the presence of secondary plant metabolites such as phenolic acids, flavonoids and carotenoids derived from various pathways; they are called phytochemicals (Hai-Lui, 2013) With the help of the primary plant metabolites including amino acids and sugars, phytochemicals provide health benefits to human, more than those that are attributed to the micro and macronutrients (Bhattacharya and Basu, 2018).). Phenols are important bioactive components of medicinal plant extracts that exhibit various pharmacological properties (Vundac *et al*., 2007). Their role is to provide mechanical strength, response to stress, and defense against pathogens (Gorshkova *et al*., 2000). Due to these pharmacological properties, plant phenolic compounds have gained increasing attention in both modern and traditional medicine as therapeutic compounds. The content of phenolic compounds and other phytochemicals present in medicinal plants is mostly influenced by genetic factors, cultivation, environmental conditions, as well as the degree of maturation and the variety of the plant (Koleva *et al*., 2002). The phytochemical analysis carried out in this study included total phenols and flavonoid content.

Flavonoids are secondary metabolites that play important roles in fruit and vegetable development. Phytochemicals such as phenolic acids, flavonoids, tocopherols, ascorbic acid and carotenoids are natural sources of antioxidants which keep monitoring the concentration of free radicals resulting from oxidative stress, which in turn lead to certain forms of cancer, cardiovascular disorders and diabetes (Bhattacharya and Basu, 2018). Okra is one of the reliable sources of phytochemicals, but information on their profiling using a more sustainable cultivation practice is still limited and segmented. The research based on phytochemical profiling of okra is important for the development of potential products and labelling (Coutinho *et al*., 2018)

The interest in the production of medicinal plants in all modern scientific research remains to discover many medicines and drugs to reduce incurable diseases (Bhattacharya and Basu, 2018). Millions of households have insufficient access to nutritionally safe food. This is largely a function of poverty, which is particularly pervasive in rural areas. Indigenous vegetable crops could play a significant role. The secondary metabolites from medicinal plants such as Flavonoids, Alkaloids, tannins and carotenoids play an important role in the treatment of many incurable diseases and to fight malnutrition (Coutinho *et al*., 2018). They are considered a major source in the discovery of modern medical drugs. Now more than ever, consumers are looking for healthy foods containing a large array of phytochemical compositions including flavonoids and phenolic compounds. Such bioactive molecules offer some protection against free radicals and reactive oxygen species produced by our bodies. Okra contributes being one of the best antioxidants and inhibits the growth of cancer cells (Bhattacharya and Basu, 2018). The antioxidant property is due to the oligomeric catechins and derivatives of flavonoids, which are potent antioxidant key factors (Kerure and Pitchaimuthu, 2019). The objective of this chapter was to determine the effect of the OMET growing technique and growing conditions on phytochemical composition in okra leaves and pods.

## 5.2 Materials and methods

### 5.2.1 Treatments and design

Harvested leaves and pods of okra grown under OMET and non-OMET growing techniques under greenhouse and micro-plot conditions (Chapter 3) were oven-dried at 40ºC until they reached a constant dried weight. Then both leaves and pods were ground into a powder.

### 5.2.2 Analysis of polar untargeted metabolites, phenolic compounds, flavonoids and antioxidants.

#### 5.2.2.1 Extraction of untargeted polar metabolites for UPLC analysis

The extraction of polar untargeted metabolites was extracted by weighing a 2 g sample into a 50 mL centrifuge tube with a screw-cap. 15 mL of 50% methanol/1% formic acid was added and the tubes were tightly capped. Thereafter the samples were vortexed for 1 minute, followed by extraction in an ultrasonic bath for 1 hour. A 2 mL of sample was then withdrawn and centrifuged at 14,000 rpm for 5 minutes. The clear supernatant was then transferred into 1.5 mL glass vials for analysis.

##### 5.2.2.1.1 Analysis of untargeted metabolites using UPLC

Liquid Chromatography-Mass Spectrometry (LCMS) Analysis, a Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. Column eluate first passed through a Photodiode Array (PDA) detector before going to the mass spectrometer, allowing simultaneous collection of UV and MS spectra. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity.

Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, one at low collision energy (4 V) and the second using a collision energy ramp (40−100 V) to obtain fragmentation data as well. Leucine enkephalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column. An injection volume of 2 µL was used and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B.

The gradient started at 100% solvent A for 1 min and changed to 28% B over 22 min in a linear way. It then went to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 mL/min and the column temperature was maintained at 55°C. A 10 µL volume of the filtered sample was injected into a Brownlee Analytical (Perkin Elmer. Bridgeport Avenue, Shelton, USA) C18 column (100 × 4.6 mm; 5 µm particle size). The chromatographic separation was performed by means of an isocratic elution of the mobile phase: a mixture of water/acetonitrile/acetic acid (78:20:2, v/v/v) filtered under vacuum through a 0.45 µm membrane before use at a flow rate of 1.0 mL $min^{-1}$ at 30°C. The column was re-equilibrated between sample injections with 10 mL of the mobile phase. Detection was performed at a wavelength of 280 nm. Compounds were quantified in a relative manner against a calibration curve established by injecting a range of catechin standards from 0.5 to 100 mg/L catechin.

5.2.2.2 Extraction of some biochemical compounds

Biochemical compounds including phenolic acid; flavonoids were extracted from both the dried leaves and pods of okra following the method described by Alasalvar *et al*. (2009). The polar targeted metabolites were extracted by weighing a volume of 0.2 g into 2 mL of 80% HPLC methanol in a thermostatic shaking water bath at 60 °C for 15 min-1. Subsequently, the mixture was centrifuged and the supernatant was filtered through Whatman® no. 1 filter paper and the pellet was repeatedly rinsed with solvent until the colour was no longer released. The pooled filtrates were dried under $N_2$ gas at 35 °C. Dried samples were re-suspended with an extraction solution and filtered

through a 0.45 µL membrane (Nylon syringe filter, Perkin Elmer™, China) prior to analysis.

5.2.2.2.1 Determination of total phenolic compounds

The total phenolic compounds of the plant decoctions were determined by using the Folin-Ciocalteu reagent method (Tambe and Bhambar, 2014), with minor modifications. The extracts (10 mg/mL) were diluted with 490 µL of distilled water to make up a final volume of 500 µL. This was followed by the addition of 0.25 mL of Folin-Ciocaltleu reagent (Sigma) in each test tube. Sodium carbonate ($Na_2CO_3$) (Sigma) (1.25 mL) was added and the mixtures were incubated in the dark at room temperature for 30 min. An ultraviolet/visible (UV/VIS) spectrophotometer was used to determine the absorbance of the mixtures at 550 nm. A blank and the standard curves were prepared in a similar manner, except that the plant extracts were replaced by distilled water for the blank. gallic acid (Sigma) was used as the standard for this procedure; whereby varying concentrations of gallic acid (1.25 - 0.08 mg/mL) were prepared. The results obtained from the linear regression formula of the gallic acid standard curve were expressed as milligram gallic acid equivalence/gram of extract (mg of GAE/g extract). The experiment was conducted in triplicates and independently repeated three times.

