Evaluation of yield, quantitative and qualitative attributes of three Amaranth species grown under Organic Medium Enclosed Trough system

By

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Declaration

I, Maeleletse Glas Mopai 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 “Evaluation of yield, quantitative and qualitative attributes of three Amaranth species grown under Organic Medium Enclosed Trough system" 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.

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Mopai M.G **Date**

Dedication

I dedicate this work to the souls I have lost, my late grandmother Nthuse “MmaLesepele” Mopai, my mother Legala Nape and my sister Mokgadi “Khekhwetsho” Mopai and, continue giving me your “eyes” until it’s my time too.

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List of abbreviations, symbols, and chemical formulae

AAS= Amino Acid Score

ACa= *A. caudatus*

ACr= *A. cruentus*

ASpp= *Amaranthus* Spp.

ALVs= African Leafy Vegetables

ARC-VIMP= Agricultural Research Council, Vegetable, Indigenous and Medicinal Plants

AOAC= Association of Official Analytical Chemist

AURI= Agricultural Utilization Research Institute

CA= Catechin Acid

CE-MS= Capillary Electrophoresis-MS

DAFF= Department of Agriculture, Fishery and Forestry

DSI= Department of Science and Innovation

ET= Evapotranspiration

FAO= Food and Agricultural Organization

FI-ICR-M= Fourier Transform Ion Cyclotron Resonance-MS

GAE= Gallic Acid

GBRCE= Green Biotechnologies Research Centre of Excellence

GC-MS= Gas Chromatography-MS

HPLC-MS-QT= High-Perfomance Liquid Chromatograph-Mass Spectrum-Quadrupole Time

ICPE-900= Inductively Coupled Plasma Optical Emission Spectrometry

LLDPE= Linear low=density polyethylene

MS= Mass Spectrometry

NASA= National Aeronautics and Space Administration

NIH= National Institute of Health

NRF= National Research Fund

Non=OMET= Non=Organic Medium Enclosed Trough

OMET= Organic Medium Enclosed Trough

OPLS-DA= Orthogonal Partial Least Square-Diseminant Analysis

PCA= Principal Component Analysis

PDA= Phodiode Array

PE= Polyethylene

PFAF= Plants For A Future

RC= Ratio Coefficient

RCBD= Randomized complete block design

RSA= Republic of South Africa

UL= University of Limpopo

UPLC= Ultra-Perfomance Liquid Chromatograph

US= United States

UV/VIS= Ultraviolet/visible

WHO= World Health Organization

Abstract

Amaranth species are a highly popular group of vegetables rich in primary and secondary metabolites, with potential to significantly reduce food insecurity and malnutrition. Yet, their production levels in South Africa (SA) remains unknown and limited to small scale farming where water scarcity is also a problem. Although the production rate of Amaranth is unknown in RSA, the consumption Amaranth has increased over the last few years. The Organic Medium Enclosed Trough (OMET) system is a non-drainable growing technique. OMET system supports and promotes organic and sustainable farming. The aim of the study was to investigate the effects of the OMET system on growth and yield in three Amaranth species, including: *A. caudatus, A. cruentus* and *Amaranthus* Spp. Data collected for the growth attributes were seedling height, stem diameter, leaf length, root length and time of flowering; whereas for yield attributes, mass of the aerial parts was determined. The nutritional composition data was acquired from determination and quantification of carbohydrates, protein, minerals (Ca, Zn, P, Se, Mg, and Mn) and amino acids. The amount of water used on the OMET and non-OMET (control) was recorded until termination. T-test at 5% level of significance was used to analyse data using computer statistical software, Statistix 10.0. This study revealed that the OMET system had significant impact on the growth and yield of all the three species as shown by the improved and increased growth and yield attributes of the Amaranth. The OMET system increased the stem diameter, plant height and leaf length of *A. caudatus* by 2.8%, 10.5% and 2.6% respectively; *A. cruentus* by 25.2%, 21.6% and 11.2% respectively, by 32.4%, 41.2% and 39.6% respectively in *Amaranthus* Spp. The OMET system increased the root length of *A. caudatus*, *A. cruentus* and *Amaranthus* Spp. by 8, 50.1 and 94.5% respectively. Furthermore, the OMET system significantly increased the mass of aerial parts of *A. caudatus*, *A. cruentus* and *Amaranthus* Spp. by24.4, 12.6 and 91.9% respectively.

The nutritional and phytochemical (targeted and untargeted) compositions of the three Amaranth species were quantified/determined and compared independently for each Amaranth species grown under OMET and control. The essential and nonessential amino acids were determined and quantified, and it has been shown that they are highly available in OMET grown *A. cruentus*. Furthermore, both *A. cruentus* grown

under non-OMET and *Amaranthus* Spp. grown under OMET system showed to be the second potential sources of amino acids compared to other Amaranth species grown under distinct treatments. The levels of phenylalanine (2.54 mg/kg), leucine (2.21 mg/kg), valine (1.45 mg/kg), isoleucine (1.29 mg/kg) and threonine (1.15 mg/kg) were dominant in all three Amaranth species but highest in *A. cruentus* grown under OMET system The results of this study indicate that the OMET system has enhanced the concentration of carotenoids and chlorophyll, although this increase is not significant inin Amaranth species grown under both treatments (OMET and non-OMET). The highest caretonoid (59.56 mg/kg) and chlorophyll (48.08 mg/kg) were obtained in *A. caudatus* grown under non-OMET and OMET system respectively. The other targeted metabolites including phenolics, flavonols and tannins were measured highest concentrations in Amaranth species that were grown under non-OMET system compared the same species grown under OMET system. The total phenolics, flavonols and tannins were specifically dominant in *A. cruentus* (276.88 mg GAE/100 g DW), *A. cruentus* (448.56 mg CA/100 g DW) and *A. caudatus* (10.5 mg CA/100 g DW).

The study also revealed that the OMET system utilised less water than the non-OMET system.In addition, OMET system improvedthe growth attributes such as stem diameter, plant height, leaf length, and fresh biomass and nutritional composition of the three Amaranth species. On the other hand, the OMET system decreased the accumulation of the secondary metabolites such as total phenolic acids, flavonols and tannins. But the non-OMET system (control) significantly increased the concentration of the above mentioned targeted metabolites in the three Amaranth species. The OMET system is a sustainable and organic growing technique that produces more yield relatively on a small area while utilizing less water, making it a brilliant strategy to conserve and utilise water sustainably and effectively. This can be important for smallholder farmers who can potentially cut production costs such as irrigation, weeding and fertilizers costs while increasing yield on a relatively small area.

CHAPTER 1: INTRODUCTION

1.1 Background

The recommended consumption of leafy vegetables in the sub-Saharan Africa mainly due to their potential in improving food security through increasing food diversification and nutritional security. As a result, twenty-one leafy vegetables including Amaranth species are included in the South African food database (Bvenura and Afolayan, 2015). Amaranth species belongs to Amaranthaceae family and specifically to the *Amaranthus* genus. Thisgenus consists of about 60 species which are distributed throughout the world. Some species including *A thunbergii, A. greazicans, A. caudatus, A. tricolor, A. arusha A. cruentus, A. spinosus, A. deflexus, A. hypochondriacus, A. viridus* and *A. hybridus* are commonly consumed as relish (Bvenura and Afolayan, 2015; Kongdang *et al*., 2021; Taia *et al*., 2021. However, other species of Amaranth remain obscure and indigenised within local communities, as a result of lack of research and scientific documentation. These species are mostly characterised by morphological attributes such as type of leaf venation or blades, count of stamens in the inflorescence as well as the presence of red spots on their leaf surfaces (Taia *et al*., 2021). Some species are characterised by showing a reticulodromous or brochidodromous leaf venation pattern with the red coloured spot on the lower or middle parts of the leaf (Taia *et al*., 2021) such as *A. tricolo* and *A. cruentus*.

Although Amaranth is commonly referred to as a weed that destructs the growth of cultivated crops, it is also an important food crop. In Southern Africa, tender young leaves of *A. caudatus, A. cruentus*, and *A. deflexus*, are often harvested and cooked as spinach (Maseko *et al*., 2017). Furthermore, the surplus is then blanched and sun-dried or frozen for use during the dry winter seasons when there is a limited supply of fresh vegetables (Maseko *et al*., 2017). Amaranth species are recognised for their balanced nutritional diet and health benefits. Both leaves and seeds contain unusually high concentrations of protein (Yarger, 2008). In fact, the consumption of 1 cup of Amaranth leaves provides 9.3 g of gluten-free diet, 46 g carbohydrates, 36 and 29% of the recommended daily intake of phosphorus and iron respectively (DAFF, 2010). Furthermore, the nutritional compositions found in Amaranth are reportedly higher than those found in exotic commonly consumed vegetables such as spinach, basella, and chard (Saunders and Becker, 1983). Therefore, Amaranth species can serve as

a cheaper food crop for alleviating food insecurity, malnutrition and poverty in Southern Africa (DAFF, 2010). In addition to its benefits for human health, Amaranth can also be used as a crop to mitigate the known accelerating effects of climate change such as high temperatures and drought (Jamalluddin *et al*., 2021). This is mainly because as a C4 plant, under extremely high temperatures and insufficient soil moisture it can reduce stomatal conductance, leading to low leaf transpiration rates which in turn result in high water use efficiency, in contrast to their C3 counterparts (Kauffman *et al*., 1990; Barba de la Rosa *et al*., 2009).

The production of Amaranth in rural areas depends on summer rains and its production is only at a small-scale level together with informal local markets in South Africa. Strategies to improve productivity of Amaranth have been investigated and reported (Sarmadi *et al*., 2016; Ngoroyemoto *et al*., 2019). Among them, sustainable strategies to improve growth, yield and post-harvest quality without compromising nutritional composition and phytochemical contents have been investigated (DAFF, 2010). These strategies were demonstrated and overlooked in certain Amaranth species. The application of chemical fertilisers (Ayodele, 2000; Kunene *et al*., 2017), growing under protected environments, irrigation regimes, bio-stimulant, and plastic mulching have been investigated to elucidate their effects on Amaranth growth attributes and yield (Lancíková *et al*., 2020). Nutritional composition and nutraceutical attributes have also been investigated to understand variations of the applied production methods on the aforementioned characteristics (Lancíková *et al*., 2020).

The Organic Medium Enclosed Trough (OMET) system is a non-drainable growing technique developed by Helmuth Rohrer in 2012 (Ferreira, 2013). The OMET system is characterised by sandwiching the soil using plastic (underlying plastic is turn-folded) to form an enclosed trough (Ferreira, 2013). Because of its characteristic design, the OMET system provides multiple benefits such as utilizing less water, no water or nutrient seepage, no high tech skills required to operate,ease of portability and, no weeding and fertilization needed. The system is also 100% organic and environmentally friendly. The once-off setup is far lower than that of a hydroponics system (Ferreira, 2013). Only the sheet mulch is replaced with a new crop while everything else is re-usable. All plant wastes are released back into the growing medium, therefore feeding the soil again. No fertiliser is applied, as the plants receive the required nutrients directly from the growing medium. Its smooth surface deters crawling insects and prevents weed growth. The OMET sheet/net has a unique wind-resistant, spider-web design with a flat roof, which allows adequate air circulation inside the tunnel (Ferreira, 2013).

1.1.1 Description of the research problem

The *Amaranthus* species occupy a strategic position in combating food and nutrition insecurity, as it is widely consumed in Africa. These species are drought tolerant with yields of up to 40 tons/ha of fresh leaves on marginal soils (DAFF, 2010). The leaves are rich in diversified nutrients that can combat food insecurity, malnutrition, and support healthy eating (Obianuju and Olubukola, 2021). Despite several benefits of different Amaranth species, the cropis underutilized in South Africa (RSA). Its consumption is concentrated in rural areas, where it is harvested from the wild (cultivation fields) during the rains (DAFF, 2010). There are no large-scale productions of Amaranth, hence the lack of data on its production. Inadequate knowledge of its uses, vegetative growth requirement, less research efforts and the implementation of the research facts are part of the reasons why Amaranth is still underutilized in RSA (Obianuju and Olubukola, 2021).

In assessing the future anticipated impacts of climate change towards crop production and agricultural water management, water availability (from rainfall, watercourses, and aquifers) will be a critical factor (Turral *et al.,* 2011). Substantial adaptation will be needed to ensure adequate supply and efficient utilization of what will, in many

instances, be a declining resource. However, the long-term climatic risk to agricultural assets and agricultural production that can be linked to water cannot be known with any certainty (Turral *et al.,* 2011). The irrigated area of the world has increased dramatically due to less rainfall and rapid population growth, resulting in high demand for food (Turral *et al.,* 2011). Research regarding breeding of Amaranth species that yield high in low input farming including limited irrigation water supply has gained attention lately because of climate change, food, and nutritional insecurity (Turral *et al.,* 2011). The increased production of Amaranth can be improved by adopting new sustainable growing techniques such as OMET system. Lack of scientific information based on the effects of OMET system on Amaranth species probably hinders the cultivation on small and large scale using OMET system. This system can be adopted in relatively low-input farming including in areas of limited irrigation water supply.Irrigation water has become most scarce agricultural commodity due to the impact of climate change and/or global warming.

1.1.2 Impact of the research problem

In RSA, indigenous vegetables including Amaranth species, are currently recognised aspart of the national food database (Bvenura and Afolayan, 2015). The COVID-19 pandemic has had serious implications on the global economy, with food and nutritional security being particularly impacted, denying many South Africans their right to adequate food (Stats. SA, 2022). Like many countries around the globe, RSA has not been spared. According to a report released by the Department of Statistics South Africa (Stats. SA) (2022), almost 23,6% of South Africans in 2020 were affected by moderate to severe food insecurity, and approximately 14.9% experienced severe food insecurity. This is mainly due to abundant content of primary metabolites which have been identified as active ingredients that can contribute to the food supply of human essential dietary needs to tackle hidden hunger and/or food insecurity issues (Stats. SA, 2022). Although varieties of exotic vegetables like kale and collard greens, regarded as high value crops, have been investigated as food solutions to nutritional security, they are inaccessible to the common man. Moreover, food diversification has been reduced, which leads to food and nutritional insecurity (Stats. SA, 2022).

RSA is a water stressed country that faces challenges of rapid population growth including food and nutrition insecurity (Oelofse and van Averbeke, 2012). Most smallholder communities live in marginal areas where crops struggle to survive because of poor growing conditions. Ultimately residents and farmers face food insecurity and malnutrition challenges due to poor growing conditions and scarcity of factors of production such as arable land, capital and skilled labour (Oelofse and van Averbeke, 2012). Furthermore, commercial or irrigated crop production takes place under areas subjected to water scarcity, with water availability likely to drop below the benchmark of 1000 m^3 person/year (Annandale *et al*., 2011). African leafy vegetables (ALVs) including Amaranth species offer alternatives both to smallholder and commercial farmers because they are nutrient dense and tolerant to several abiotic and biotic stresses (DAFF, 2004; van Averbeke *et al*., 2012).

There is an insufficient scientific information on the effect of water scarcity and the use of OMET system on quality and quantity attributes of indigenous vegetables including Amaranth species (including the ones investigated in this study) such as growth, yields and accumulation of vital primary and secondary metabolites. Furthermore, the information on enhancement and evaluation of growth, yield, nutrients, and phytochemical compositions of Amaranth species using climate-smart farming techniques such as the OMET system has not been investigated and documented. The effect of OMET system on growth, yield and metabolites was unknown hence the investigation is conducted in this study. This is a clear indication that there exists a paucity of information regarding the effects of OMET system on Amaranth species. This study was aimed at investigating the quality and quantity of Amaranth species produced under the non-drainable vegetable growing technique, the OMET system. This system relatively utilises less water and reduce water and nutrient loss through seepage while producing moreon a relatively small area.

1.1.3 Possible causes of the research problem

The effects of human-caused global climate change are becoming more and more apparent as we see more record-breaking weather phenomenon such as heat waves, intense droughts, shifts in rainfall patterns and a rise in average temperatures. These environmental changes are linked to every part of crop production (Zhongming *et al*.,

2021). Around the world, agricultural practices have developed as a function of topography, soil type, crop type, annual rainfall, and tradition. The amount of water available for irrigation is already affected by climate change impacts (Zhongming *et al*., 2021). Nutrient loss and/or depletion in the soil through leaching is increasing daily. Groundwater levels are also sensitive to changes in climate such as persistent drought and excessive rain. NASA (2018) reported that where groundwater is used for agriculture, groundwater levels are generally decreasing. Additionally, plants access water in the soil, which in hotter regions and a hotter futureis more prone to evaporation, leaving less for plants to absorb. Access to water and nutrients has direct effects on crop growth, health, and yield. Therefore, favourable growing conditions whereby the plant has direct access to water and nutrients needs to be established, investigated, and implemented (Zhongming *et al*., 2021).