5.2.2.2.2 Determination of flavonoids

The total flavonoid content was determined using the aluminium chloride method (Tambe and Bhambar, 2014). Briefly, 100 µL of 10 mg/mL of the decoctions was added to 4.9 mL of distilled water in a clean test tube. To this reaction mixture, 300 µL of 5% sodium nitrite ($NaNO_2$) (Rochelle) dissolved in distilled water was added and the mixture was left at room temperature for 5 min. This was followed by the addition of 300 µL of 10% aluminium chloride ($AlCl_3$) (Rochelle) (dissolved in distilled water). The reaction was allowed to stand for 5 min at room temperature. After the elapsed time, 2 mL of sodium hydroxide (NaOH) (Rochelle) was added to the solution. The mixture in the test tube was then made up to 10 mL with distilled water. Catechin (Sigma) was used as a standard; whereby different concentrations (500 - 31.5 µg/mL) were prepared.

The absorbance of the experimental samples and the standard were determined using a UV/VIS spectrophotometer at a wavelength of 510 nm. The blank was prepared in the same manner as the experimental samples with 100 µL of distilled water added instead of the extracts. The total flavonoid content of the samples was expressed as milligram catechin equivalence/ gram of extract (mg CA/g extract)

5.2.2.3 Determination of antioxidant: Scavenging activity (DPPH)

The free radical scavenging activity of the extracts was determined by using the DPPH method (Chigayo *et al.,* 2016), with modifications. Briefly, different concentrations of the extracts (250 - 15.63 µg/mL) were prepared to a volume of 1 mL of the solution. L-ascorbic acid (Sigma) was used as a standard by preparing the same concentration range as the extracts. To these solutions, 2 mL of 0.2 mmol/L DPPH solution dissolved in methanol was added and vortexed thoroughly. The solutions were left to stand in the dark for 30 min at room temperature. The control solution was prepared by adding 2 mL of 0.2 mmol/L DPPH to 1 mL of distilled water. After the elapsed time, the solutions were analysed with a UV/VIS spectrophotometer set at a wavelength of 517 nm. The experiment was run in duplicate and repeated three times. Free radical scavenging activity of the extracts was expressed as percentage inhibition of DPPH from the control solution. The results were expressed as $EC_{50}$ (the sample required the reduction of the absorbance of the radical 50%) in mg of Gallic acid equivalent per gram of the leaves and pods.

The following equation was used to calculate the percentage of DPPH radical scavenging activity:

$$\text{DPPH scavenging (\%)} = \frac{Acontrol - Asample}{Acontrol} \times 100$$

In this equation, A sample is the absorbance of the sample after reaching the plateau and A control is DPPH absorbance. The compounds' concentrations inhibiting 50% of the total DPPH radicals ($EC_{50}$ inhibition values) were estimated as a positive control. In this estimation, lower $EC_{50}$ inhibition values indicate higher antioxidant activity.

## 5.3 Statistical analysis

Untargeted metabolites data were processed for both the leaves and pods with a factorial type and completely randomized design using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) 1,2 SWATH-MS/MS and DIA-MS: MS-DIAL: data independent MS/MS deconvolution for comprehensive metabolome analysis. Nature Methods, 12, 523–526, 2015 Identifying metabolites by integrating metabolome databases with mass spectrometry cheminformatics. Nature Methods, 15, 53-56, 2018 and analysed using explorative principle analysis (PCA). Biochemical data were subjected to statistical analysis using GenStat 18th Edition (VSN International, Hempstead, UK). The mean separation for significant treatments was achieved using a T-test at the significance level of 5%.

## 5.4 Results and discussion

5.4.1. Identification and quantification of phenolic metabolites identified in okra pods and leaves grown on OMET and different growing conditions.

Phenolic compounds including phenolic acids and flavonoids contribute to plant adaptation to different growing conditions. The predominating phenolic acid identified were members of the glucuronic acid derivatives including 2-O-caffeoylglucaric acid, n-O-caffeoylglucaric acid, and 2-O-sinapoyl-D-glucaric acid which contributed 35% of the total phenolic compounds in the studied okra samples. In these phenolic acids, 2-O-caffeoylglucaric acid was detected at retention time: 6.735 and *mz*: 6.735. The results showed that n-O-caffeoylglucaric acid was detected at 9.568 with *mz*: 371.062, whilst the 2-O-sinapoyl-D-glucaric acid was detected at rt: 12.863; and *mz*: 415.087. Based on the ESI MS of peak eluting at rt: 13.51 with *mz*: 191.01869, tentatively the peak was identified as Chlorogenic acid. Results further corroborate the presence of ferulic acid isomer detected at rt: 13.102 and mz: 457.13428 tentatively identified as 2'-(E)-Feruloyl-3-(arabinosylxylose).

Other phenolic compounds that have contributed to the total phenolic compounds include the O-glycosyl derivatives of Icariside F2; Benzyl beta-D-Apiofuranosyl-O-beta-D-glucopyranoside (rt: 13.663 and *mz*: 401.144) and isotan B (rt: 14.22 and *mz*: 308.079). The Phenethyl rutinoside: a member of the O-glycosyl compound was detected at rt: 19.429 and *mz*: 429.176. These O-glycosyl compounds attributed to 15% of the total phenolic compounds in the studied okra (Table 5.1). The majority of phenolic compound (45%) constituents were members of the flavonoid-O-glycosides which included the quercetin moieties tentatively identified as quercetin 3-galactoside-7-glucoside (rt: 16.108 and *mz*: 625.141), quercetin 3-lathyroside (rt: 16.494 and *mz*: 595.1322), and quercetin 3-galactoside (rt: 18.188 and *mz*: 463.087). Other derivatives of the flavonoid-O-glycosides were the tentatively identified Myricetin 3-(3'', 4''-diacetylrhamnoside) rt: 18.528 and *mz:* 547.1043 and neocarlinoside eluting at rt: 18.528 and *mz*: 579.1342. The remaining 5% were constituted by the terpene glycoside derivative eluting at rt: 14.546 and *mz*: 431.1918 tentatively identified as citroside A.

The tentatively identified 'Mollugoside': and Iridoid O-glycosides that emerged at rt: 11.843 and mz: 417.10382 were recorded. These metabolites were all present in all the studied samples, however, quantities varied based on the treatments. To summarize these virtual metabolites' profiling distinctions or similarities, i.e., to highlight metabolome fingerprint change induced by the OMET growth technique and growing conditions, multivariate analyses were performed. These statistical analyses were employed to generate multidimensional information which will indicate the biochemical changes related to the study. Unsupervised principal component analysis (PCA) (Figure 5.1) provided a dimensional explorative non-biased overview of OMET treatments and growing environment treatments. Results revealed three clusters based on the plant tissue and growing condition-related sample (Figure 5.1). This was reflected by the first cluster which indicated similarities of metabolites in okra leaves grown under greenhouse conditions irrespective of the growth technique (OMET or non-OMET), the second cluster grouped okra leaves grown under micro-plot either grown on OMET or non-OMET. The third cluster showed a clear indication of metabolites similarities in okra pods grown either in OMET or non-OMET or irrespective of the growing environment (greenhouse and micro-plot condition) (Figure 5.1).

There were significant differences between okra leaves and pods grown in an OMET growth technique and different growing environments in terms of phenolic compounds . In a greenhouse condition, Isotan B (62.0 mg/kg) was highly maintained, whilst euphosalicin (1.1 mg/kg) was the least found in okra pods grown under the OMET growth technique. Other compounds including (in ascending order) mollogoside and citroside A were recorded and were upregulated in okra pods grown under the OMET growth technique than non-OMET except for euphosalicin. On the other hand, leaves grown under OMET growth technique demonstrated the highest content of mollugoside (96.2 mg/kg), followed by isotan B (47.3 mg/kg), euphosalicin (13.2 mg/kg) and myricertin (27.6 mg/kg). A similar trend that illustrated higher organic acids in OMET leaves samples than non-OMET was observed. However, the non-significant difference between OMET and non-OMET was declared for citriside A. The results showed that organic acids such as mollugoside, citroside A, and euphosalicin were upregulated in the leaves than pods, whilst isotan B were higher in pods than leaves in a greenhouse condition irrespective of the growth techniques. A different trend was observed in micro-plot conditions, where mollugoside outperformed other identified metabolites in okra pods, such that it was two times higher than the isotan B content found in OMET and four times higher used in non-OMET okra pods. In addition, citroside A and euphosalicin were upregulated in nom-OMET than in OMET pods (Table 5.1). Unlike in Okra pods, the okra leaves grown under OMET conditions maintained the Isotan B most than the other organic acids. Whereas, citroside A was not affected by the OMET growth technique. The results further demonstrate the impact of OMET down-regulating some organic acids such as molloguside, and euphosalicin when compared to their counterparts: non-OMET (Table 5.1).

Glycosides such as Icariside F2 and Ethyl (S)-3-hydroxybutyrate were highly maintained in non-OMET (194.9) and OMET (51.2) leaves grown under greenhouse conditions. Besides the OMET (21.8 and 3.5) and non-OMET (13.6 and 30.5) pods grown in a greenhouse condition, these glycosides were highly maintained in non-OMET than in OMET in okra leaves grown under a greenhouse and in pods and leave grown under micro-plot conditions.

Polyphenols such as quercetin 3-galactoside-7-glucoside, Quercetin 3-galactoside, Quercetin 3-lathyroside, n-O-caffeoylglucaric acid, 2-O-caffeoylglucaric acid, 2,3,5-

trihydroxy-7-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-4H-chromen-4-one,7-hydroxy-4-{3-oxo-3H-benzo[f]chromen-2-yl}-2H-chromen-2-one, 2-O-sinapoyl-D-glucaric acid, 2'-(E)-Feruloyl-3-(arabinosylxylose), Chlorogenic acid, Neocarlinoside, Phenethyl rutinoside and Ethyl 7-epi-12-hydroxyjasmonate glucoside were detected and quantified in okra pods and leaves irrespective of the growing technique and growing environment (Table 5.1).

The results suggested that the okra plant (pods and leaves) is a source of flavonoids metabolites and quercetin isomers including quercetin 3-galactoside-7-glucoside, quercetin 3-galactoside, and quercetin 3-lathyroside were to most predominant okra pod markers. However, there was a significant variation between OMET and non-OMET samples grown neither in a greenhouse nor micro-plot conditions for all polyphenols metabolites with the exception of chlorogenic acid, neocarlinoside, and phenethyl rutinoside in okra leaves grown under a greenhouse (Table 5.1).

From the results, it was clearly observed that all of these polyphenols excluding 2-O-sinapoyl-D-glucaric acid were upregulated by the OMET growing technique than non-OMET in okra pods grown under greenhouse and micro-plot growing conditions. These results showed an opposite trend which indicated higher metabolites expression in non-OMET leaves grown under both greenhouse and micro-plot conditions.

Secondary metabolites play a critical role in the plant adaptation mechanisms against different abiotic stresses. The results of this study have shown the impact of the OMET growing technique and different growing methods on polar and phenolic compounds metabolites. These metabolites are members of the Shikimic pathway and phenylpropanoid pathways which contribute to improving plant defence mechanisms. The results have clearly appointed the OMET growth technique to conserve most of the detected metabolites in comparison to non-OMET. Although this study was the first to report about secondary metabolites versus OMET and growing conditions, similar metabolites detected and quantified in this okra leaves and pods including quercertin-3-galactoside, 2'-(E)-Feruloyl-3-(arabinosylxylose) and phenethyl rutinoside were also detected in *Momordica balsamina* L and pumpkin leaves (Mashiane *et al*., 2022; Mashitoa *et al*., 2021). It was noteworthy to observe that

metabolites were induced more in the micro-plot than in the greenhouse condition. These results were found to be similar to those reported by Guijarro-Real (2019). Therefore, this may be elucidated by stressful environmental factors such as light intensity, and harsh waether which further promote the trans-evaporations of plants in an unregulated chamber (Mashitoa *et al.*, 2021).

Table 5.1: Tentative peak assignment and quantification of the phenolic metabolites present in okra leaves and pods grown under OMET growing technique and different growing conditions