The ever growing population is facingfood insecurity and malnutrition crisis. The underutilization of indigenous vegetable crops such as Amaranth is growing enormously probably due to lack of information about the benefits of consuming these highly nutritive indigenous vegetables (Padulosi and Hoeschle-Zeledon, 2004). Amaranth is one of the most less cultivated and underutilized indigenous vegetable species in RSA (DAFF, 2010). Padulosi and Hoeschle-Zeledon (2004) defined underutilized species as those non-commodity crops, which are part of a larger biodiversity portfolio, once more popular and today neglected by users'for a variety of agronomic, genetic, economic, social, and cultural factors. Nowadays farmers cultivate them less than in the past because these species are no longer competitive with the crops that have come to dominate the world food supply and are supported by seed supply systems, production and post-harvest technologies and extension services. In addition, their markets are well established, and consumers are accustomed to using them (Padulosi and Hoeschle-Zeledon, 2004). Potential ALVs need to be investigated and scientific information about their benefits needs to be diffused to the society to enhance diversification and utilization of these nutrient rich crops.

1.1.4 Proposed solutions to the research problem

Although Amaranth species are highly underutilised and not usually cultivated in RSA, they arecultivated, by small scale farmers i.e., in Venda, Vhembe District, Limpopo Province. These indigenous leafy vegetables have the potential to reduce the food and nutritiona insecurity crisis (DAFF, 2010). In response to the problem statement, innovative strategies to enhance Amaranth production, utilization and improve their market must be implemented. To enhance the cultivation and utilisation of indigenous vegetables including Amaranth, their competitiveness must be addressed and new opportunities such as new food and lifestyle trends and the developments taking place in production and post-harvest technologies must be explored. This is, however, not the whole story. The lack of competitiveness may be an important factor for underutilisation, but this tells us little about the geographical social and economic reasons associated with the decline of local crops (Padulosi and Hoeschle-Zeledon, 2004). For instance, regarding geographical distribution, a species might be underutilised in some regions, but not in others.

There are several strategic factors that need to be considered if we are to successfully promote underutilised species such as (I) focusing on local values, indigenous knowledge and uses. Such an approach has the potential to strengthen the link between diversity and sustainable uses and is important in considering marketability; (II) recognizing underutilized species as a public good to ensure the continued availability and accessibility of plant genetic material to present and future generations (Padulosi and Hoeschle-Zeledon, 2004). The development of sustainable value chains for Amaranth from cultivation on the field to production of different value-added products and understanding of its numerous health benefits could enable a significant intervention to uplift millions of rural and poor urban households in developing countries where malnutrition is glaring with its attendant health consequences (Aderibigbe *et al*., 2020). Thus, Amaranth appears to be an economically viable underutilized crop with great potential (Aderibigbe *et al*., 2020).

The present study involves the investigation of the OMET system, a promising growing technique. It is promising to enhance growth, yield and accumulation and concentration of primary and secondary metabolites in Amaranth species. The effectiveness of this study will aid small scale farmers to enhance the production and yield of indigenous vegetables including Amaranth species. The study also provides

scientific information regarding the benefits of consuming and/or utilising Amaranth which can potentially reduce the food and nutrition insecurity crisis. This study addresses the crisis of agricultural practices that do not ensure sustainability and safety of the environment including conservation of nutrients, water, and the preservation of the high value indigenous vegetables.

1.1.5 General focus of the study

The spotlight of the research is on the following: food insecurity, malnutrition, soil water and nutrients loss, water scarcity, sustainability and environmentally friendly vegetable growing techniques, low input farming, climate change adaptation strategies and enhancing cultivation and/or production and utilization of Amaranth species and other nutrient-rich indigenous leafy vegetables.

1.2 Problem statement

Food insecurity and the loss of soil nutrients and productive capacity are serious problems considering a rapidly growing world population (DAFF, 2010). Indigenous food crops like Amaranth species are neglected and/or underutilized, yet they can be the source of necessary nutrients required to achieve nutritional security and help combat the food insecurity crisis that SA faces (DAFF, 2010). Species of Amaranth are some of the commonly consumed leafy vegetables in RSA. They are rich sources of both primary and secondary metabolites. However, their availability is dependent to the occurrence of rainfall since they mainly grow voluntarily as weeds (DAFF, 2010). The production of Amaranth leafy vegetables in RSA is based on only small-scale farming, which results in lower yields owing to lack of irrigation water and weed competition (DAFF, 2010. Therefore, with such evidence leading to challenges related to food insecurity and malnutrition most likely in poor rural populations. The strategy for minimising weed competition and improved yields in Amaranth production includeshydroponic system (Maboko and Duplooy, 2011), yet there is still a lack of significant commercial production of Amaranth species in RSA.

There is a lack of scientific information on the optimum or enhanced production systems for cultivation of Amaranth species in African countries, including commercialization and consumption patterns (Grubben, 2004). Amaranth is not

usually planted in RSA but occurs as a volunteer crop after the first rains; it is harvested from the wild (cultivation fields) (DAFF, 2010). The cultivation of this plant is not varying extensively in RSA, the main reason for cultivation being for household food security and replenishment of the seed bank. The production levels of Amaranth are not known (DAFF, 2010). However, recent research indicates that under cultivated conditions, Amaranth produces fresh leaf yields of up to 40 t/ha. The yield of grain Amaranth is highly variable with 1 000 kg/ha considered a good yield (DAFF, 2010).

Agriculture is both a victim and a cause of water scarcity. Climate change and unsustainable agricultural water use practices threatens the sustainability of livelihoods dependent on water and agriculture (Ghosh *et al*., 2022). Fresh water is becoming the scarcest most critical agricultural commodity nationally and internationally (Ferreira, 2013), necessitating the need to introduce a growing technique that utilizes less water at small-scale and commercial level. A need to investigate other innovative strategies for Amaranth production on maximised yields and metabolites is necessitated by recent changes in climate which leads to water scarcity and soil nutrient losses, and arapidly growing global population which needs to be fed. Growing of Amaranth on an OMET system can be a promising tool for improved yield. However, since this technique is a newly developed idea, there is a lack of information on the actual rates of yield and metabolite accumulation on Amaranth species grown under this OMET system. Freshwater is becoming the most scarcely critical agricultural commodity worldwide (Ferreira, 2013), thus necessitating the need to introduce a water-use efficient planting technique such as OMET system for both small-scale and commercial farming.

1.3 Rationale

The burden of malnutrition and food insecurity in Africa calls for deeper exploration of underutilized species which are rich in nutrients and have the potential to reduce food and nutrition insecurity (Aderibigbe *et al*., 2020). The common staple crops are not able to meet daily requirements for both macro- and micro-nutrients. To lessen this burden; protein, calorie and micronutrient deficiencies must be properly addressed for optimal growth and development to be attained (Aderibigbe *et al*., 2020). African indigenous underutilized vegetables can play a significant role in food security of

vulnerable groups like under-five children and women in both urban and rural settings. The potential of Amaranth in meeting the nutrition needs of humans has thus remained a subject of interest and scientific research (Aderibigbe *et al*., 2020).

Amaranth species are significantly highly nutritious, cheap, and easy to produce and adapt easily to local environmental conditions (Wambugu and Muthamia, 2009). The protein score, defined by WHO as a measure of protein quality, of Amaranth is 74, by comparison, wheat is scored 47, soya bean 68–89, rice 69 and maize 35 (AURI, 2003). Amaranth protein is high in the amino acid lysine, which is the key component found in insufficient amounts in staple such as maize, wheat, rice and other cereals, and the sulphur-containing amino acids, which are normally limited in legumes, thus making Amaranth ideal for supporting human dietary needs (AURI, 2003). The high content of lysine, arginine and histidine makes Amaranth seeds useful as a dietary supplement for treating malnourished children. The high levels of calcium, iron, sodium, and vitamins support the intake of recommended daily levels of these micronutrients (AURI, 2003). Total protein and fat content per amount of dry matter is significantly higher in Amaranth than in wheat, maize, and sorghum. The overall nutritional value of Amaranth is regarded significantly higher than milk, soya bean, wheat, and maize (AURI, 2003).

According to Onyango (2010), Amaranth enhancement via research and development can help develop a simple and cost-effective way of eradicating malnutrition and improve human health as well as eliminating food insecurity, especially in vulnerable and disadvantaged communities. Amaranth species has the earliest maturity period in the pseudo-cereal class ranging between 6 to 10 weeks (Jacob, 2005). The cultivation of the Amaranth crop does not vary extensively in RSA, the main reason for cultivation being for household food security and replenishment of the seed bank (AURI, 2003). The production levels of Amaranth are not known. However, recent research indicates that under cultivated conditions, Amaranth produces fresh leaf yields of up to 40 t/ha. The yield of Amaranth is highly variable with 1 000 kg/ha, considered a good yield (DAFF, 2010). In RSA, Amaranth occurs voluntarily as a weed during rainy seasons and at some point, is cultivated in small scale farming whereby maximum production is limited due to high costs of cultivation practices such as weeding, soil amendments practices and irrigation programmes. The main producing areas of Amaranth in RSA are Limpopo, North West, Mpumalanga, and KwaZulu-Natal provinces (AURI, 2003).

Innovative strategies to conserve water and reduce production costs without compromising the nutritional, phytochemical and bio-chemical quality of indigenous leafy vegetables including Amaranth species should be investigated. OMET system is the non-drainable growing technique designed to prohibit water and nutrients loss through deep drainage and/or seepage. Since water is a scarce agricultural commodity, the sheet mulch used in the OMET system virtually eliminates evaporation, and dramatically reduces the crop water requirements (Ferreira, 2013). This system reduces the cost of cultivation practices such as weeding and fertilization. The underlying sheet of this growing technique eliminates the problem of soil nutrient loss and loss of soil water through drainage, seepage or deep percolation. Soil fertilization programmes are not necessary as the nutrients that feed the plants, naturally cycle back in the growing medium to feed the soil again (Ferreira, 2013).

1.4 Purpose of the study

1.4.1 Aim

To investigate the effectsof the OMET system on growth, yield, and metabolites of three Amaranth species namely *Amaranthus caudatus*, *Amaranthus cruentus* and an unidentified *Amaranthus* Spp.

1.4.2 Objectives

- To assess the impact of the OMET system on the growth attributes of three Amaranth species
- To investigate the effects of OMET system on the yield components of three Amaranth species
- To evaluate the influence of the OMET system on the nutrients and phytochemicals of three Amaranth species

1.5 Reliability, validity, and objectivity

For the presentstudy, reliability was based on using appropriate statistical analysis at significance level of 5% (P=0.05). The treatments were used to increase the range of validity. Validity was achieved through replication of treatments. Objectivity was achieved by discussing the findings based on empirical evidence as shown by

statistical analyses, with findings checked for similarities and differences with other findings in other studies, thereby eliminating all forms of subjectivity.

1.6 Bias

Biasness in this study was eliminated by random sampling. Replication was done to increase precision and validity of this research.

1.7 Scientific contribution

The findings of this investigation will aid the indigenous crop farmers to have a concrete scientific knowledge base and background regarding the degree of sustainability, effectiveness, and efficiency of the OMET system on production of Amaranth species and other potential indigenous food crops. Increased yield has the potential to significantly contribute to enhanced food and nutritional security among vulnerable communities. The phytochemicals found in Amaranth species have been reported to possesshealth benefits that can potentially enhance the human immune system. Some of them serve as antibiotics. The study will also potentially help farmers to familiarise themselves with a sustainable, organic, and environmentally friendly growing technique that allows less water use while producing more on a relatively small area.

1.8 Structure of the dissertation

The structure of this dissertation is similar to that of research papers or articles. Chapter 1 which is the introduction, outlines the background, research problem, the imopacts and causes of the problem. The proposed solutions for the research problem and ageneral focul of the study are also fully explained. The problem statement and rationally which include the aim and objectives are thoroughly outlined and finally the scientifiv contribution of the study.

Chapter 2 consists of a literature review which furnishes an overview of the published, available data on the issues relevant to this topic. These include the growth habits and yield rates of Amaranth species and the importance of primary and secondary metabolites found in Amaranth species studied. Furthermore, the role of the

indigenous vegetable, Amaranth in reducing food and nutrition insecurity as well as their value as a therapeutic source among the rural population.

Chapter 3 consists of the detailed materials and methods employed to carry out the growth and yield attributes and statistical analysis. The results, discussion, recommendation and conclusion regarding the growth and yield attributes are all recorded in this chapter.

Chapter 4 comprise of the detailed materials and methods employed to carry out the primary metabolites' experiments. The results, discussions, recommendations, and conclusion for primary metabolites are all recorded in this chapter.

Chapter 5 include the materials and methods employed to conduct secondary metabolites' experiments. The methods of analysis are all outlined. The results, discussions, recommendations, and conclusion for secondary metabolites are all outlined and recorded in this chapter.

In text reference cited in every chapter are acknowledged at the end of that chapter, following the Harvard style of author-alphabet as approved by the Senate of UL.

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CHAPTER 2: LITERATURE REVIEW

Abstract: Amaranth species are commonly consumed indigenous vegetables in South Africa. Some species are locally sold at informal markets while some are harvested from cultivated lands during rainy seasons. There are approximately 60 species of Amaranthus including *A. thunbergii*, *A. greazicans*, *A. caudatus*, *A. tricolor*, *A. arusha*, *A. cruentus*, *A. spinosus*, *A. deflexus*, *A. hypochondriacus*, *A. viridus* and *A. hybridus*, which are commonly consumed. Other species of Amaranthus remains obscured and indigenised within local communities, because of lack of research and scientific documentation. The objectives of this study were to 1) Investigate overlooked Amaranth species that have potential for improved productivity and strategies used to improve the productivity of Amaranth species. 2) To create a comprehensive documentation about nutritional and phytochemical composition in different Amaranthus species and their response to different cultural practices. Fertiliser application, irrigation scheduling and time of planting have been studied extensively and were demonstrated to affect nutrition and phytochemical composition in different species of Amaranth. These cultural practices are critical components affecting growth and yield in amaranth production.

2.1 Potential and benefits of African Leafy Vegetables (ALVs)

African leafy vegetables refer to a group of plant species used as leafy vegetables. In South Africa, the Xhosa speaking people call them imifino and Pedi and Sotho speaking people call them morogo (Van Rensburg *et al*., 2007). ALVs form part of the daily staple diet of millions of South African households, especially in rural areas and are rich in nutrients such as vitamin A and iron (Faber and Wenhold, 2007; Uusiku *et al*., 2010). The young, succulent stems, flowers and very young fruit, are consumed as vegetables. Although neglected and underutilised in South Africa, indigenous vegetables can diversify farming systems, ensure food security and help to alleviate poverty, whilst increasing income and improving human health (Kahane *et al*., 2013). Indigenous vegetables are tolerant of harsh environmental conditions, having already adapted to local Southern African conditions (as most grow wild) and

are therefore suited to non-irrigated production (Modi and Mabhaudhi, 2013). In addition, these crops are also part of the region's cultural heritage.

Amaranth, spider plant (*Cleome gynandra*), *Corchorus spp*., Nightshade (*Solanum retroflexum*), Pumpkins and bitter melon (*Cucumis* spp.), Cowpea (*Vigna unguiculata*) and Taro (*Colocasia esculenta*) are some of the common indigenous vegetables (Louw, 2022). *Amaranth spp., Corchorus* and *C. gynandra* are traditional leafy vegetable crops with high nutritional value. They are also known to contain high levels of calcium, iron and vitamins A and C. Amaranth and *Corchorus* are also rich in protein and fibre (Louw, 2022). Although these crops are low management crops and can grow in poor soils, research shows that yield can be increased by adding fertilizer or enriching the soil with compost (Louw, 2022).