| Retention time (min) | Chemical formula | Generated ESI MS *m/z* | Tentative Identification | Samples detection (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Micro-plot | | | | Greenhouse | | | |
| | | | **Glucuronic acid derivatives** | OMET Leaves | Non-OMET Leaves | OMET Pods | Non-OMET pods | OMET Leaves | Non-OMET Leaves | OMET Pods | Non-OMET pods |
| 6.735 | $C_{15}H_{16}O_{11}$ | 743.13 | 2-O-caffeoylglucaric acid | 4.5±0.73[a] | 2.2±0.53[b] | 71.6±13.70[c] | 226.1±33.20[d] | 0.2±0.09[e] | 0.1±0.02[e] | 127.2±23.10[g] | 286.1±59.57[h] |
| 9.568 | $C_{15}H_{16}O_{11}$ | 371.06296 | n-O-caffeoylglucaric acid | 23.1±2.01[a] | 18.0±1.01[b] | 66.9±10.07[c] | 100.3±17.39[d] | 6.6±2.087[e] | 8.0±1.087[e] | 87.9±19.03[g] | 107.4±21.30[h] |
| 12.863 | $C_{17}H_{20}O_{12}$ | 415.08722 | 2-O-sinapoyl-D-glucaric acid | 0.1±0.04[a] | 0.2±0.09[a] | 3.8±1.10[c] | 14.2±2.09[d] | 0.0±0.00[e] | 0.1±0.00[e] | 3.6±1.200[g] | 6.4±3.900[h] |
| | | | **Tricarboxylic acids and derivatives** | | | | | | | | |
| 13.51 | $C_6H_8O_7$ | 191.01869 | Chlorogenic acid | 3.3±0.90[a] | 1.8±0.39[b] | 9.9±2.20[c] | 22.8±6.02[d] | 1.2±0.14[e] | 1.2±0.14[e] | 11.9±7.01[g] | 31.5±11.01[h] |
| 13.102 | $C_{20}H_{26}O_{12}$ | 457.13428 | 2'-(E)-Feruloyl-3-(arabinosylxylose) | 3.2±0.12[a] | 1.3±0.72[b] | 46.8±11.03[c] | 55.8±13.03[d] | 3.6±0.22[e] | 3.3±0.22[e] | 145.4±33.04[g] | 153.7±53.04[h] |
| | | | **O-glycosyl derivatives** | | | | | | | | |
| 13.663 | $C_{18}H_{26}O_{10}$ | 401.14426 | Icariside F2; -O-beta-D-glucopyranoside | 49.2±8.01[a] | 23.5±1.01[b] | 22.0±.7.01[c] | 19.7±3.09[d] | 194.9±74.10[e] | 140.2±29.80[f] | 99.2±9.04[g] | 80.6±27.04[h] |
| 14.22 | $C_{14}H_{15}NO_7$ | 308.08 | Isotan B | 62.0±11.04[a] | 32.5±7.08[b] | 47.3±8.09[c] | 25.9±8.22[d] | 6.2±1.00[e] | 3.1±0.90[f] | 52.7±11.02[g] | 36.5±18.02[h] |
| | | | **O-glycosyl compound** | | | | | | | | |
| 19.429 | $C_{20}H_{30}O_{10}$ | 429.18 | Phenethyl rutinoside | 0.4±0.02[a] | 0.1±0.002[b] | ND | ND | 0.2±0.072[e] | 0.2±0.032[e] | 0.9±0.01[g] | 4.0±1.30[h] |

| Retention time (min) | Chemical formula | Generated ESI MS *m/z* | Tentative Identification | Samples detection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Micro-plot | | | | Greenhouse | | | |
| | | | **Flavonoid-O-glycosides** | OMET Leaves | Non-OMET Leaves | OMET Pods | Non-OMET pods | OMET Leaves | Non-OMET Leaves | OMET Pods | Non-OMET pods |
| 16.108 | $C_{27}H_{30}O_{17}$ | 625.1402 | Quercetin 3-galactoside-7-glucoside | 124.7±0.01[a] | 128.±0.01[a] | 150.0±15.2[c] | 62.8±0.10[d] | 14.7±0.40[e] | 2.0±0.13[f] | 0.8±00.01[g] | 0.4±0.04[h] |
| 16.494 | $C_{26}H_{28}O_{16}$ | 595.13226 | Quercetin 3-lathyroside | 42.4±1.20[a] | 26.1±0.55[b] | 54.5±4.04[c] | 88.2±2.00[d] | 34.1±0.14[e] | 39.7±0.14[e] | 34.7±0.10[g] | 393.9±40.0h |
| 18.188 | $C_{21}H_{20}O_{12}$ | 463.08798 | Quercetin 3-galactoside | 72.9±0.70[a] | 63.6±0.04[b] | 22.0±0.21[c] | 11.7±1.01[d] | 87.8±0.02[e] | 50.3±0.02[f] | 163.3±9.01[g] | 201.2±15.1h |
| 18.528 | $C_{25}H_{24}O_{14}$ | 547.10437 | Myricetin 3-(3'',4''-diacetylrhamnoside) | 2.7±2.13[a] | 1.2±0.03[b] | 13.2±2.03[c] | 7.2±1.93[d] | 1.0±0.11[e] | 0.8±0.11[e] | 12.2±1.09[g] | 14.7±0.19g |
| 18.091 | $C_{26}H_{28}O_{15}$ | 579.13428 | Neocarlinoside | 0.5±0.83[a] | 0.3±77[a] | 0.4±0.19[c] | 0.4±0.19[c] | 16.8±0.00[e] | 30.0±0.00[f] | 80.2±4.01[g] | 112.2±16.0h |
| | | | **Iridoid O-glycosides** | | | | | | | | |
| 11.843 | $C_{17}H_{22}O_{12}$ | 417.10382 | Mollugoside | 20.5±0.20[a] | 6.5±0.08[b] | 96.2±1.03[c] | 75.4±1.23[d] | 13.9±0.09[e] | 13.4±0.09[e] | 27.7±7.03[g] | 42.0±8.05[h] |
| | | | Terpene glycosides | | | | | | | | |
| 14.546 | $C_{19}H_{30}O_8$ | 431.19186 | Citroside A | 16.6±1.03[a] | 10.6±0.23[b] | 41.0±0.32[c] | 41.0±0.47[c] | 6.0±0.[04e] | 10.1±0.04[f] | 41.0±12.2[g] | 41.0±14.72[g] |

*Values are expressed as means ± standard error, column with different alphabetic letters a-h is significantly different ($p \leq 0.05$). ND= Not Detected.*