2.2 Morphology of various Amaranth species

2.2.1 Amaranthus caudatus L.

Amaranthus caudatus L. is a bushy, erect annual (PFAF, 2019) or biennial (RHS, 2021) plant that is generally believed to have originated from Central to South America. *A. caudatus* also grows best in full sun but can tolerate a variety of conditions, both humid and arid. It rarely occurs as an escape from cultivation, persisting near the places of cultivation (FNAEC, 2016). It is an annual herb, erect to 1.5 m tall, commonly reddish, or purple throughout, stems and leaves are less or not hairy, long-petiolate, lamina broadly ovate to rhomboid-ovate (Figure 2.1). It has a 2.5-15 cm long, 1-8 cm broad, obtuse to subacute at the mucronulate apex, shortly cuneate at base, flowers in axillary or terminal red or green spikes formed of cymose clusters, the terminal inflorescence often tail-like, pendulous spike to 30 cm or more long; bracts and bracteoles deltoid-ovate, acuminate, with a rigid arista; perianth segments 5; capsule 2-2.5 mm long, ovoid-globose, circumscissile; seeds black, shiny (Table 2.2; Figure 2.1) (Liogier, 1985).

Figure 2.1: Example of *Amaranthus caudatus* plant (*gobotany.newngland.org*, 2015)

Accessed: 22 March 2022

2.2.2 *Amaranthus cruentus* L.

Amaranthus cruentus L. is an annual herbaceous plant originating from Central America and cultivated since ancient times for its grain (PFAF, 2019). Introduced to many countries, it is now widespread and naturalized in many parts of the world. Plants are not hairy or slightly hairy, especially when young (FNAEC, 2016). Stems are erect, green or reddish purple, branched distally, 0.4-2 m (Figure 2.2). Leaves have petiole 1/2 as long as to ± equalling blade; blade rhombic-ovate or ovate to broadly lanceolate, 3-20 × 1.5-15 cm, occasionally larger in robust plants, base cuneate to broadly cuneate, margins entire, plane, apex acute or sub-obtuse to slightly emarginate, with macro inflorescences terminal and axillary, erect, reflexed, or nodding, usually dark red, purple, or deep beet-red, less commonly almost green, or greenish red, leafless at least distally, large and robust (FNAEC, 2016) (Table 2.2; Figure 2.2). Bracts narrowly spathulate, 2-3 mm, equalling or slightly longer than tepals, apex short-spinescent. Pistillate flowers: tepals 5, oblong to lanceolate, not clawed, equal or subequal, 1.5-3 mm, apex acute; style branches erect or slightly reflexed; stigmas 3. Staminate flowers at tips of inflorescences; tepals 5; stamens (4-5) (FNAEC, 2016) (Figure 2.2). Utricles are obovoid to elongate-obovoid, 2-2.5 mm, smooth or slightly rugose distally, dehiscence regularly circumscissile. Seeds are usually white or ivory, with reddish or yellowish tint, sometimes dark brown to dark reddish brown, broadly

lenticular to elliptic-lenticular, 1.2-1.6 mm diameter, smooth or indistinctly punctate (FNAEC, 2016).

Figure 2.2: Example of *Amaranthus cruentus* plant (*gobotany.newngland.org,* 2012)

Accessed: 22 March 2022

Table 2.1: Morphological differences between Amaranth species

Plant species	**Colour of stem**	**Colour of leaves**	**Colour of the root**	**Colour of inflorescence**	**Shape of leave**	**Presence of spines**	**Arrangement of leave on stem**	**Seed colour**	**Reference**
A. hybridus	Green; Purple/Green	Green	Brown	Green	Ovate	Absent	Alternate	Cream	Alege and Daudu, 2014
A. caudatus	Purple/Green; Purple & green Pink base & green mid-stem to tip	Purple; Purple & green	Purple	Purple Green	Ovate; ovate to rhomboid	Absent	Alternate	Cream/ black/Pink; Reddish to dark brown	Alege and Daudu, 2014
A. viridis	Green	Green	Brown	Green	Obovate	Absent	Alternate	ND	Alege and Daudu, 2014
A. spinosus	Green	Green	Brown	Green	Obovate	Present	Alternate	ND	
A. dubius	Purple	Green	Purple	Green	Obovate	Absent	Alternate	ND	
A. cruentus	Green	Green/purple	ND	Green/Pink	ND	ND	ND	Yellowish white to	Grubben, 2004a

								pale brown	
A. hybridus	Pink	Green; Pink reddish	ND	Green, wine	ND	ND	ND	Cream, Black, cream, and black	Akin-Idowu *et al*. 2016
A. hypochondriacus	Green/Pink	ND	ND	Green/wine	Ovate to rhomboid	ND	ND	ND	

ND= Not determined

2.3 Economic benefits of indigenous vegetables

Amaranth seeds yield was about 450–700 kg/ha under der-land cultivation, while under irrigated or high rainfall production the yield ranged from 900 to 2000 kg/ha in East Africa (AURI, 2003). Due to low production inputs in Africa, the maximum yield obtained was 3000 kg/ha, while the values increased in Mexico and Peru (7000 kg/ha). Therefore, including a wide range of varieties for selection and the use of good agronomic practices contributes to achieving higher yields (AURI, 2003). The large variability reported for Amaranth seed yield in different parts of the world, genetic diversity, and phenotypic plasticity, offer opportunities for further research and more targeted selection of regionally suitable cultivars (Alemayehu *et al*., 2015). In 1996, the value of the indigenous vegetable production in Cameroon was estimated to be US$ 22 million (Gockowski *et al*., 2003). This value is expected to grow because most urban dwellers are of rural origin and prefer to consume traditional food (Gockowski *et al*., 2003). In Africa, and RSA in particular, Amaranth is not usually cultivated but it occurs voluntarily during summer rainfall, and the yield rates are unknown (DAFF, 2010). Although Amaranth is placed among the five most important vegetables, it is usually grown by small holder farmers (Schippers, 2000). The high yield reported in Mexico and Peru is most likely due to Amaranth production having been more common in that region and local varieties that already have undergone a selection process for improved seed yield, which has not been carried out to the same degree in Africa (Brenner *et al*., 2000; Gimplinger *et al*., 2008). However, both growth attribute and biomass are subjected to variation based on the Amaranth species, fertilisation, irrigation regimes, and sowing date (Brenner *et al*., 2000; Gimplinger *et al*., 2008).

2.4 Growth attributes of Amaranth species

In the production perspective, growth is measured based on primary objective indices including the leaf length, root length, plant height, stem diameter and, number of leaves per plant (Poorter *et al*., 2012). These growth parameters are often associated with the yield components measured as biomass and dry mass (Sinclair and Muchow, 2001). In fact, these growth attributes play a key role in the absorption and transportation of water and nutrients from the ground to various parts of the plant (Sinclair and Muchow, 2001). Improved leaf length or surfaces are generally associated with improved gaseous exchange and photosynthesis rates which

ultimately improves plant biomass and yield (Yan *et al*., 2021). Similarly, thick long stem diameter and roots are associated with effective transportation of water and nutrients from the roots to the leaves, leading to well-nourished plants (Yan *et al*., 2021).

2.5 Application of fertiliser and biostimulant impact on yield of Amaranth species

Application of both organic and inorganic fertilizers at an optimum quantity promotes growth and yield in Amaranth species (Oyedeji *et al*., 2014). Inorganic nitrogen (N) fertilizer application at 15, 30, 45 and 60 kg ha dose, increased vegetative yield in an Amaranth species known as 'grain Amaranth (Onyango *et al*., 2012). Furthermore, application of 150 N kg/ha demonstrated an increased biomass yield (Iqbal *et al*., 2014). In fact, the plant height, stem length, leaves per plant and fresh or dried mass increased with increasing N doseage (Onyango *et al*., 2012). Similar results were observed when higher concentrations (50 and 100 kg/ha) of N fertiliser were applied in *A. hypochondriacus* and *A. cruentus* (Matshona *et al*., 2016). On the other hand, a combination of Maize-Stover (3.0 t ha) with N fertilizer (30 kg N/ha) demonstrated an improved yield and vegetative growth including plant height, stem diameter, leaves per plant, aerial and dried mass in *A. cruentus* species (Matshona *et al*., 2016). These improvements in yield and plant growth were in association with improved N and phosphorus uptake by the roots (Sinclair, and Vadez, 2002).

In addition, application of zinc fertiliser (200 mg/kg) improved the length of roots and shoots of red Amaranth species (Fageria *et al*., 2002). However, further increase of zinc fertiliser atrates of 300, and 400 proved to retard growth in Amaranth red species (Martens, and Westermann, 1991; Fageria *et al*., 2002). A combination of an organic (vermicompost: 2.5; 5; 10 $t.ha^{-1}$) and Inorganic (NPK: 50%) fertiliser was optimal in improving growth and yield in red Amaranth species (Alam *et al*., 2007). In a study aimed at evaluating organic manure and inorganic fertiliser (diammonium phosphate) effect on growth and yield attributes, plants supplied with organic manure produced lowest yields in comparison to those fed by diammonium phosphate (Onyango *et al*., 2012).

Biostimulant with biologically active compounds such as seaweed or silicon extracts are known to enhance crop yield and quality and can improve the plants defence

mechanism against abiotic stress such as salinity or water deficit (Abdelgawad *et al*., 2018; Cozzolino *et al*., 2020; Hidalgo-Santiago *et al*., 2021). Furthermore, Ngoroyemoto *et al*. (2019), reported that biostimulant improved growth of *A. hybridus* by altering the fresh and dried shoot/root ratio, leaf number per plant, leaf area and stem diameter, except in Kelpack® and Eckol® (Khandaker *et al*., 2011). Furthermore, application of salicylic acid at 10^{-5} m demonstrated the highest yield and plant growth (plant height, stem length, leaf length, number of leaves, root length and fresh or dried biomass) in *A. tricolor* (Khandaker *et al*., 2011).

2.6 Effect of planting date on biological yield of Amaranth species

Planting date is a critical component in the production of Amaranth because it informs the day and night temperatures, day length and overall climate under which crop growth will be taking place (Chepkoech *et al*., 2018). Although Amaranth is a highly adaptable crop, planting date still plays a significant role in the overall growth of Amaranth crops (Yarnia *et al*., 2010). Research studies demonstrated that Amaranth sown in early December (summer) in SA exhibit higher plant height, number of inflorescences and number of seed per plant, which resulted in higher yield when compared to those sown in April and July (winter) in SA (Yarnia *et al*., 2010).

2.7 Effect of irrigation regimes and water quality on yield of Amaranth

Although *Amaranthus* species are regarded as drought tolerant, different water regimes affects yield (Ribeiro *et al*., 2017). For instance, a water deficit of 25 and 50% led to reduced leaf area and relative water content which contributed to an overall reduction of biomass in *A. tricolor* (Ribeiro *et al*., 2017). In fact, yield components including straw, panicle and length of the internode in *A. tricolor* decreased with water deficit (Ribeiro *et al*., 2017). On the other hand, biological yield was not affected by different irrigation regimes in *A. tricolor* (Pulvento *et al*., 2021). When a comparison for *Amaranthus* species; *A. viridis* and *retroflexus* was made over deficit irrigation rates (25, 50, 75 and 100%), results showed no difference in growth attributes (Chen *et al*., 2022). There was a similar trend in the reduction of growth attributes (shoot: root biomass ratio) in response to increasing water deficitin *Amaranthus* species; *viridis* and *retroflexus* (Chen *et al*., 2022). Moreover, deficit irrigation has been associated

with reduced height, stem diameter, and the number of leaves in *A. retroflexus* (Weller *et al*., 2021). However, the quality of water influenced grain yield (Pulvento *et al*., 2021). Therefore, biological yield was reduced due to reduced photosynthetic rate. Furthermore, *A tricolor* showed to be more sensitive to salinity as compared to the *A. cruentus* and *A. hypochondriacus* (Sarker and Oba, 2019; Hoang *et al*., 2020). However, a lower concentration of salts (NaCl) (30 mmol/L) improved growth of *A. cruentus* (Luyckx *et al*., 2021).

2.8 Nutritional composition of vegetable crops and human health

Plant nutrition is the study of elements and compounds necessary for plant growth, metabolism, and external supply. A plant cannot complete its life cycle in their absence. Nutrients are the components found in our food such as carbohydrates, vitamins, minerals, fats, among others. These components are necessary for good health and survival.

2.8.1 Carbohydrates

Carbohydrates are biomolecules made up of three elements: carbon, hydrogen, and oxygen. The dietary carbohydrates are a diverse group of substances with a range of chemical, physical and physiological properties. These properties have implications for our overall health (Cummings and Stephen, 2007). The general empirical formula of carbohydrate is $C_x(H_2O)_y$. They are the rapid energy source for our body and hence, are one of the essential food nutrients (Cummings and Stephen, 2007). Carbohydrates are one of the three macronutrients that the body needs (Sahay, 2022). Carbohydrates come in three primary categories: starches, fibre, and sugars. Complex carbohydrates are frequently used to describe starches. They can be found in grains, legumes, and starchy vegetables like corn and potatoes (Sahay, 2022).

In brief, carbohydrates are a group of organic substances that are the most abundant biomolecules on earth, with a wide range of physical and physiological properties and lots of health benefits (Ramesh and Tharanathan, 2003; Sahay, 2022). The main function of carbohydrates is to provide energy. However, they also

play an essential role in the structure and function of cells, tissues, organs, and metabolism (Vanderhoof 1998). They are also used in making drugs for treating diseases (Sahay, 2022). Nowadays, synthetic chemicals resembling natural carbohydrates are replaced to meet their demand and avoid diet issues related to calories (Wawrzyńska and Sirko, 2014; Sahay, 2022). Food rich in carbohydrates should be included in the human diet, such as bread, fruits, vegetables, among others, that act as fuel for thebody (Sahay, 2022).

A chemical approach classifies carbohydrates into three main groups, sugars (DP1–2), oligosaccharides (short-chain carbohydrates) (DP3–9) and polysaccharides (DP≥10). Sugars comprise (i) monosaccharides, (ii) disaccharides and (iii) polyols (sugar alcohols) (Cummings *et al*., 1997; FAO, 1998). Oligosaccharides are either (a) malto-oligosaccharides (α-glucans), principally occurring from the hydrolysis of starch and (b) non-α-glucan such as raffinose and stachyose (α galactosides), fructo- and galacto-oligosaccharides or other oligosaccharides (Table 2.1) (Cummings *et al*., 1997; FAO, 1998). Polysaccharides may be divided into starch (α-1:4 and 1:6 glucans) and non-starch polysaccharides (NSPs) (Table 2.1), of which the major components are the polysaccharides of the plant cell wall such as cellulose, hemicellulose and pectin. Some carbohydrates, like inulin, do not fit neatly into this scheme because they exist in nature in multiple molecular forms (Cummings *et al*., 1997; FAO, 1998).

2.8.2 Protein

Proteins are biological macromolecules of major importance, both quantitatively and qualitatively, in all living organisms (Cozzone, 2002). They are constituted from basic units, called amino acids, which are covalently linked together to form the primary structure. The amino acid sequences determine the higher structural levels of proteins (secondary, tertiary, and quaternary) and specify their biological properties (Cozzone, 2002). Proteins are important biological polymers formed from building blocks called amino acids. The three-dimensional structure and biological activity of proteins depend on the physicochemical properties of their constituent amino acids (Cozzone, 2002).

Proteins are classified into four levels of structures namely: Primary, secondary, tertiary and quaternary structure. The primary level of structure refers to the linear sequence of amino acids along a protein chain and to the location of covalent bonds, namely disulfide bonds, between chains or within a chain. The primary structure identifies a protein unambiguously, determines its chemical and biological characteristics, and specifies the higher levels of protein structure (Cozzone, 2002; Sahay, 2022).

In principle, a polypeptide chain could assume great flexibility owing to the free rotation of the atoms around different bonds along the chain (Cozzone, 2002). If it did so, it would behave like a random coil and could theoretically adopt a myriad of conformations of similar energies (Cozzone, 2002). In fact, in biological conditions, each protein adopts essentially only one conformation because the sidechains of its amino acid residues associate locally with one another and with the solvent to yield a global structure of maximum stability (Cozzone, 2002). The correct folding of proteins sometimes requires the assistance of a particular class of cellular proteins called molecular chaperones. Several folding patterns occur repeatedly in parts of protein molecules. They are known collectively as secondary structure, which constitutes the next level of protein structure after the primary structure (Cozzone, 2002; Sahay, 2022). These regular arrangements of the linear polypeptide chains with repeating values of the F and c torsions angles and main-chain hydrogen bonding are of two major types: a helices, with repeating patterns of local hydrogen bonding, and b sheets, with repeating patterns between distant parts of the polypeptide chain (Cozzone, 2002). *A. caudatus*

The tertiary level of structure refers to the spatial arrangement of a polypeptide chain through folding and coiling to produce a compact globular shape. For some proteins the participation of molecular chaperones is required (Cozzone, 2002). The tertiary structure is essentially determined by the packing of the secondary structures, a helices and b sheets, which combine to form one or several units called ‘domains (Cozzone, 2002; Koprivova and Kopriva, 2014). These combinations are limited in number, and some of them are especially frequent in proteins. They represent the fundamental elements of globular polypeptide chains in terms of three-dimensional structure as well as in terms of function. On average, a single domain consists of 100–150 amino acid residues, corresponding to a globule of about 25 A˚ diameter. Some

domains can be isolated as fragments by limited proteolytic cleavage of the linking peptide chain (Cozzone, 2002). Such fragments keep the original conformation they have in the native protein; they are stable and can fold/refold like autonomous structures (Cozzone, 2002).

Quaternary Structure Many proteins are made up from two or more polypeptide chains, called subunits or monomers, which may have identical or different amino acid sequences (Cozzone, 2002; Koprivova and Kopriva, 2014). Such polypeptide aggregation, which represents the quaternary structure, is generally of critical importance to the proper functioning of these oligomeric proteins. Each subunit is usually folded independently, then interacts with the other subunits because they display complementary surfaces as far as shapes and physical interactions are concerned (Cozzone, 2002; Wawrzyńska and Sirko, 2014).

2.8.3 Macronutrients and micronutrients

Plant growth and development are largely determined by nutrient availability; therefore, to ensure better productivity of crop plants, it becomes essential to understand the dynamics of nutrient uptake, transport, assimilation, and their biological interactions (Wawrzyńska and Sirko, 2014). Although a large number of elements are naturally available in the soil, 17 elements are currently known to be important for the proper growth and development of crop plants. While nitrogen (N), phosphorus (P), potassium (K), calcium, sulphur (S), and magnesium are known as macro-nutrients (required in comparatively larger amounts), Iron (Fe), Zinc (Zn), Copper, Boron, Manganese Molybdenum, Chloride, and others are the micro-nutrients (required in a smaller quantity) for the growth and development of crop plants (Kumar, 2013). Nitrogen is one of the nutrients essentially required for the vegetative growth of crop plants as it is needed for the synthesis of starch in leaf, production of amino acids for protein synthesis, and thus yield of the crop. P is an essential constituent of nucleic acids, cellular membranes, and enzymes. It is needed for diverse cellular processes like photosynthesis, carbohydrate metabolism, energy production, redox-homeostasis, and signalling. P works as an activator for more than 60 enzymes in plants, regulates water content, and reduces the adverse effects of salts in plants. Similarly, sulfur is essentially required for the synthesis of amino acids like cysteine

and methionine, as a cofactor/prosthetic group in Fe-S centre, thiamine, S-adenosyl methionine, and in several primary and secondary metabolites (Wirtz and Hell, 2006; Khan *et al*., 2010; Koprivova and Kopriva, 2014).

The micro-nutrients like Fe and Zn play very important roles in the physiological processes of crop plants; however, they are required in very little amounts. Fe is required for chlorophyll synthesis and maintenance of chloroplast structure and functions. It is generally present in higher quantities in the soil, but its bioavailability becomes limited in aerobic and neutral pH environments (Colombo *et al*., 2014). In aerobic soils, Fe is found predominantly in the Fe^{+3} form, with extremely low solubility, which does not fulfil the plant's iron requirement. Hence, Fe-deficiency becomes a common nutritional disorder in many crop plants, resulting in interveinal-chlorosis in young leaves, stunted root growth, poor yield, and reduced nutritional quality. Similarly, Zn is required for optimum plant growth, as it influences several biological processes including cell proliferation, carbohydrate metabolism, and P-Zn interactions (Rehman *et al*., 2012). Zn is the only metal required for all the six classes (hydrolases, oxidoreductases, lyases, transferases, ligases, and isomerases) of enzymes (Coleman, 1998). Although it plays a structural role in some of the regulatory proteins (Berg and Shi, 1996), its higher concentration is toxic for the cell (Sresty and Rao, 1999; Xu *et al*., 2013). Zn deficiency in a plant result in deformed chlorotic leaves, interveinal necrosis, decreased photosynthesis, and reduced biomass production leading to reduced plant growth, lesser yield, and poor nutritional quality of the produce (Zhao and Wu, 2017).

2.8.4 Minerals

Minerals are naturally occurring inorganic substances with a definite chemical composition and an ordered atomic arrangement. Among plants, vegetables are excellent sources of minerals and contribute to RDA of essential nutrients (Sonni, 2002). Minerals are very important ingredients for normal metabolic activities of body tissues. Out of 92 naturally occurring minerals 25 are present in living organisms (Sonni, 2002). They are constituents of bones, teeth, blood, muscles, hair, and nerve cells (Sonni, 2002). Minerals are important for vital body functions such as acid-base and water balance. Calcium is one of the largest minerals present in the structure of

the body and in bones. Na and K are used as electron carriers in the body, and iron (Fe) is an important constituent of Haemoglobin. Vegetables contribute these minerals and enhance their availability in daily life (Omale and Ugwu, 2011).

2.9 Nutritional composition in *Amaranthus* species

Amaranth consumption is mostly on the fresh leaves in African. As a result, the nutritional characteristics of *Amaranthus* leaves have been wildly reported to contain high levels of both macro and micronutrients, essential for a well-balanced diet (Sarkar *et al*., 2022).

For example, Sarker and Oba (2019) reported leaves of different *A. cruentus* accessions to be rich in dietary fibre, protein, fat, carbohydrates, energy, and ash. In a different study, the protein compositions of varying *Amaranthus* species were found to be unique from other indigenous leafy vegetables (Ajayi *et al*., 2018). This was further confirmed by Martinez-Lopez *et al*. (2020), who reported the protein quality score of Amaranth to be greater (74%), than that of wheat (47%), soybean (68–89%), rice (69%) and maize (35%) (Table 2.3). Amaranth protein is high in the amino acid lysine, which is the key component lacking in maize, wheat, rice, and other cereals (Soriano-García and Aguirre-Díaz, 2019). In fact, Amaranth has sulphur-containing amino acids, which are normally limited in other protein rich crops such as grain legumes, thus making Amaranth ideal for supporting human dietary needs (Jensen *et al*., 2019). The high content of lysine, arginine and histidine makes Amaranth useful as a dietary supplement for treating malnutrition in children (Gorinstein *et al*., 2002). Moreover, calcium, iron, sodium, and vitamins which are also found in high quantities in Amaranth species help meet the daily recommendation (Table 2.1) (Gogo *et al*., 2017; Manyelo *et al*., 2020).

Table 2.2: Comparison of the mineral composition of five (5) selected leafy vegetables

Species	Macro-elements (mg/g FW)				Micro-elements (mg/g FW)			
	Ca	**K**	**Mg**	**P**	**Cu**	**Fe**	**Mn**	**Zn**
A. hybridus	1.4-3.5	4.6-9.2	1.5-4.5	0.43-1.0	0.56-2.4	6.6-26	2.2-17	6.7-20
Spider flower (*Gynandropsis gynandra*)	1.1-2.3	3.4-7.3	0.76-1.5	0.97-1.4	1.3-2.5	12-25	3.9-6.3	9.3-16
Black nightshade (*Solanum scabrum)*	1.4	4.3-4.6	1.0-1.1	0.57-0.58	1.3	11-12	8.1-8.6	4.5-4.9
Spinach (*Spinacia oleracea)*	0.99	5.6	0.79	0.49	1.1	27	5.9	3.5-5.3
Kale (*Brassica oleracea)*	1.5	4.9	0.47	0.92	0.40	4.0-15	3.0	2.9-5.6

Source: Jimenez-Aguilar and Grusak, 2017

Fresh weight (FW), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn)

Table 2.3: Comparison of the nutritional composition of four (4) selected leafy vegetables

Primary metabolites	Amaranth	Spinach	Malabar spinach (*Basella*)	Chard
Ash	2.6	1.5	1.4	1.6
Protein	3.5	3.2	1.8	2.4
Fat	0.5	0.3	0.3	0.3
Starch	6.5	4.3	3.4	4.6
Fibre	1.3	0.6	0.7	0.8

Source: Saunders and Becker, 1983

Table 2.4: Comparison of the nutritional composition of maize, wheat, sorghum, and *A. hypochondriacus*

Primary metabolites (g)	Maize	Wheat	Sorghum	*A. hypochondriacus*
Ash	1.2	1.7	1.7	2.5
Protein	10	13.2	12.7	14.5
Fat	5.2	2.7	4	10.2
Starch	72.8	65.7	70.1	62.7
Dietary fibre	9.3	12.1	8.5	8.8

Source: Pederson *et al*., 1987

2.10 Amino acid composition of leafy vegetables

Amino acids are natural vegetable components. They contribute to the maintenance of overall vegetable quality and nutritional value. Amino acids are the building blocks of proteins in living organisms (Świder *et al*., 2019). They occur in vegetables in distinct forms, either in a free form or more commonly bound in proteins and non-protein

compounds (Glew *et al*., 1997). They contain single carboxylic acid within the amino group on the alpha carbon and are characterised by the ionisation and the position of the side group (Omoyeni *et al*., 2015). More than 500 amino acids have been found in nature. However, the human genetic code directly encodes only 20 amino acids which are classified as either essential or nonessential and further as acidic, aromatic, hydroxylic, aliphatic, and basic and sulphur containing (Setoyama *et al*., 2017). The essential amino acids [Isoleucine (ILe), Leucine (Leu), Valine (Val), Phenylalanine (Phe), Tryptophan (Try), Histidines (His) and Methionine (Met)] are those that cannot be synthesized by the human body and thus should be obtained from the diet, whereas nonessential amino acids [Alanine (Ala), Glycine (Gly), Proline (Pro), Aspatic acid (Asp-a), Aspartate (Asp), Glutamic acid (Glu-a), Arginine (Arg), Serine (Ser), Cysteine (Cys), Asparagine (Asn), Tyrosine (Tyr) and Glutamine (Gln)] can be synthesised by the body through normal a metabolism (Keutgen and Pawelzik, 2008).

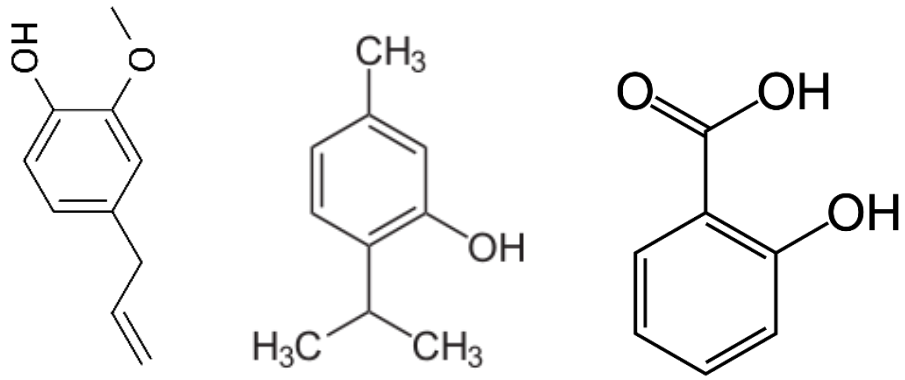
Amino acids are important compounds for vegetable taste and can be grouped into various classes of taste namely: tasteless (Arginine, Asparagine, Isoleucine, Threonine, Valine, Lysine and Serine), bitter (Leucine, Tyrosine, Phenylalanine and Tryptophan) and sweet (Proline and Alanine) (Breslin and Spector, 2008). The amino acids also possess various properties in the human body. Arginine contains a signalling molecule and possesses the anti-proliferation property (Mncwangi *et al*., 2012).

2.11 Phytochemical composition of leafy vegetables

Phytochemicals are non-nutritive secondary metabolites which own preventative and therapeutic actions against degenerative non-communicable diseases (Alamgir, 2017). In fact, the role of phytochemicals in human health is based on improving immunity which ultimately improves defence against an ‘offender’. However, the body acquires these phytochemicals through diets rich in fruits and vegetable. These phytochemicals play a similar role in plants, whereby they promote adaptability of crops to specific non-optimum growing conditions including low soil fertility, drought, and saline conditions (Alamgir, 2017).

2.11.1 Phenolic compounds

Phenols are compounds that have one or more hydroxyl groups attached to a benzene ring. Phenols 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 (Tungmunnithum *et al*., 2018). More than 8000 phenolic compounds as naturally occurring substances from plants have been reported (Kumar and Pandey, 2013; Ahmed *et al*., 2016). It is very interesting to note that half of these phenolic compounds are flavonoids presenting as aglycone, glycosides and methylated derivatives (Kumar and Pandey, 2013; Ahmed *et al*., 2016). Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action (Bendary *et al*., 2013). Phenolic compounds such as phenolic acids and flavonoids are reported to be involved in various biochemical activities such as antioxidant, antimicrobial, antithrombotic, antiatherogenic, anti-inflammatory, anticarcinogenic and antimutagenic (Alpinar *et al*., 2009). Furthermore, some of them stimulate the synthesis of endogenous antioxidant molecules in the cell (Côté *et al*., 2010). According to multiple reports in the literature, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden (Oberoi and Sandhu, 2015). Although many of the essential oils are terpenes, some are phenolic compounds, for example thymol from *Thymus* spp. (thyme) (Figure 21.1). Many simple phenols are responsible for taste, for example eugenol in cloves.



A **B** **C**

Figure 2.3: The structure of simple phenols, A- eugenol, B- thymol and C- salicylic acid

2.11.2 Flavonoids

Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Dixon *et al*., 1983). Their activities are structure dependent. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization (Heim *et al*., 2002). Flavonoids consist of a large group of polyphenolic compounds having a benzo-*γ*-pyrone structure and are ubiquitously present in plants. They are synthesized by phenylpropanoid pathways. Available reports tend to show that secondary metabolites of a phenolic nature including flavonoids are responsible for a variety of pharmacological activities (Mahomoodally *et al*., 2005; Pandey, 2007). They can be divided into a variety of classes such as flavones (e.g., flavone, apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin), and others. Their general structures are shown in Figure 2.4. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings (Middleton, 1998).

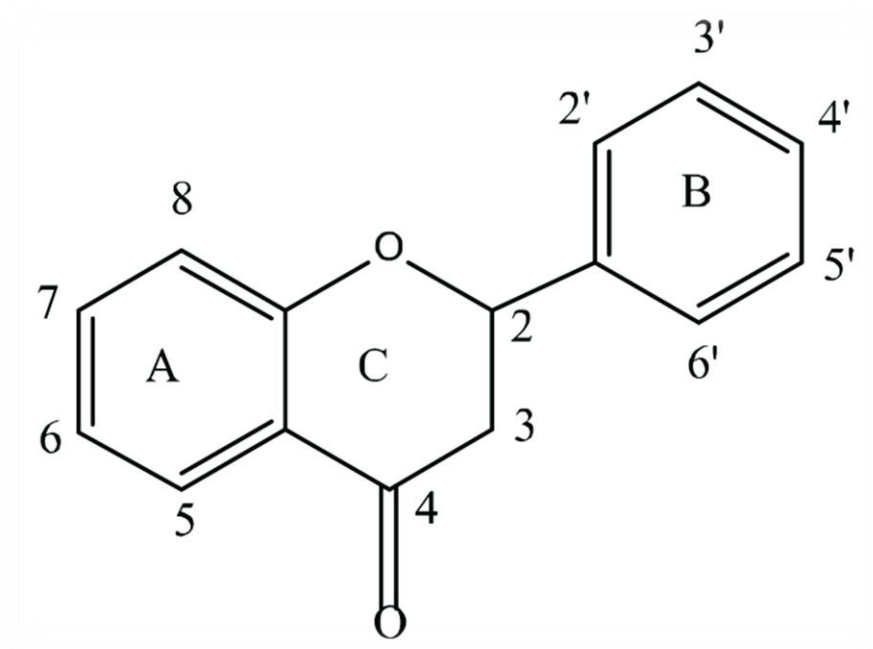


Figure 2.4: Basic structure of flavonoids with three flavone rings

Flavonoids occur as aglycones, glycosides, and methylated derivatives. The basic flavonoid structure is aglycone. Six-member ring condensed with the benzene ring is either a *α*-pyrone (flavonols and flavanones) or its dihydroderivative (flavonols and

flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavanones by a hydroxyl group at the 3-position and a C2–C3 double bond (Narayana *et al*., 2001). Flavonoids are often hydroxylated in positions 3, 5, 7, 2, 3′, 4′, and 5′. Methyl ethers and acetyl esters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose (Harborne, 2013).