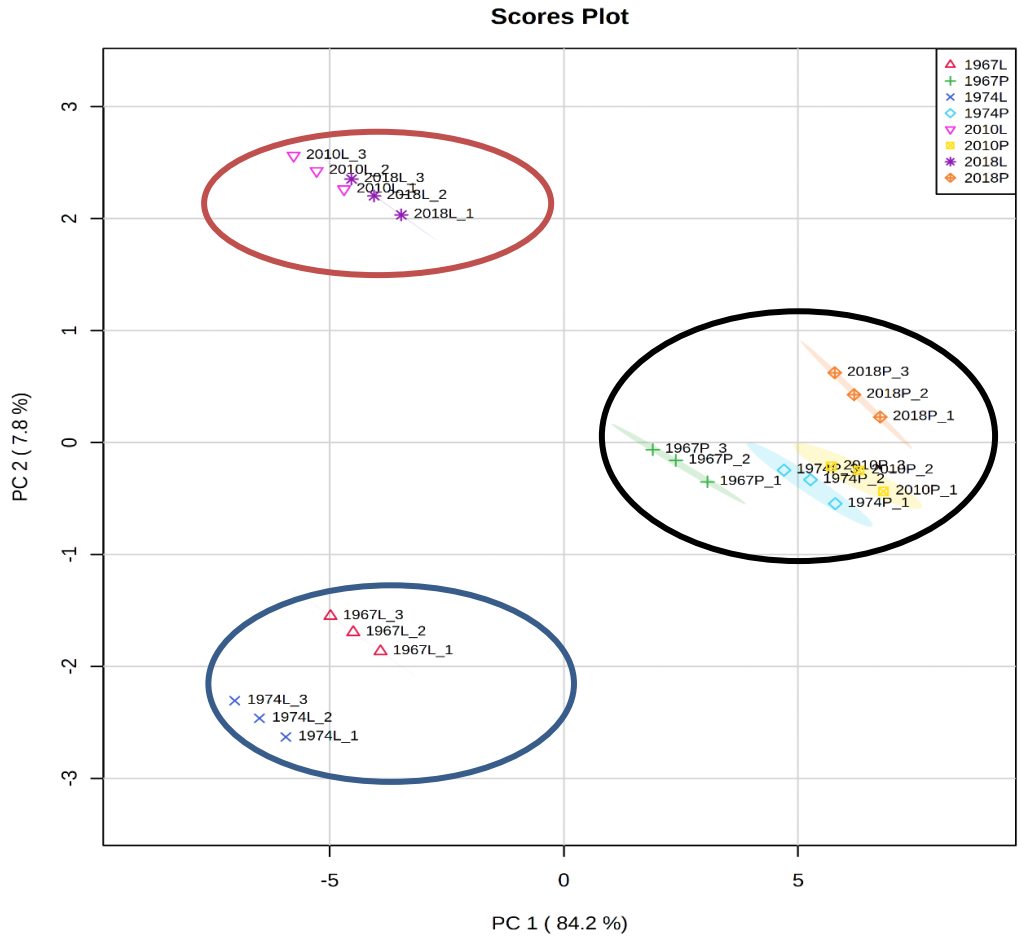


Figure 5.1 (Principle Component Analysis) plot (1967L = greenhouse OMET leaves, 1967P= greenhouse non-OMET pods, 1974L= greenhouse non-OMET leaves, 1974P= greenhouse OMET-pods, 2010L= micro-plot non-OMET leaves, 2010P= micro-plot non-OMET pods, 2018L= micro-plot OMET leaves and 2018P= micro-plot OMET pods.

5.4.2 The effect of the OMET growing technique and growing condition on biochemical compounds.

The concentration of total phenolics of okra leaves and pods grown under greenhouse and micro-plot conditions are presented in Figure 5.2. A and B respectively. Under greenhouse conditions, the OMET growing technique significantly affected the concentration of total phenolics ($p \leq 0.05$) in both the leaves and pods of okra. In the OMET-grown okra leaves the total phenolics were higher with 165.33 mg GAE/g compared to 89.45 mg of GAE/g of the non-OMET grown leaves. The OMET-grown leaves resulted in the accumulation of 54% of the total phenolics more than the non-OMET. When observing the pods, the OMET growing technique resulted in a higher accumulation of the total phenolics compounds as compared to non-OMET grown pods (200.17 and 105.55 mg of GAE/g) respectively. The total phenolics compounds measured in this study using the OMET growing technique under the greenhouse conditions ranged from (188 to 201 mg of GAE/g) for the leaves and (215 to 233 mg of GAE/g)

When evaluating the concentration of the total phenolics compounds in the micro-plot growing condition, the OMET growing technique significantly affected the total phenols ($p \leq 0.05$) in both the leaves and pods of okra. The OMET growing technique resulted in a higher accumulation of the total phenolics compounds as compared to the non-OMET. The total phenolics concentration measured in this study using the OMET growing technique ranged from (687 to 707 mg of GAE/g) for the leaves and (189 to 233 mg of GAE/g) for the pods.

The highest total phenolics compounds were obtained under OMET micro-plot conditions in comparison to the greenhouse experiment. Phytochemicals like phenolics are important in plant defence against different environmental stress conditions which include but are not limited to wounding, infection, and excessive light or UV irradiation (Berger *et al*., 2007; Bergguist *et al*., 2007). Light intensity enhances the biosynthesis of phenolic substances in plant chloroplast (Muzafarov and Zolotareva, 1989). In this study, the reduced synthesis and accumulation of the total phenolics in the greenhouse experiment

may be attributed to the reduced stimulation of phenolic biosynthesis by light. And also probably the result of endogenous degradation of some of the phenolic compounds, occurring after air exposure, as well as to be the result of an increase in temperature or light exposure during field experiments (Santos-Gomes *et al.,* 2002).

The concentrations of flavonoids in both the leaves and pods of okra extracts grown under greenhouse and micro-plot using the OMET and non-OMET growing technique, measured as milligram catechin equivalence per gram (mg CA/g), are presented in Figure 5.3 A and B respectively.

Under greenhouse conditions, the OMET growing technique significantly affected ($p \leq 0.05$) the concentration of flavonoids. The OMET growing technique resulted in higher flavonoid concentration in both the leaves and pods which was 70 and 68 mg of CA/g respectively as compared to the non-OMET. The micro-plot samples followed a similar trend, both the leaves and the pods of the OMET-grown okra resulted in a higher concentration of the flavonoids which was three-fold higher than the non-OMET.

The number of active components in plants can be influenced by cultivation practices such as the growing technique and the growing environment (Hernandez *et al.,* 2009). The use of a growing technique that restricts water and nutrient seepage can improve soil fertility and it is a logical step towards increasing the production of medicinal plants. Flavonoids significantly increased when using the OMET growing technique, which implies that the physical properties of the soil or soil nutritional status were improved. The synthesis and accumulation of flavonoids can be influenced by other factors such as genotype (species and variety) and ecological conditions such as locality and harvesting period (Jiang *et al.,* 2007). Flavonoids may be responsible for the protective effect against many disease processes, such as cancer (Wang and Mazza, 2002), cardiovascular and circulatory diseases (Stoclet *et al.,* 2004) and diabetes (Ishige *et al.,* 2001; Abdille *et al.,* 2005). There were different concentrations in the total of phenols detected in both the leaves and pods in both the treatments and the same trend was also noticed in the flavonoid content. Different levels of expression of plant secondary metabolites like phenols suggest the differences in the ability of different crop in establishing themselves in changes in the growing condition. Changes in the growing condition may exert stress

on the plants and this may result in the expression of more plant secondary metabolites. Plants with high total phenols composition are regularly used as a basis for the production of valuable synthetic compounds such as pharmaceuticals, cosmetics, or more recently, nutraceuticals (Bourgaud *et al.,* 2001).