2.11.3 Tannins

Tannins are complex and heterogeneous polyphenolic secondary metabolites, biosynthesized by higher plants, with molecular weights ranging from 500 to over 20,000 Da (Frutos *et al*., 2004). Vegetable tannins can be divided into three main groups: hydrolyzable, condensed, and complex tannins. The structure of the hydrolyzable tannins is built from the basic molecule, 1,2,3-trihydroxybenzene (pyrogallol) (Khanbabaee and van Ree, 2001). In the molecular structure of the hydrolyzable tanning agents, sugar molecules are bonded to pyrogallols. The hydrolyzable tannins are hydrolyzed by acids (or enzymes) into a sugar molecule or a related polyhydric alcohol and a phenolic carboxylic acid. Depending on the nature of this phenolic carboxylic acid, the hydrolyzable tannins (HTs) are usually subdivided into gallotannins and ellagitannins (Khanbabaee and van Ree, 2001; Frutos *et al*., 2004; Carşote *et al*., 2016). Condensed tannins (CTs) contain a group of polyhydroxyflavan-3-ol oligomers and polymers linked by C-C bonds between flavanol subunits (Schofield *et al*., 2001; Pasch *et al*., 2001). The mean degree polymerization (mDP) of CTs can vary widely, ranging from dimers to polymers of up to 30 or more subunits depending on the plant species (Barbehenn and Constable, 2011). Even within one genus, there can be dramatic variation in chain length. Tannins are among the most widely occurring secondary metabolites of plants, although they are not evenly distributed. They have been reported within the ferns, fern allies, gymnosperms, and many dicots and monocots plants (Mole, 1993). While the CTs can be found in all these groups, HTs are restricted to the dicot plants (Iwashina, 2000; Okuda *et al*., 2009).

2.11.4 Carotenoids

Carotenoids are a widespread group of naturally occurring fat-soluble colorants (Morganti, 2009). Carotenoids form a group of isoprenoid metabolites vital for life. They are tetra-terpene pigments, which exhibit yellow, orange, red and purple colours (Kebede *et al*., 2014). Carotenoids are an essential component of all photosynthetic organisms due to their eminent photo-protective and antioxidant properties (Khachatourians, 2017). Carotenoids also provide precursors for the biosynthesis of phyto-hormones abscisic acid (ABA) and strigo-lactones (SLs) (Teofanova *et al*. 2019). In addition, carotenoid derivatives can act as signalling molecules in response to environmental and developmental cues or serve as regulators of plant growth. However, their occurrence is not restricted to plants, algae, and cyanobacteria, as some fungi and non-photosynthetic bacteria can synthesize carotenoids as well, and many animals rely on food-borne carotenoids as visual pigments, antioxidants, or colorants (Britton *et al*., 2004). Most carotenoids consist of eight isoprene units with a 40-carbon skeleton. Their general structures commonly consist of a polyene chain with nine conjugated double bonds and an end group at both ends of the polyene chain (Britton *et al*., 2004).

Carotenoids are divided into two groups: carotenes and xanthophylls. Carotenes, such as *α*-carotene, *β*-carotene, *β*, *ψ*-carotene (*γ*-carotene), and lycopene, are hydrocarbons. About 50 kinds of carotenes are present in nature (Britton *et al*., 2004). On the other hand, xanthophylls, such as *β*-cryptoxanthin, lutein, zeaxanthin, astaxanthin, fucoxanthin, and peridinin, are carotenoids containing oxygen atoms as hydroxy, carbonyl, aldehyde, carboxylic, epoxide, and furanoxide groups in these molecules. Molecular compound structure of 6 various carotenoids is illustrated in Figure 2.5. Some xanthophylls are present as fatty acid esters, glycosides, sulfates, and protein complexes. Structures of xanthophylls show marked diversity. About 800 kinds of xanthophylls have been reported in nature up until 2018 (Britton *et al*., 2004; Maoka, 2009). Most carotenoids have 40-carbon skeleton (C40 carotenoid). Some carotenoids have a 45- or 50-carbon skeleton, which are called higher carotenoids. About 40 kinds of higher carotenoids are present in some species of archaea. On the other hand, carotenoids composed of carbon skeletons with fewer than 40 carbons are called apocarotenoids. About 120 kinds of apocarotenoids are present in some

species of plants and animals as degradation products of C40 carotenoids ((Britton *et al*., 2004; Maoka, 2009).

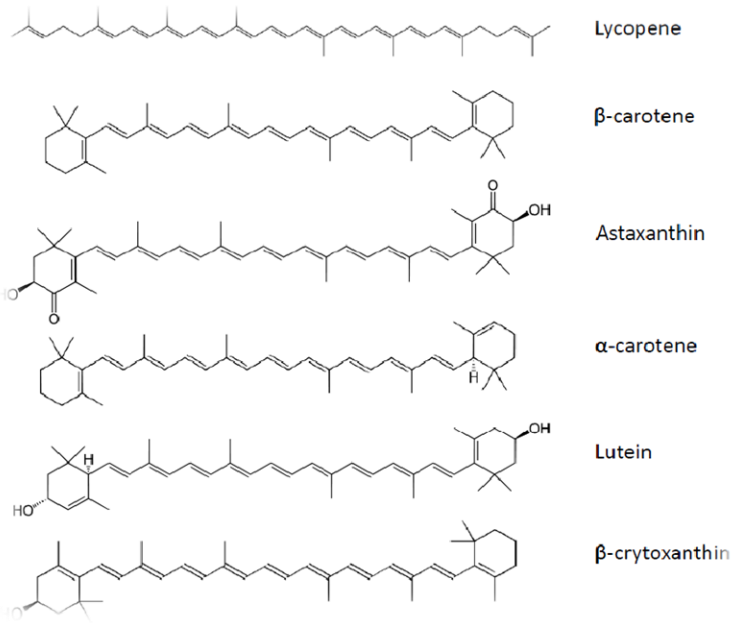


Figure 2.5: Molecular compound structure of six (6) various carotenoids

2.11.5 Chlorophyll

Green plants have the ability to make their own food called autotrophs. They do this through a process called photosynthesis, which uses a green pigment called chlorophyll (Chl) also referred to as the foundation of life in the “food webs” (Steele *et al*., 2008). Leaf chlorophyll content (Chl) is a key indicator of the physiological status of a plant (Steele *et al*., 2008). Both forms of chlorophyll, Chl a and Chl b, are essential pigments for the conversion of light energy to store chemical energy in land plants and the amount of solar radiation absorbed by a leaf is a function of the total photosynthetic pigment content (Curran *et al*., 1990; Filella *et al*., 1995). Thus, Chl content is linked directly to photosynthetic potential and primary production (Curran *et al*., 1990; Filella *et al*., 1995). In addition, chlorophyll gives an indirect estimation of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll pigments (Filella *et al*., 1995). Chlorophyll gives plants their green colour because it does not absorb the green wavelengths of white light. That particular light wavelength is reflected from the plant, so it appears green (Hörtensteiner and Kräutler, 2011). There are many different types of pigments in nature, but chlorophyll is unique in its ability to enable plants to

absorb the energy they need to build tissues (Hörtensteiner and Kräutler, 2011). Chlorophyll, however, because of its light-absorbing properties Chl is a dangerous molecule and a potential cellular phototoxin (Hörtensteiner and Kräutler, 2011). This is seen in situations where the photosynthetic apparatus of plants is overexcited, for example in high light conditions. Absorbed energy can then be transferred to oxygen, resulting in the production of reactive oxygen species (ROS). Likewise, inhibition of chlorophyll (Chl) biosynthesis or degradation can lead to ROS production and cell death. Because of this, metabolism of Chl is highly regulated during plant development (Hörtensteiner and Kräutler, 2011).

2.11.6 Antioxidants and Antinutritive compounds

An antioxidants isany molecule that can significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate (Halliwell, 2007). Antioxidants can be synthesized *in vivo* (e.g., reduced glutathione (GSH), superoxide dismutase (SOD), among others) or taken as dietary antioxidants (Sies, 1997; Halliwell, 2007). In vivo synthesis of antioxidants is a well-known chemical process that allows the removal of electrons or hydrogen from a substance (Rao, 2016). Free radicals are produced during the biological oxidation reaction. Because the radicals are reactive, they start the chain reaction simultaneously. This can lead to the damage or even the death of a cell (Rao, 2016). Hence, antioxidant agents are capable of terminating a chain reaction by eliminating free radical intermediates. They perform the antioxidant behaviour by being oxidized, hence antioxidants can be considered reducing agents. Some examples are ascorbic acid, thiols, or polyphenols (Rao, 2016). Relative antioxidant efficiencies vary markedly from one oxidizing lipid substrate to another. In the same lipid substrate, the relative activities of antioxidants often depend on the antioxidant concentrations (Frankel, 2014).

2.12 Phytochemical composition of Amaranth species

Phytochemical composition depends on the species, plant part and the growing conditions. Mateos-Maces *et al*. (2020) demonstrated different phytochemical composition in different Amaranth species including *A. cruentus, A. hypochondriacus, A. caudatus, A. hybridus* and *A. viridis*. Samples of *A. cruentus* were predominated by

total phenolic compounds (hydoxycinnamic and hydroxybenzoic acid) and flavonoids (Table 2.4) (Adebooye *et al.*, 2008; Li *et al*., 2015). There were traces of alkaloids, flavonoids, saponins, tannins, phenols, and hydrocyanic acid in *A. hybridus* (Akubugwo *et al*., 2007). There content of phenolic compounds (phenolic acid and flavonoids) and pigments chlorophyll and carotenoids compounds, were studied in *A. spinosus* and *A. viridis* leaves (Sarker and Oba, 2020) (Table 2.4). Furthermore, identification of phytochemicals such as vitamin C, and different phenolic compounds mainly ferulic, chlorogenic, caffeic, gallic, chlorogenic, vanillic, p-hydroxybenzoic, p-coumaric syringic acids, rutin, phloridzin, myricetin, quercetin; naringenin, phloretin, galangin and apigenin, have been detected in *A. tricolor, A. acanthochiton, A. deflexus A. viridis* and *A. hybridus* (Table 2.4) (Jiménez-Aguilar *et al*., 2017; Santiago-Saenz *et al*., 2018). In addition, the phytochemical compositions in species with red leaves was found to outperform those of species that possess a green colour. In fact, the contents for chlorophyll a, chlorophyll b, β-cyanins, total flavonoids, total phenols, β-carotene, and vitamin C were found higher in red Amaranth in comparison to those of green Amaranth (Sarker and Oba, 2019). Furthermore, these phytochemicals have been attributed to higher scavenging antioxidant activity in red Amaranth than in those of green Amaranth species (Table 2.4) (Sarker and Oba, 2019).

Table 2.5: The phytochemical composition of *A. hypochondriacus*

Phytochemical composition	**Amaranth species and plant parts**	**Reference**
Total phenolics (gallic acid), total flavonoids (catechin) and tannins; Betalain; Amaranthine; iso-Amaranthine; betanin; iso-betanin-gallic, protocatechuic, chlorogenic, gentistic, 2,4-dihydroxybenzoic, ferulic, ellagic and salicylic acids; rutin; kaempferol-3-rutinoside; and quercetin	*A. hypochondriacus, A. caudatus and A. cruentus*: leaves, stalks, flowers, sprouts, and seeds	Adebooye *et al*., 2008; Li *et al*., 2015
Alkaloids, flavonoids, saponins, tannins, phenols, hydrocyanic acid	*A. hybridus: leaves*	Akubugwo *et al*., 2007
Chlorophyll, total carotenoids, β-cyanin and β-xanthin content, β-carotene, vitamin C, total polyphenols, and total flavonoids	*A. spinosus and A. viridis:* leaves	Sarker *et al*., 2020
Vitamin C, total phenolics (gallic acid), flavonoids (catechin)	*A. acanthochiton, A. deflexus and A. viridis:* leaves	Jiménez-Aguilar *et al*., 2017
Chlorophyll; carotenoids; ferulic, chlorogenic, caffeic, gallic, chlorogenic, vanillic, p-hydroxybenzoic, p-coumaric and syringic acids; rutin; phloridzin; myricetin; quercetin; naringenin; phloretin; galangin; apigenin	*A. hybridus*, *A. tricolor:* leaves	Santiago-Saenz, *et al*., 2018; Sarker and Oba, 2020

Mateos-Maces *et al*., 2020

2.13 Application of metabolomics and chemo-metrics in crop production to elucidate cultural practice

Metabolomics is the study of low molecular weight molecules or metabolites found within cells and biological systems. The metabolome is a measure of the inputs and outputs of biological pathways and, as such, is often considered more representative of the functional state of a cell than other metabolomics measures such as genomics or proteomics (NIH, 2016). Plant metabolomics is the large-scale study of metabolites within plant tissues, contributing to the understanding of plant physiology and biology since metabolites reflect the endpoint of biological activities (Hall *et al*., 2008). Methodologies and instrumentations of plant metabolomics have been developing rapidly. A series of integrated technologies such, such as nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS) including gas chromatography-MS (GC-MS) and liquid chromatography-MS (LC-MS), and capillary electrophoresis-MS (CE-MS) and Fourier transform ion cyclotron resonance-MS (FI-ICR-MS) enable us to perform a comprehensive and unbiased analysis of the chemical inventory of a plant cell (An *et al*., 2018).

Metabolomics strategies can be divided into untargeted and targeted approaches. Untargeted metabolomics is a global analysis of all the measurable compounds in a sample whereas targeted metabolomics is the measurement of targeted groups of chemically characterized and biochemically annotated metabolites (An *et al*., 2018). Untargeted analysis can detect a novel metabolite and cover all the measurable analytes in a sample, while targeted metabolomics offers more accurate characteristics and quantitative analyses. Untargeted metabolomics allows for the investigation of metabolomics phenotypes involving up to thousands of metabolites in a single run (An *et al*., 2018). It enables an unbiased screening of all metabolites but results in more complex data steps. In contrary, plant targeted metabolomics provide a high-resolution tool for the identification and quantitative analysis of targeted metabolites in plants, measuring their responses to environmental and genetic changes in an unbiased and specific way (An *et al*., 2018).

Metabolomics is fast becoming a valuable tool in the area of food and agriculture and has emerged as a worthy technology for profiling crop varieties, enhancing the compositional quality of crops, examining metabolite accumulation during plant growth

and maturity, and to determining the metabolic response to diverse biotic and abiotic stresses (Table 2.6) by investigating the mechanism involved (An *et al*., 2018). The main objective of making use of agricultural biotechnology is to discover and utilize the synthesis of a product considered to be desirable, such as bio-products, and to minimize as much as possible the undesirable products such as agrochemicals (Beckles and Roessner, 2012).

Table 2.6: Application of metabolomics in seven (7) selected crops

Techniques	Crop	Findings	References
GC-MS	Strawberry	The results showed significant alterations in primary metabolites, which include sugars such as fructose and glucose, organic acids like malic and citric acid, and amino acids such as alanine, threonine, and aspartic acid. Sugars and amino acids showed the most exciting metabolites because they defined the organoleptic quality of the fruit. These primary metabolites helped in growth and development as a result of their participation in a broad array of physiological reactions and in resistance of the plant to biotic and abiotic environmental stress under different agronomic conditions.	Akhatou *et al*., 2016
GC-MS	Sunflower	A discovery of variations in carbon primary metabolism in the sunflower capitulum, a tissue which is the main entry point of *Sclerotinia sclerotiorum* infection thereby preventing negative impact on crop yield.	Peluffo *et al*., 2010
GC-MS	African eggplant	Proline, glutamate, sucrose, fructose, and tricarboxylic and cycle metabolites were discovered to have a correlation with drought stress. There were significant changes in the metabolites with respect to drought stress effects and tolerance	Mibei *et al*., 2018
GC-MS	Sunflower	NAC TFs, a candidate gene associated with senescence was identified, thus making us understand the mechanisms involved in leaf senescence in sunflower, which has far-reaching consequences on crop yield.	Moschen *et al*., 2016
NMR	Soybean	Soybean metabolome was examined with response to flooding stress. The research proved flooding affects both primary and secondary metabolism of soybean plant. Alanine,	Coutinho *et al*., 2018

		sucrose, citrate, acetate, and succinate accumulation were observed under flooding when compared to the leaves where these compounds were reduced.	
NMR	Mung beans	The research proved that NMR-based metabolomics is a crucial tool to identify the key metabolites in energy-regulated germination and sprouting processes of mung bean. This will help to actively check the developmental process and also enhance the quality of mung bean.	Chen *et al*., 2019
LC-MS	Maize	Metabolomic profiling revealed great differences in gene expression and hormonal patterns in the roots and leaves of maize. This explains that different organs employ distinct chemical defence systems to maize anthracnose caused by *Colletotrichum graminicola*, thus, helping in breeding for maize anthracnose.	Balmer *et al*., 2013

2.13.2 Chemo-metrics and its application in metabolomics and crop production

Chemo-metrics has been a fundamental discipline for the development of metabolomics, while symbiotically growing with it (Brereton, 2018). From design of experiments, through data processing, to data analysis, chemo-metrics tools are used to design, process, visualize, explore and analyse metabolomics data. Chemo-metrics is a discipline dedicated to chemistry, particularly to analytical chemistry, which is an area with a remarkable importance in science, interacting with other areas, such as biochemistry, organic chemistry, and physical chemistry (Koel and Kaljurand, 2019). Analytical chemistry is mainly focused on the development and implementation of methodologies that can provide quantitative and qualitative scientific information from analytical systems in the most efficiently way as possible (Koel and Kaljurand, 2019).

Analytical systems (i.e., analytical instrumentation employed in the detection or identification of chemical compounds in samples) are evolving constantly, fulfilling the ongoing demands from academic and professional analytical research (Shabir *et al*., 2007). Such developments in analytical instrumentation, however, tend to increase the complexity in the analytical data acquired from samples. In one hand, more scientific information can be obtained from such complicated analytical data v. On the other, such data may be too complicated to interpret by inadequate data analysis methods, and the potential of such instrumental approaches may be underused (Shabir *et al*., 2007).

Classical approaches in analytical chemistry, univariate or multivariate, tend to become less efficient in the analysis and interpretation of complicated datasets from evolving analytical instrumental systems (Brereton *et al*., 2018). Thus, up-to-date computational data analysis methodologies are sought, aiming to maximize the utility of novel instruments and the potential to obtain more scientific information from complex analytical systems. Chemo-metrics is dedicated to solving such problems arising from complicated analytical systems, combining statistics, mathematics, and computational programming languages (Otto, 2016). Large datasets with many variables (dimensions) tend to be complicated to interpret (Wold *et al*., 2001). Principal component analysis (PCA) is a technique that can reduce the dimensionality of data, according to correlations between variables, without a significant loss of statistical

information (Mishra *et al*., 2017). The application of PCA can facilitate the interpretation of complicated analytical data, e.g., by providing a visualization of the structure of the data, to detect outliers in data, or to assess the quality of sample replicates in data (Caicedo *et al*., 2017). PCA is considered one of the most important techniques in multivariate data analysis, and it constitutes the basis of numerous multivariate data analysis methods in several disciplines (chemistry, biology, geology, etc.) (Mishra *et al*., 2017).

2.14 Conclusion and recommendation

Amaranth species are one of the indigenous vegetable crops that contain significant amounts of primary and secondary metabolites with the potential of maintaining the daily dietary needs of humans as defines by the WHO. The accumulation and concentration of metabolites differs per species. Therefore, the OMET system offers a better growing environment to avoid such unfavourable growing conditions.

Recent innovations in biological research have resulted in new and exciting discoveries which, when applied in food and agriculture, will cushion the many challenges faced today. One such innovation is metabolomics, which has great promise to advance agricultural research. It is a constantly evolving field of research, and necessary attention needs to be given to it by scientists to overcome current limitations. The metabolomics approach can be the impetus to solve several biologically important questions such as climate change, biotic and abiotic stresses, breeding, nutritional properties of crops and genetic engineering.

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CHAPTER 3: THE EFFECT OF OMET SYSTEM ON GROWTH AND YIELD OF AMARANTH SPECIES

Abstract: Amaranth species are a highly popular group of indigenous vegetables cultivated for their green leaves. The organic medium enclosed trough (OMET) system is a non-drainable vegetable growing technique. The aim of the study was to investigate the effects of the OMET system on growth and yield of three Amaranth species; *A. caudatus*, *A. cruentus* and unidentified *Amaranthus* Spp. There was a significant difference ($p \leq 0.05$) in growth and yield attributes between the same Amaranth species grown under both OMET and non-OMET (control) system. Data collected for growth attributes are seedling height, stem diameter and leaf length; whereas for yield attributes are, mass of the aerial parts and longest root length. Time of flowering was closely observed and recorded. The amount of water used on OMET and non-OMET was also recorded weekly for eight weeks (termination week). T-test at 5% level of significance was used to analyse data using computer statistical software, Statistix 10.0. The study revealed that the OMET system increased the stem diameter and plant height of *A. caudatus* by 2.8% *and* 10.5%; *A. cruentus* by 25.2% *and* 21.6%, while *for Amaranthus Spp.*, it increased by 32.4% *and* 41.2% respectively. The leaf length of OMET grown Amaranth species were two-fold longer than the length of those grown under non-OMET system. The mass of the aerial parts and root length increased significantly for *A. caudatus* by 24.4% *and* 8% and *A. cruentus* by 12.6% *and* 50.1% respectively, while for *Amaranthus* Spp., increased by 91.9% *and* 94.5% respectively on OMET system. The study has also shown that the OMET system utilized less water, 500 ml lesser than the non-OMET system, making it a brilliant strategy to conserve water and utilize it sustainably, effectively and efficiently.

3.1 Introduction

Amaranth species possess different morphological traits which distinguish certain species among others. In the production perspective, growth is measured based on primary objective indices including the leaf length, root length, plant height, stem diameter and, number of leaves per plant (AURI, 2003). These growth parameters are often associated with the yield components measured as biomass and dried mass. In

fact, these growth attributes play a key role in the absorption and transportation of water and nutrients from the ground to various parts of the plant. Improved leaf length or surfaces are likely associated with improved photosynthesis rate which ultimately improve plant biomass and more specifically, grain yield (AURI, 2003). However, the degree at which these components respond to growing conditions differs (AURI, 2003).

A widespread agricultural practice across the world consists of covering the soil around plants with plastic films. The introduction of this technique in agriculture dates back to the 1970s, and its success is still linked to multiple benefits (Cozzolino *et al.,* 2020). The popularity of using plastic films has also led to the development and introduction of non-drainable vegetable growing technique known as the OMET system (Ferrari, 2013), which is approximately similar to plastic mulch. The linear low-density polyethylene (LLDPE), especially black polyethylene (PE), is mainly used due to its easy processing, excellent chemical resistance, high durability, and flexibility and odourlessness as compared to other polymers (Wright, 1968; Espí *et al*., 2006).

Mulching is an effective method of manipulating crop growing environment to increase yield and improve product quality by controlling weed growth, ameliorating soil temperature, conserving soil moisture, reducing soil erosion, the development and establishment of plant soil-borne diseases, improving soil structure and enhancing/conserving organic matter content and available nutrients (Green *et al*., 2003; Awodoyin and Ogunyemi, 2005; Scarascia-Mugnozza *et al*., 2006), which in turn influence crop productivity. Plastic mulches directly affect the microclimate around the plant by modifying the radiation budget of the surface and decreasing the soil water loss, just like OMET system (Liakatas *et al*., 1986). The decrease in soil water evaporation results in a more uniform soil moisture content and a reduction in the amount of irrigation water, which is very important in summer crops in dry areas and areas under limited irrigation water supply (Chalker-Scott, 2007).

Plastic mulch is impermeable to the gassy movement which act as a greater wall for the process of solarisation and fumigants. It can also show an astonishing role in increasing soil health and control of pests (Chalker-Scott, 2007). Consequently, it supports in keeping the nutrient around the root of plant for effective use of nutrient and also helps in reduction of leaching of fertilizer. Mulching prevents the fluctuations

in temperature up to 30 cm depth in soils (Fausett and Rom, 2001). This favours root development, and the raised soil temperature in the planting bed, promoting faster crop development and early maturity and harvest (Fausett and Rom, 2001; Wood, 1994). Mulches permit the desired crop plant to extend its root system far away from the main trunk as compared to the un-mulched barren soil. In this way, mulched plants get more biomass and more plant height (Burgess *et al*., 1997; Watson, 1988). Mulching materials conserve soil fertility status, directly improving vegetative growth of crops (Scharenbroch and Lloyd, 2006).

The OMET system is a vegetable growing technique similar to plastic mulching, possessing similar and/or more benefits. The benefits include conserving nutrients and water raised temperatures, eliminated erosion, weed growth and soil borne diseases and pests (Ferreira, 2013). The sheet met of the system also promotes soil health and controls pests. Just like mulching, OMET system manipulates the crop's growing conditions to be favourable, resulting into improved growth and enhanced productivity. This system raises and optimize the growing medium temperatures which promotes greater root development (Ferreira, 2013). The underlying plastic of OMET system promotes accumulation and concentration of nutrients and water around the root system of the plant for adequate uptake. Availability of nutrients and water to the crop root system combat the nutrient deficiencies and wilting, while influencing the crop growth and yield. The OMET system allows and support the plants to have more fibrous roots or root hairs for optimum nutrients and water uptake, which accommodates the plants to have greater biomass (Ferreira, 2013). In high value vegetable crop production, plastic mulches have shown to improve yields for many decades (Tittonel and Giller, 2013). Therefore, the objective of this study it was to evaluate the influence of OMET system ongrowth and biomass of three Amaranth species: *A. caudatus, A. cruentus,* and *Amaranthus* Spp.

3.2 Methodology and analytical procedure

3.2.1 Study site

The experiment was conducted in the greenhouse at green biotechnologies research centre of excellence (GBRCE), University of Limpopo, Limpopo Province in RSA. The altitudes and longitude of the location are (23º53′10″S, 29º44′15″E). The greenhouse is covered with polyethylene. Ambient day/night temperatures during season

averaged 28/21°C, with maximum temperatures controlled using thermostatically activated fans and wet wall.

3.2.2 Experimental design

The randomized complete block design (RCBD) was used to layout the experiment due to variation that is in the greenhouse, brought about by the fans and wet walls. The experiment comprised of three plots for each Amaranth species both on the OMET system and the non-OMET system, with 12 plants/plot.

3.2.3 Procedures

The experiment was conducted in the greenhouse under two prepared treatments: the OMET and non-OMET (control) system. The OMET system was prepared on the greenhouse bench(s) as follows; a layer of organic medium (25 cm thick) was sandwiched using a sterilized black plastic to form an enclosed trough. Holes for planting the seedlings under OMET were made on the top enclosing sheet with row spacing of 20 cm x 20 cm between plants. The non-OMET system was also done in the 25 cm planting pots with the same growing medium as the OMET system. Uniform seedlings of the three Amaranth species were transplanted on the Hygromix growth medium, the OMET system and another set on a non-OMET system as it is schematically represented in Figure 3.1, 3.2 and 3.3.

The seeds for the three Amaranth species namely *A. caudatus, A. cruentus,* and unidentified *Amaranthus* Spp*.,* were obtained from the Agricultural Research Council, Vegetables, Industrial, Medicinal Plants (ARC-VIMP), Pretoria. Seedlings for each Amaranth species were grown/established in seedling trays filled with Hygromix (Hygrotech, Pretoria North, RSA). All the seedling trays were irrigated to field capacity. Four weeks old Amaranth seedlings, at four leaf stage were hardened off outside of the greenhouse for four days before transplanting. Uniform seedlings were transplanted on the holes for OMET and planting pots for non-OMET system, each comprising of steam pasteurized loam soil, river sand and Hygromix at a ratio of 2:1:1 respectively (Figure 3.1 and 3.2). Multi-feeder was applied as per need in both OMET and non-OMET system through fertigation. The transplanted seedlings were irrigated as per need with 250 ml of tap water.