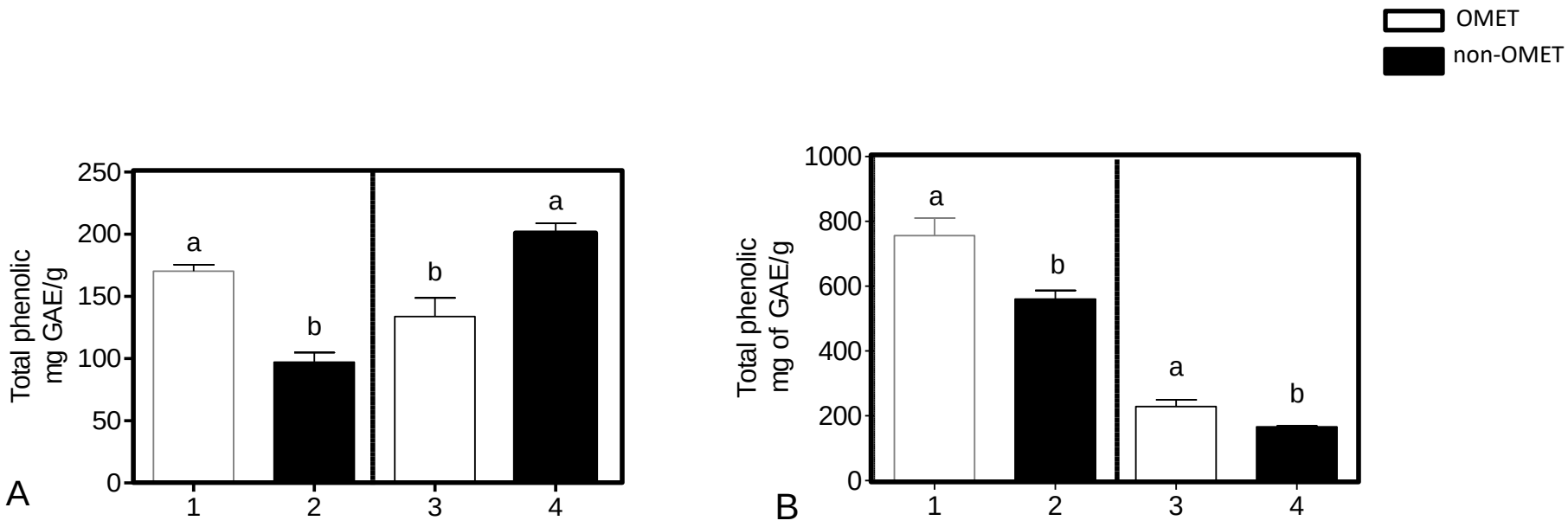


Figure 5.2: Total phenolic concentrations of okra leaves and pods grown under greenhouse (A) and micro-plot (B) conditions (milligram gallic acid equivalence/gram of extract (mg of GAE/g)) respectively; Bars (± SE) with different letters are significantly different ($p \leq 0.05$). Separation of means was done using a parametric T-test. 1= OMET leaves, 2= non-OMET leaves, 3= OMET pods and 4= non-OMET pods)

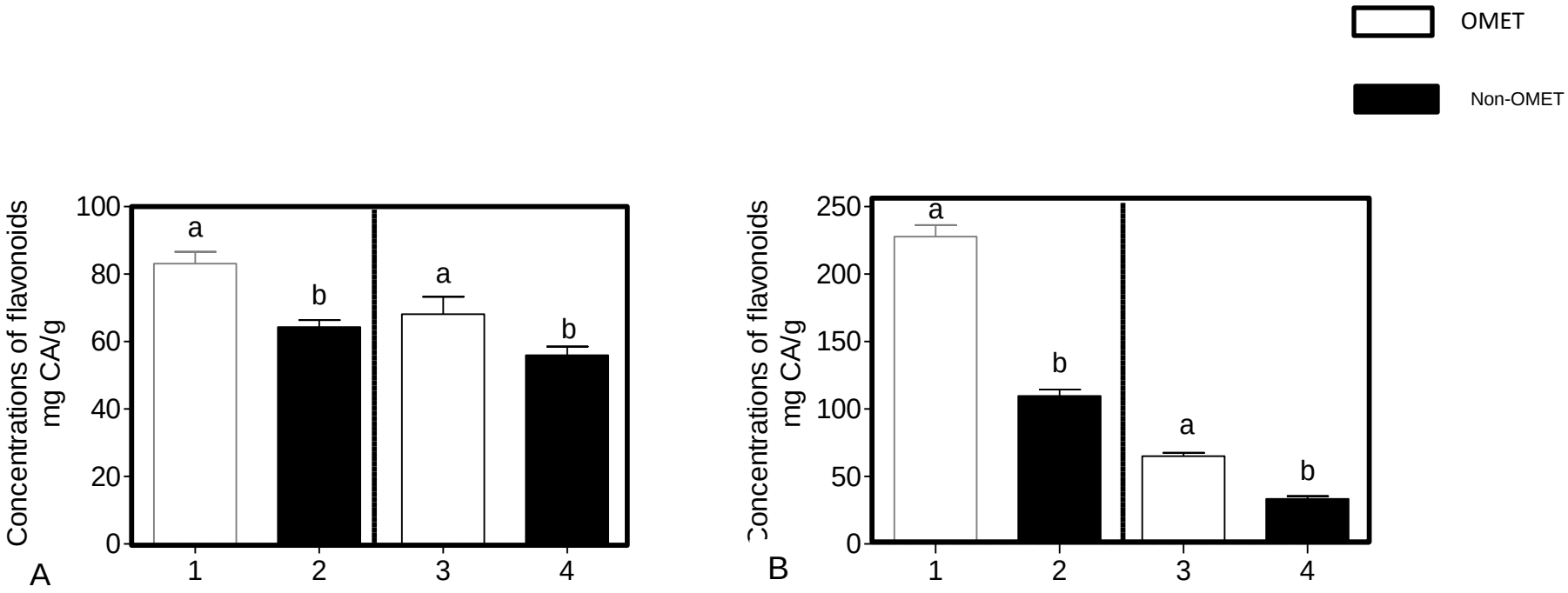


Figure 5.3: Concentrations of flavonoids (milligram catechin equivalence/ gram (mg CA/g)) of okra leaves and pods grown under greenhouse (A) and micro-plot (B) condition respectively; Bars (± SE) with different letters are significantly different ($p \leq 0.05$). Separation of means was done using a parametric T-test. . 1= OMET leaves, 2= non-OMET leaves, 3= OMET pods and 4= non-OMET pods).