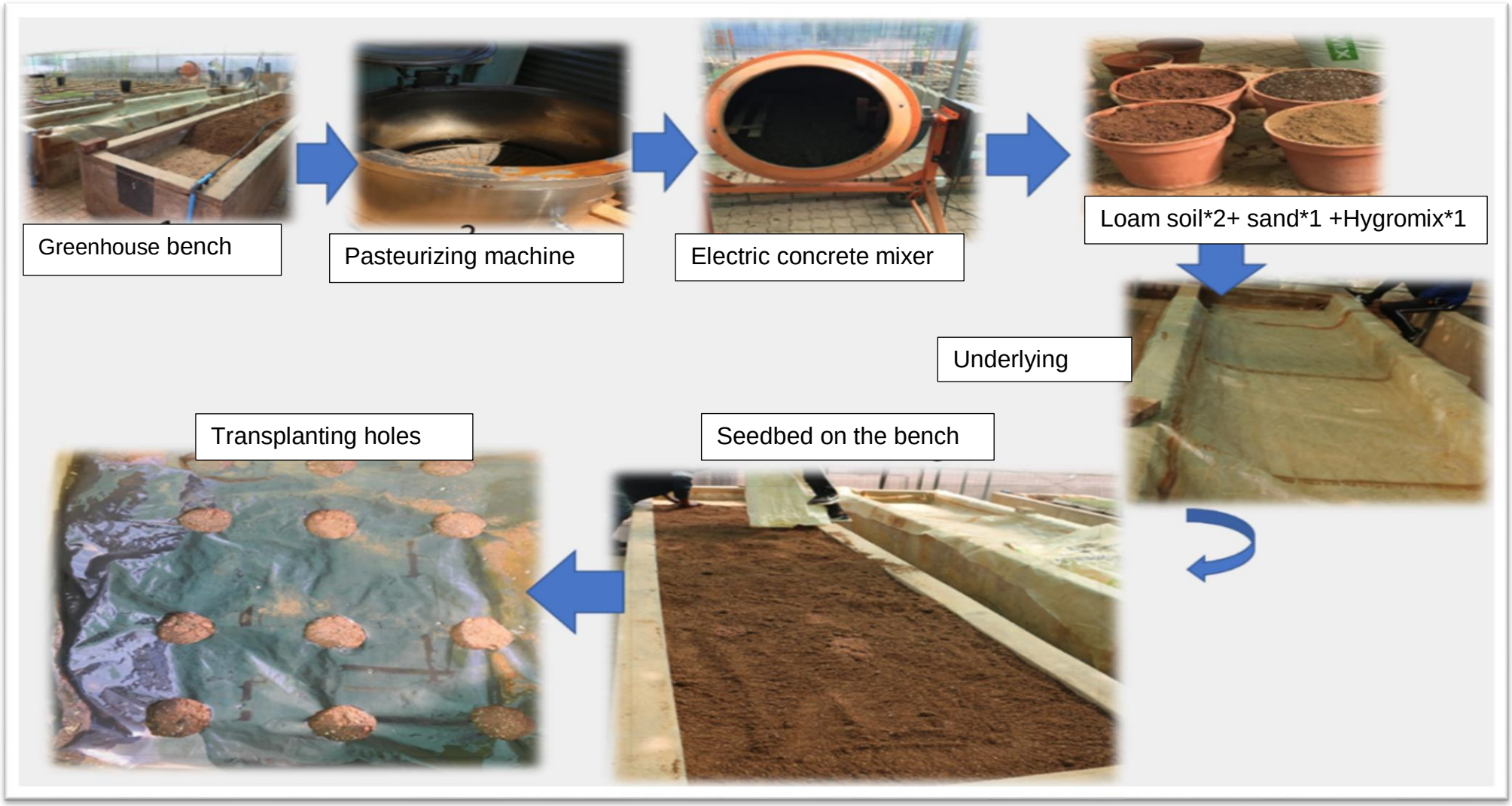

Figure 3.1: The procedure and process of OMET system preparation

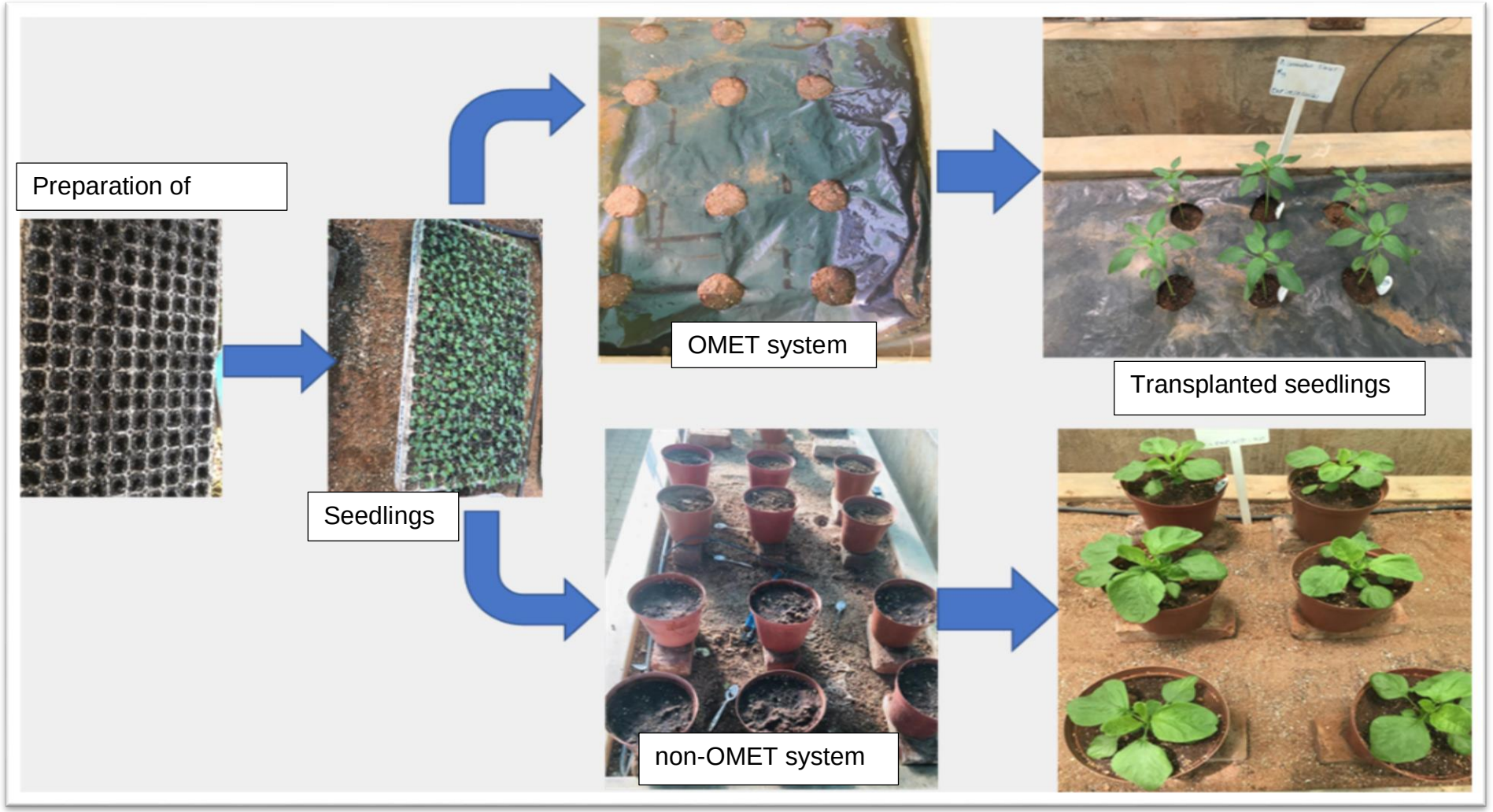

Figure 3.2: Preparation of seedlings and transplantation under both OMET and non-OMET system

Figure 3.3: Amaranth plants grown under the OMET system at week three after transplanting

3.2.4 Data collection

The growth parameters were measured weekly for eight weeks as the plant grew. The stem diameter was measured 5 cm above the ground using a digital vernier calliper; plant height and leaf length were measured using a tape measurer and meter ruler respectively; time of flowering was recorded as per observation.

At harvest, data collected included fresh biomass and longest root length. They were determined at the termination of the trial, eight (8) weeks after transplanting. Fresh biomass (mass of the aerial parts) and the longest root length were measured using laboratory weighing balance and tape measure or meter ruler respectively.

3.2.5 Statistical analysis

The study adopted a single factorial analysis (OMET) in each Amaranth species. Mean separation for significant treatments was achieved through a T-test at the significance level of 5% (P=0.05) using computer statistical software, Statistix 10.0.

3.3 Results and discussion

The results for growth and yield attributes are independent for each species, comparison is between the OMET and non-OMET system separately for each species.

Table 3.1: Effect of OMET system on growth attributes, flowering time, yield and amount of water used eight (8) weeks after transplantation

A. caudatus	**Stem diameter**	**Plant height**	**Leaf length**	**Root length**	**Flowering time**	**Fresh biomass**	**Cumulative water used**
OMET	*	**	*	ns	*	**	*
non-OMET	*	**	*	ns	*	**	*
A. cruentus							
OMET	*	**	*	*	ns	**	*
non-OMET	*	**	*	*	ns	**	*
***Amaranthus* Spp.**							
OMET	*	**	*	*	*	**	*
non-OMET	*	**	*	*	*	**	*

** and * in the same column= significant difference at p≤0.01 and p≤0.05, respectively; ns= not significant

3.3.1 Effect of OMET system on growth attributes of three Amaranth species

Figure 3.4 illustrates the increase in stem diameter of three Amaranth species over eight (8) weeks after transplanting. There was a significant difference ($p \leq 0.05$) in stem diameter of OMET and non-OMET grown Amaranth species. It is evident that the OMET system promoted an increase in the stem diameter compared to the non-OMET system as evidenced by higher stem diameter from week three (3) under OMET system until the end of the experiment (week eight) except for OMET system grown *A. cruentus,* which had its stem diameter significantly improved early at week two (2) compared to the non-OMET system (refer to Figure 3.4). The results obtained proved that the OMET system can enhance the stem diameter of *A. caudatus, A. cruentus* and *Amaranthus* Spp*.*

The OMET system has significantly ($p \leq 0.05$) enhanced stem diameter and plant height of all the three Amaranth species namely *A. caudatus and A. cruentus*, and *Amaranthus* Spp*.* The leaf length of all Amaranth species was also significant ($p \leq 0.05$). The longer leaf length is obtained in *A. cruentus* followed by *A. caudatus and Amaranthus* Spp*.* respectively (refer to Figure 3.6). In general, species with larger leaves have longer internodes, larger flowers, and thicker twigs (Westoby and Wright 2003). Mulch is effective for vegetable growth and yield by improving moisture content of soil, heat energy. In addition, organic mulch add organic N and other mineral to improve nutrient status of the soil (Saeed and Ahmad, 2009).

The effectiveness of OMET system on increased stem diameter and plant height was probably due to the potential to conserve water and nutrients, which cause a moderate availability of water resulting in good nutrient dissolution and absorption by the plants. The same results were reported by Ngala *et al*. (2019). These authors reported that growth parameters including plant height, stem diameter and leaf length of Amaranth were significantly and positively affected by neem leaves and sea weeds mulch. Ferrini *et al*. (2008) also found that mulching with neem leaves and seaweeds significantly improved trunk girth and plant height of ornamental trees. This was attributed to the increase in physiological activities due to improved soil physio-chemical properties.

The largest stem diameter and tallest plant height obtained in OMET grown Amaranth species was due to the process of cell division and elongation as it was also revealed by Vurayai *et al*. (2011). The rate and extent of elongation is subject to a variety of

controls, including nutrition, hormones, and environmental factors such as light and temperature. The results are similar to the thosereported by (Weller *et al*., 2021) who reported increased stem diameter and plant height for adequately irrigated treatments than those with reduced amounts of available irrigation water.

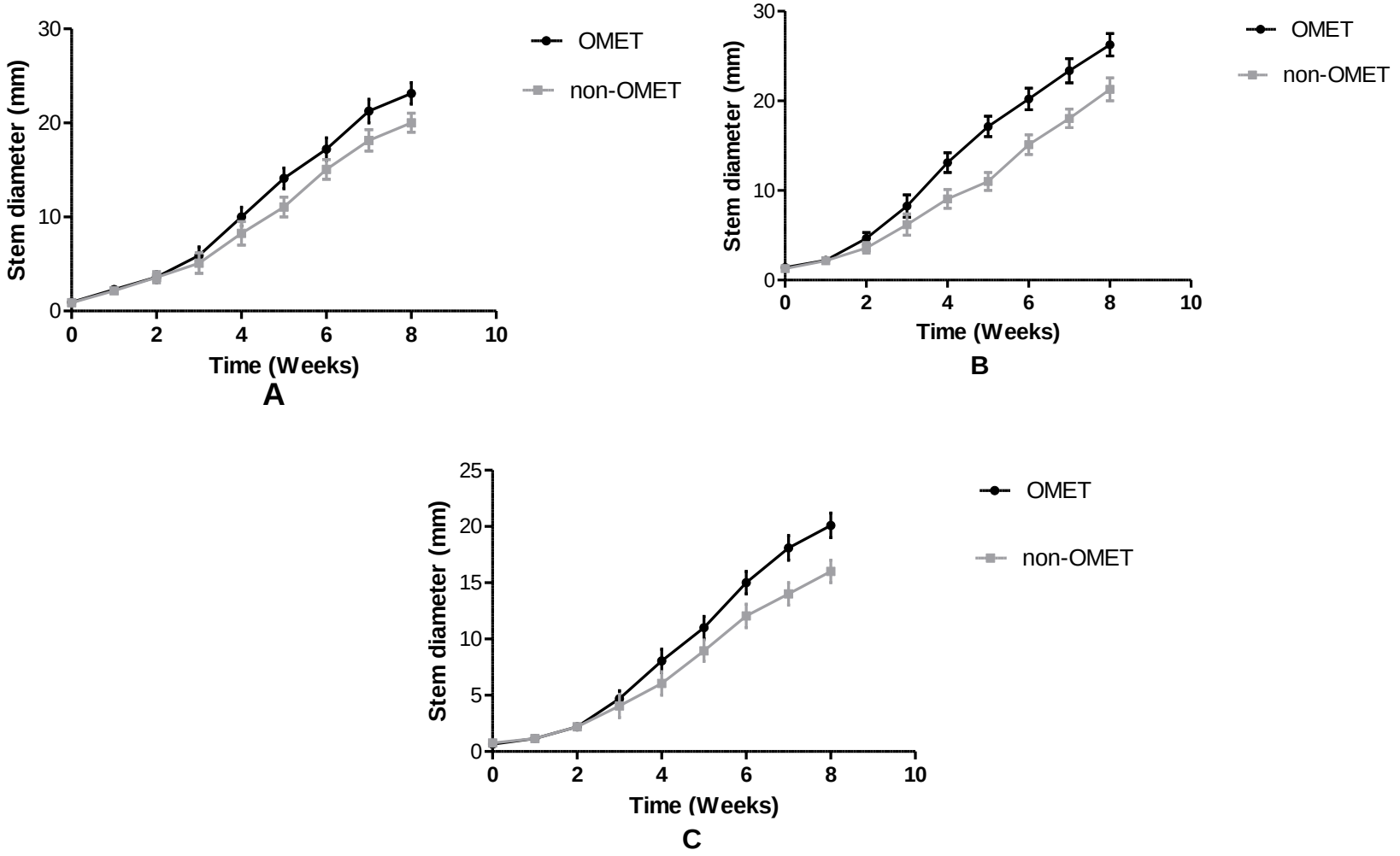

Figure 3.4: The influence of OMET system on stem diameter in A- *A. caudatus*, B- *A. cruentus* and C- *Amaranthus* Spp. eight (8) weeks after transplanting. Results are expressed as the mean values ± standard error (n=12).

The plant height of three Amaranth species grown under both OMEt and non-OMET (control) system was significantly different ($p<0.05$). Figure 3.5 below indicate the plant height trend of all the three Amaranth species over a period of eight (8) weeks. There was a significant difference ($p\leq0.01$) in plant height of Amaranth species grown under OMET and non-OMET system. As observed in Figure 3.5, the OMET system improved plant height of all three Amaranth species compared to the non-OMET system. All the three Amaranth species under OMET and non-OMET system measured relatively the same plant height until week 4. The difference in plant height for *A. caudatus* was clearly observed at week four (4) and for *A. cruentus* and *Amaranthus* Spp. was at week five (5) until termination week (week 8) (refer to Figure 3.5).

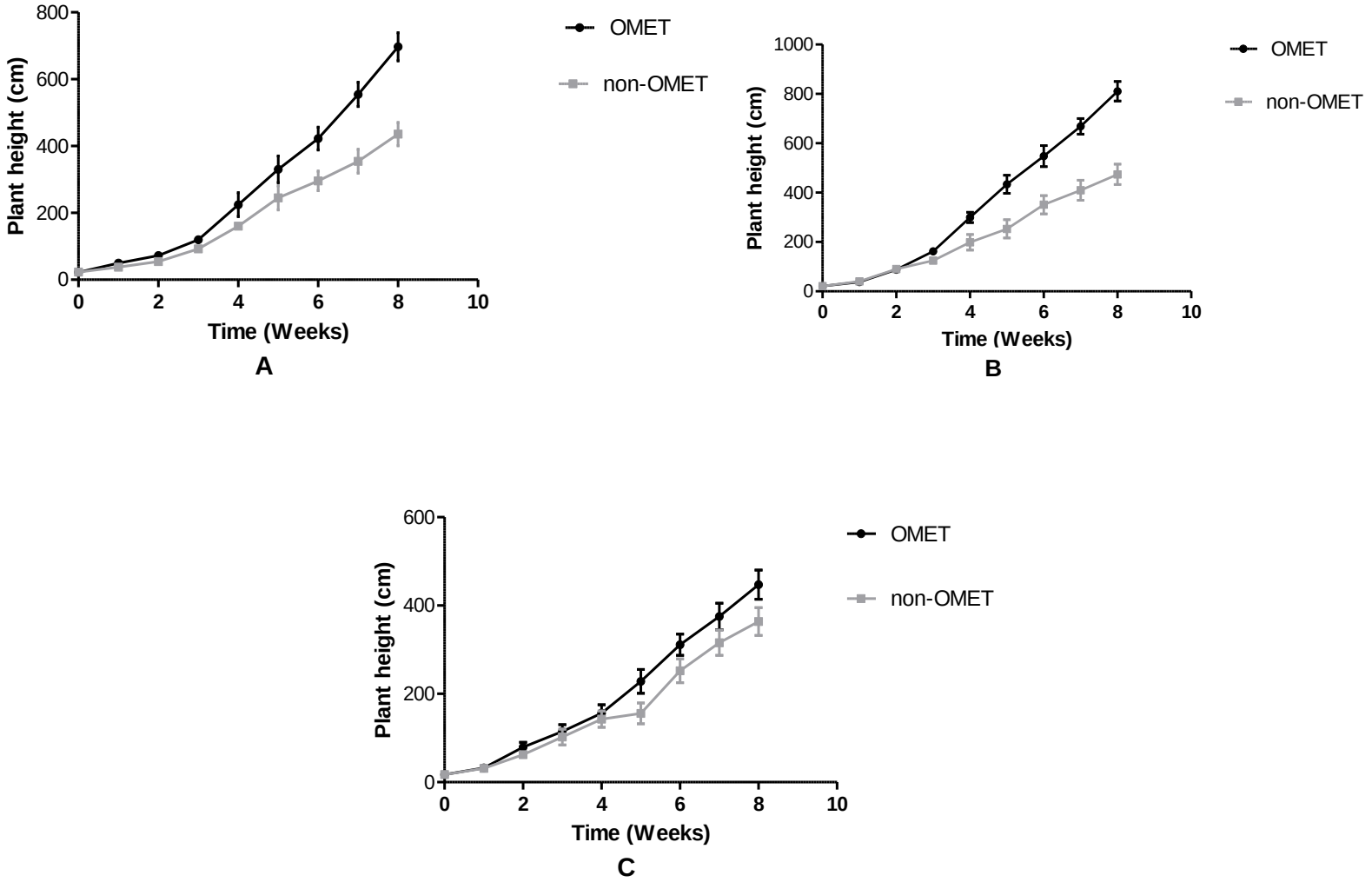

Figure 3.5: The effect of OMET system onplant height of (A) *A. caudatus*, (B) *A. cruentus* and (C) *Amaranthus* Spp. eight (8) weeks after transplanting. Results are expressed as the mean values ± standard error (n=12)

The leaf lenth of the three Amaranth species grown under OMET and non-OMEt system was measured weekely for 8 weeks and the results are illustrated below. Figure 3.6 illustrates the leaf lengths of three Amaranth species after eight (8) weeks after transplanting. The OMET system improved the leaf lengths of the three Amaranth species compared to the non-OMET system.