5.4.3. The effect of OMET growing technique and growing condition on DPPH radical scavenging activity of okra leaves and pods

The $EC_{50}$ values for the DPPH radical scavenging potentials of okra leaves and pods grown under greenhouse and micro-plot conditions are shown in Figure 5.4 (A and B) respectively. The widely used parameter to measure antioxidant activity is the concentration of a test sample needed to decrease the initial DPPH concentration by 50% which is denoted as $EC_{50}$ (Atoui *et al.,* 2005). The half maximal effective concentration ($EC_{50}$) to inhibit DPPH was determined to elucidate the effectiveness of the free radical scavenging activity of the extracts. Low $EC_{50}$ values indicate that a small amount of the extract is required to inhibit half the amount of total free radicals in a solution. Higher $EC_{50}$ values indicate the converse.

The results of the okra grown under greenhouse condition radical scavenging activity showed that, the OMET growing technique significantly influenced the antioxidant activity. The leaves of OMET-grown okra had the lowest $EC_{50}$ of 32.73 mg/mL compared to 46 mg/mL of the non-OMET, which meant that there was high antioxidant activity in the OMET-grown leaves as compared to the non-OMET. The pods followed the same trend, with the OMET grown pods extracts resulting in a 40.91 mg/mL $EC_{50}$ value as compared to 84.55 mg/mL of the non-OMET pod's extracts.

When evaluating the micro-plot experiment, the OMET growing technique also significantly influenced the free radical scavenging activity for both the leaves and pod extracts. Both the OMET gown leaves and pods showed the highest antioxidant activity with an $EC_{50}$ of 60.00. and 36.73 mg/mL compared to the non-OMET 81.82 and 42.87 mg/mL respectively. Suggesting that the OMET growing technique resulted in higher antioxidant activity as compared to the non-OMET.

Extracts of medicinal plants rich in phytochemical compounds are increasingly used as a natural source of antioxidants in the manufacture of food or are consumed directly as raw ingredients (Exarchou *et al.,* 2002). The results generated from this study were in contrast

to those reported by Valšíková *et al.* (2018) whereby they reported a decreasing trend in antioxidant activity when investigating the effect of plastic mulch on tomatoes. The total antioxidant capacity was similarly reduced also in (Melgarejo *et al*., (2012), where average lower values of the total antioxidant capacity were found after the application of the plastic mulch. Guilherme *et al*. (2020) reported that higher phenols and flavanols compounds in green peppers significantly affected higher DPPH antioxidant activity and that corresponded with our results, as the OMET-grown okra leaves and pods were significantly higher in both the total phenols and flavonoids which contributed to the antioxidant activity.

Progressing climate change necessitates the search for solutions for plant protection against the effects of water deficit. Maintaining water balance in the face of changing environmental conditions is a crucial survival strategy for plants (Mahdieh *et al.,* 2008). It is well known that plastic mulch which is a similar growing technique to the OMET system protects plants against the adverse effects of periodic water shortage in the soil, improving their hydration level and water use efficiency (Zhu and Gong, 2014).

Phytochemical analysis conducted on both the leaves and pods plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofoura, 1993). Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, flavonoids, glycosides and organic acids. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc. (Ali *et al*., 2008). Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial

substances against a wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Salah et al., 1995). They also are effective antioxidants and show strong anticancer activities (Del-Rio *et al.,* 1997; Okwa, 2004). Glycosides are known to lower blood pressure according to many reports (Nyarko and Addy, 1990). The results obtained in this study may be a valuable tool to fight food insecurity and malnutrition.

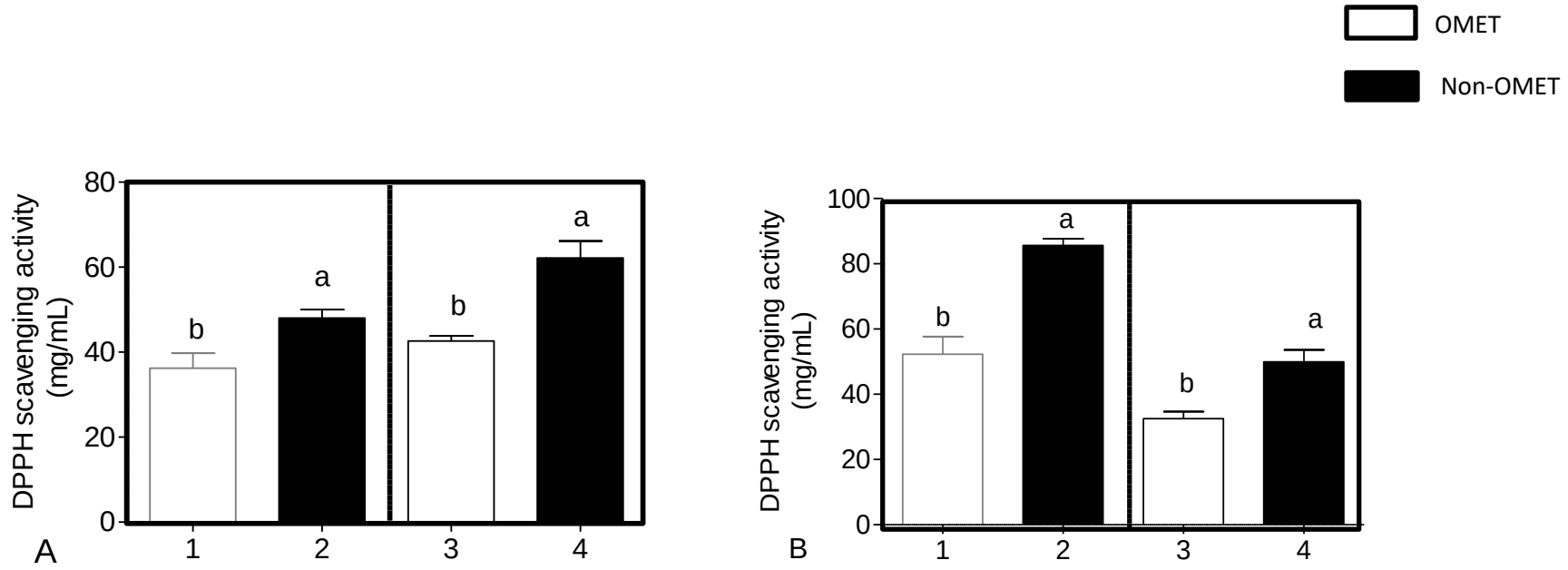


Figure 5.4: The results of DPPH scavenging activity (mg/mL) of both the leaves and pods of okra grown under greenhouse (A) and micro-plot (B) conditions respectively expressed as $EC_{50}$. Bars (± SE) with different letters are significantly different ($p \leq 0.05$).

## 5.5 Conclusion

Plant secondary metabolites act as bulwark mechanisms against herbivores and pathogenic attacks. The effects of OMET and non-OMET growing techniques on secondary metabolites were shown to vary greatly. This designates the type of bioactive compounds that could be responsible for therapeutic benefits. The non-OMET growing technique significantly increased the accumulation of 2-O-caffeoylglucaric acid and n-O-caffeoylglucaric acid in the pods with a concentration of 226.1 and 100.3 mg/kg under greenhouse and 286.1 and 107 mg/kg under micro-plot conditions respectively. Isotan B showed higher concentration in the leaves grown using the OMET growing technique under greenhouse conditions. Total phenols and flavonoid levels were higher in OMET and lowest in non-OMET grown okra plants. High phenolic concentration in OMET-grown okra could suggest this growing technique enhances the plant to produce more secondary metabolites. Total concentrations of phenols and flavonoids significantly increased under OMET growing technique regardless of the growing condition. The results indicate the importance of the continuity of phytochemical and cultivation present, especially in the evaluation of biological activities. Generally, the concentration of the compounds measured among the different genotypes varied across the two environments. This observation might be a result of environmental differences, inherent genetic factors as well as growing condition andenvironment interaction. Comparatively, commercial production could be more suitable under field conditions to achieve the higher antioxidant activity.

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# Chapter 6:

# Summary of findings, significance, recommendations and conclusions.

## 6.1 Summary of findings

The findings of this study provided new empirical information on the effectiveness of the OMET-growing technique to improve the yield and growth, nutritional levels and phytochemical profiling of okra regardless of the growing condition under the disposal of lower irrigation using sustainable agriculture. Plant growth and yield attributes, macro and micro nutritional elements, amino acids composition, phytochemical composition and antioxidant activity capacity were demonstrably higher in the OMET-grown okra regardless of the growing environment. The study revealed that growing okra using the OMET growing technique results in a two-fold higher increase in the biomass (g), no of branches, no of pods per plant, fresh pod weight (mm), pod diameter length (mm), pod diameter width (mm) and fresh root mass (g). The nutritional composition and phytochemicals were also increased by the use of the OMET growing technique. Under the greenhouse growing condition, the use of the OMET growing technique significantly improved the essential amino acid composition of okra leaves with Thr (0.57 mg/kg), Val (0.70 mg/kg) Leu (0.90 mg/kg) and Phe (1.03 mg/kg) being higher than the non-OMET grown okra leaves. The pods showed that the OMET growing technique significantly improved the accumulation of all the tested non-essential amino acids with Lys being the highest. It was observed that the micro-plot experiment resulted in the OMET growing technique significantly enhancing the accumulation of all the tested essential amino acids in both the leaves and pods with Phe and Lys (1.53 and 0.70 mg/kg) being the highest in the leaves and pods respectively. The non-essential amino acid composition was also significantly improved using the OMET growing technique in both the leaves and pod with Glu (2.73 and 4.05 mg/kg) being the highest respectively.

## 6.2 Significance

The rusults documented will be used in the future to improve the production of neglected crops under climate-smart agriculture. Furthermore, this information will also be shared with small-scale farmers and growers of neglected crops to improve yield without compromising the nutritional and phytochemical composition. The information generated from this study is important because it will contribute to bridging the scientific gap on the information that has never been documented on the effectiveness of climate-smart OMET growing technique. This information will be useful to rural farmers to increase their yields while improving their nutritional diet. The results presented in this study are the first information on enhancing growth and yield, nutritional composition and phytochemical levels in okra using a climate-smart OMET growing technique. The current study demonstrated that growth and yield attributes, nutritional content and phytochemical levels accumulation in okra are influenced by the growing technique and also the growing condition. The results showed that growing okra in OMET growing technique can result in increased growth and yield without compromising the nutritional quality.. The results showed that growing okra in OMET growing technique can result in increased growth and yield without compromising the nutritional quality.OMET growth technique resulted in a potential to maintain daily recommended amino acids ratio coefficient equal to 1. Whereby Lys, Met, Thr in the pods, and Thr, Lys, Tyr+Phe, Leu, Ile, and Met were maintained in the leaves. In this, study, OMET growing technique maintained higher contents of mineral nutrients than in non-OMET.

In the current study, the responses of nutrient elements were restricted to macro (Ca, Mg and K) and microelements (Fe, Mn, Na, P, Se and Zn) alone. In future studies, the scope of the studies could be expanded to other nutrient elements in order to have a comprehensive view of the effect of the OMET growing technique under different irrigation water regimes. Such a comprehensive view would enhance the use of the OMET system to improve the growth and yield of under-utilized indigenous crops to fight food insecurity and nutrients deficiency in the diets. `.

## 6.3 Recommendations

Based on the findings of this study, it is recommended to use the OMET growing technique in water-scarce environments to enhance the growth, yield, phytochemical and nutritional composition of okra because OMET growing technique has proven to be effective in enhancing the aforementioned attributes. The use of this technique can come in handy for small-scale farmers in semi-arid and arid regions as it minimizes the use of water. The results of this study should therefore stimulate further studies on the use of the OMET growing technique on other medicinal crops under deficit irrigation to increase plant growth and yield attributes, nutritional and phytochemical composition, ensuring the effectiveness and successful commercialization of medicinal plants under the disposal of lower irrigation.

## 6.4 Conclusions

Okra is one of the low-fat foods with unique nutrients and phytochemical profiles and is particularly rich in minerals, amino acids, nutrients as well as bioactive components, such as flavonoids. The report from the current study showed that the OMET growing technique can significantly improve the growth and yield of okra. Also, when screening the aqueous and ethanol extract of okra leaves and pods, it was found that the OMET system increased the presence of amino acids, nutritional minerals, and bioactive compounds and improved the biological scavenging activity of okra leaves and pods. It can be concluded that okra leaf and pod extract are rich in chemical composition exhibiting the highest activities.

The result available in this work shows the potential nutritional importance of okra as well as its role in health and nutrition improvement. Incorporation of okra into a diet may help in improving its quality and subsequently reduce the risk for the development of chronic non-communicable diseases. The study revealed that growing okra using the OMET growing technique is simple and an affordable source of nutrients. As a result of complex in the nature of disease etiology and different factors linked to their occurrence, it is

pertinent that researchers continue unravel the mechanism and principle involved in disease control and braced up on how bioactive from OMET grown okra can influence human health. The variation in phytochemical compounds found in the present study is another breakthrough and revelation on the medicinal attributes of okra and a pointer to pharmacological applications of okra in treating human diseases and several ailments. Apart from the above highlighted nutritional and medical characteristics of okra, the phytochemical compound detected in this work is another scientific point towards its earlier use in many herbal formulations for the cure of various ailments, in particular the regulation of blood pressure, fat, diabetes, chronic dysentery genito-urinary disorders and ulcer.