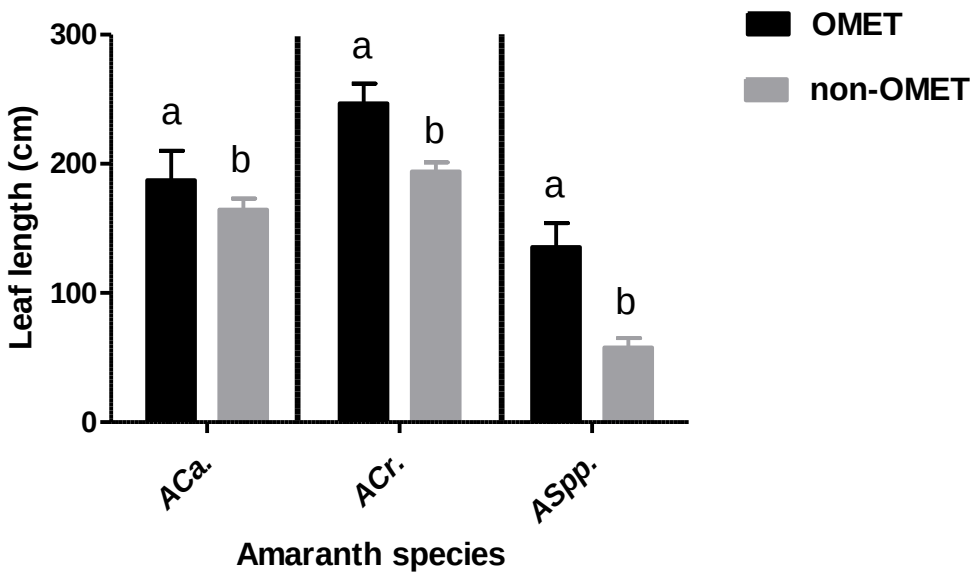

Figure 3.6: The leaf length of three Amaranth species grown under the OMET and non-OMET. Results are expressed as the mean values ± standard error (n=12) Bars in the same column marked with different letters indicate significance difference at p≤0, 05. ACa= *A. caudatus*; ACr= *A. cruentus*, ASpp= *Amaranthus* Spp.

3.3.2 The influence of OMET system on the time of flowering in three Amaranth species

Table 3.2 shows the time of flowering of the three Amaranth species. As observed in Table 3.2, *A. caudatus, A. cruentus, and Amaranthus* Spp. began flowering at week 5, 6 and 6 respectively under the OMET system whereas under the non-OMET system, *A. caudatus, A. cruentus, and Amaranthus* Spp., commenced flowering at week 4, 6 and 5 respectively. *A. caudatus* and *Amaranthus* Spp. grown under non-OMET system started flowering earlier (a week earlier) than the ones grown under the OMET system, whereas *A. cruentus* began flowering at the same mature stage (week 6) in both treatments (Table 3.2).

The time of flowering in both treatment for all Amaranth species was closely observed and recorded. The *Amaranthus* Spp. and *A. caudatus* grown under the non-OMET flowered early, week 4 and 5 respectively, then the same species grown under OMET flowered a week later (at week 5 and 6) respectively. In contrary, *A. cruentus* grown under both treatments flowered at the same week (week 6). The reason(s) for early flowering in non-OMET grown *Amaranthus* Spp. and *A. caudatus* could probably be due to water and nutrient stress. The exposure of the plant to severe drought induces early flowering and halts the production of leaves (DAFF, 2010). In this case, the non-OMET system had less to no potential to conserve the irrigation water and nutrients compared to the OMET system, resulting into high rates of water loss and nutrients through evaporation, drainage and leaching respectively. That can probably cause plant physiological and morphological stress, resulting into early flowering (DAFF, 2010).

The number of growing days during the growing season is a major determinant of Amaranth plant growth. Lower temperatures and shorter days will induce flowering with a subsequent reduction in leaf yield (DAFF, 2010). The floral initiation and development in *A. caudatus* are enhanced by short day effects (Table 3.2). This study was conducted in the greenhouse during spring season, resulting in lower temperatures and shorter days than summer season, which induced early flowering of the two species; *Amaranthus* Spp. and *A. caudatus*, under non-OMET compared to the ones grown under the OMET system. *A. cruentus* presented a late flowering ecotype that was well adapted to flower under summer field conditions, hence it flowered late than both *Amaranthus Spp.* and *A. caudatus*, and *A. caudatus* under OMET and under both the treatments respectively (Table 3.2).

Table 3.2: Time of flowering in three Amaranth species grown under OMET and non-OMET system

Treatment	**Amaranth species**	**Time of flowering (Weeks)**							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
OMET system	*A. caudatus*					**X**			

	A. cruentus						**X**		
	Amaranthus Spp.						**X**		
non-OMET system	*A. caudatus*				**X**				
	A. cruentus						**X**		
	Amaranthus Spp.					**X**			

OMET= Organic Medium Enclosed Trough

3.3.3 The effect of OMET system on at harvest attributes (yield and root length)

Figure 3.7 indicates the fresh biomass/aerial mass (yield) of the three Amaranth species. Fresh root mass was also quantified. The biomass of the Amaranth grown under OMET system was higher than thatgrown under the non-OMET (control) system. The fresh biomass of each OMET grown Amaranth species is two-fold higher than the non-OMET (control) grown Amaranth of the same species.

The OMET system significantly ($p \leq 0.01$) improved the biomass of all the three Amaranth species. As presented in Figure 3.7, the yield of Amaranth grown under the OMET for each species was two-fold higher than the yield of the ones grown under the non-OMET system (Figure 3.7). This can be due to several factors associated with plant growth and development under each treatment. Although Amaranth is drought resistant, it performs optimally under irrigation (DAFF, 2010) and sufficient supply of nutrients especially Nitrogen (N). Under irrigation, Amaranth yields a harvest of leaves every two weeks during summer. In sandy soils, an irrigation frequency of four to five days is maintained in the summer season, while in the rainy season the irrigation frequency is based on soil moisture levels (DAFF, 2010). Amaranth species are C4 plants, drought tolerant and can grow under limited irrigation water supply and low input farming, but still, they require conducive growing techniques/conditions that will not subject them to any physiological stress by supplying them with sufficient water and nutrients to achieve high yield and quality (DAFF, 2010), thus including the OMET system. The increased yield of Amaranth was probably due to the ability and potential

of OMET to supply frequent and enough water, as Amaranth is said to yield optimally under sufficient irrigation.

The OMET system is an efficient low technological growing strategy in terms of increasing the growth (plant height, stem diameter, plant canopy, leaf lengths and number of leaves per plant and yield components (aerial biomass and root length) of Amaranth species as it was investigated and evaluated in this study (refer to Figure 3.8).

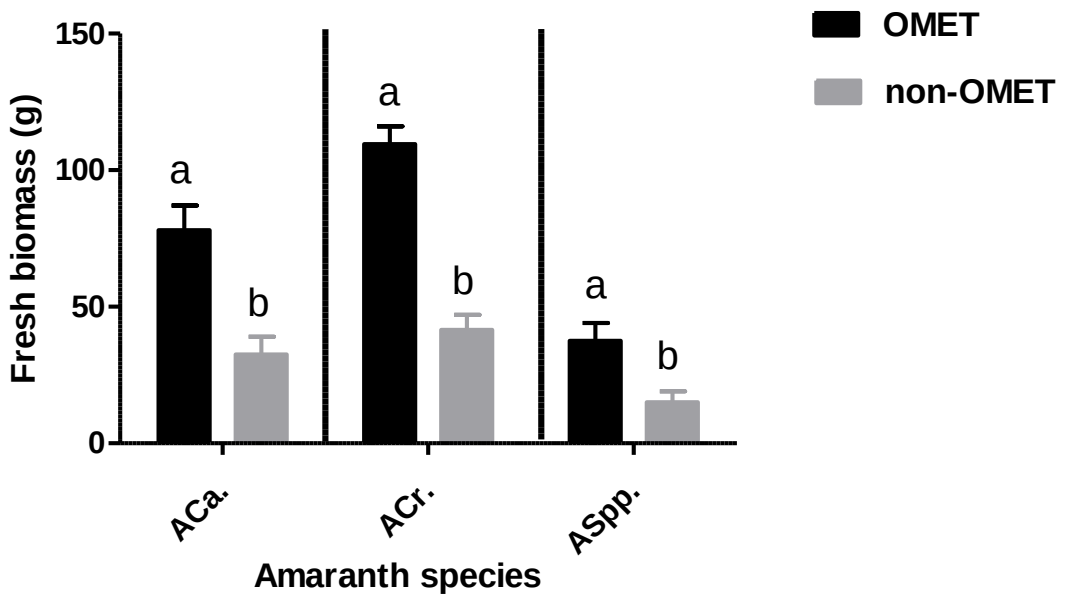

Figure 3.7: Fresh biomass of three Amaranth species grown under both the OMET and non-OMET system. Results are expressed as the mean values ± standard error (n=12). Bars in the same column marked with different letters indicate significant difference at p≤0.05. ACa= *A. caudatus*; ACr= *A. cruentus*, ASpp= *Amaranthus* Spp.

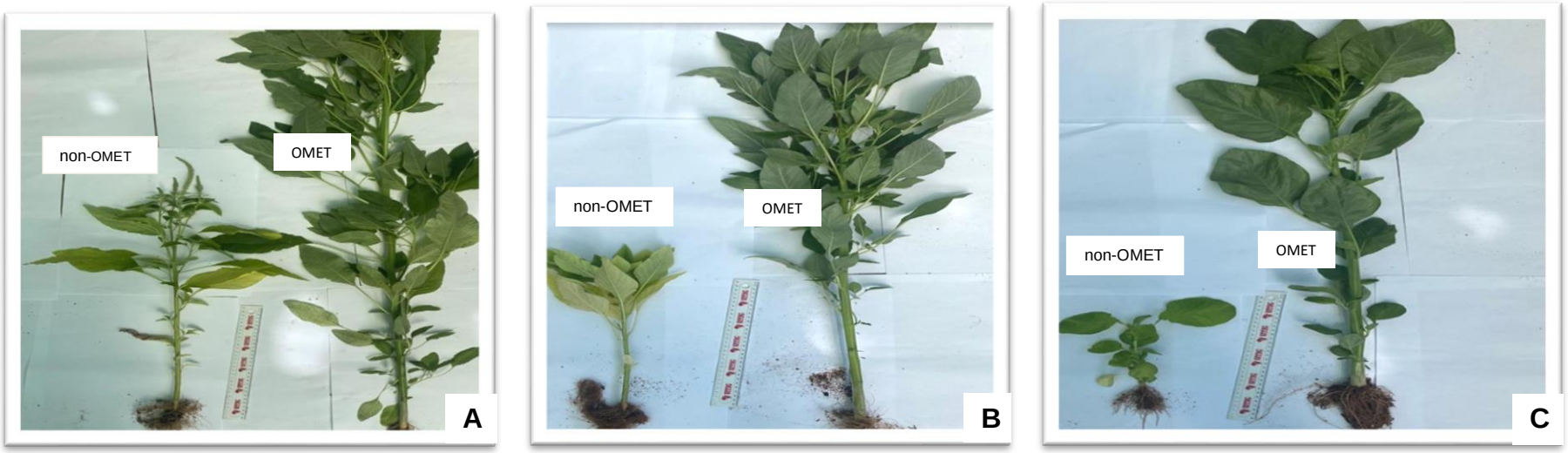

Figure 3.8: Comparison between A- *A. caudatus*, B- *A. cruentus* and C- *Amaranthus* Spp. grown under OMET (right) and non-OMET (left) system

The Amaranth grown under the OMET system had longer leaf lengths resulting in increased plant surface. Enhanced plant surface is associated with an increase in the rates of photosynthesis which ultimately improve the biomass of the plant (AURI, 2003). One of the essential elements, and one which participates directly as an indispensable requirement for normal plant growth, is N. Application of the multi-feeder made N and other vital nutrients become readily available for uptake by the plants. Probably due to the potential of OMET system to conserve irrigation water and nutrients, it prolonged the availability and uptake of water, N and other nutrients by the plants, thus prompting vigorous vegetative growth and high yielding in Amaranth species. According to Ngala *et al*., (2019), neem leaf mulch increased the fresh biomass of Amaranth. Ferrini *et al*. (2008) found that mulching neem leaves significantly improved the fresh weight of ornamental trees. Previous studies have also shown that organic materials increase soil organic matter by directly improving soil properties (Scharenbroch and Lloyd, 2006), increasing photosynthesis, and by having an impact on above ground C allocation (Scharenbroch, 2009).

3.3.4 Effect of OMET system on the root length of three Amaranth species at harvest

The non-OMET system significantly ($p \leq 0.05$) improved the rooting length of *A. caudatus*, except for *A. cruentus* and *Amaranthus* Spp. (Figure 3.9). The rooting length of Amaranth species under non-OMET system was probably due to the rapid extension of the plant roots to access downward water. Higher soil water content in the surface layer reduces the mechanical resistance to growing roots, which helps in their proliferation (Chalker-Scott, 2007). The non-fluctuating raised temperatures and available water content at the surface layers of medium, in this case under OMET system, stimulated the rapid growth and development of longer roots (Lamont, 1993; Wood, 1994; Fausett and Rom, 2001; Chalker-Scott, 2007) for *A. cruentus* and *Amaranthus* Spp. Root elongation and branching are iterative processes in root development (Malamy and Benfey, 1997; Nibau *et al*., 2008; Atkinson *et al*., 2014).

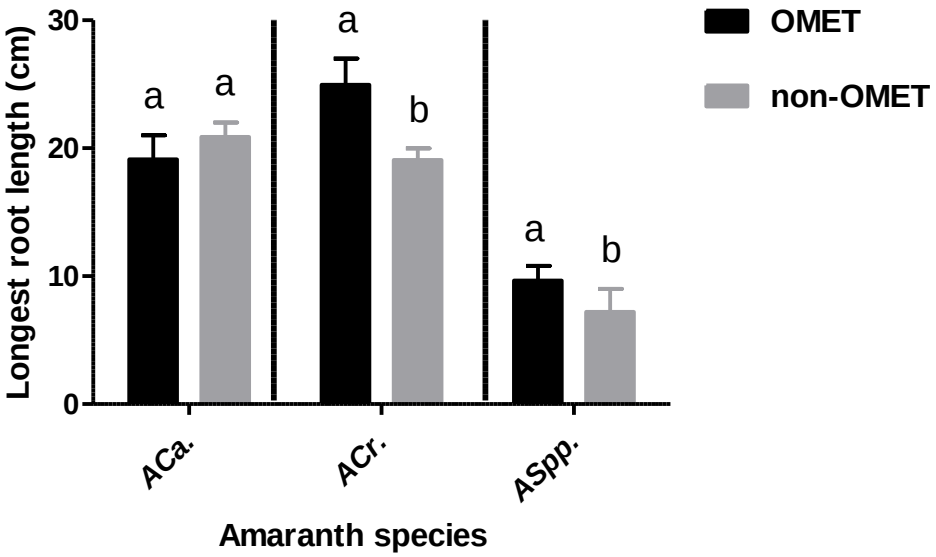

Figure 3.9: The effect of OMET system on the rooting length of three Amaranth species grown under both OMET and non-OMET system. Results are expressed as the mean values ± standard error (n=12). Bars in the same column marked with different letters shows significant difference at p≤0.05, independently on each Amaranth species. ACa= *A. caudatus*; ACr= *A. cruentus*, ASpp= *Amaranthus* Spp.

3.3.5 The effects of OMET system on amount of irrigation water utilised in three Amaranth species

Figure 3.10 below indicates the amount of water used over eight (8) weeks after transplanting. The OMET system used less water compared to the non-OMET (control) system. The amount of water used was constant (250 ml per irrigation day) and increased between weeks as observed in Figure 3.10. Generally, OMET system utilised 2500 ml of irrigation water whereas non-OMET (control) system utilized 3000 ml. OMET system has utilized 500 ml lesser than the non-OMET system while achieving high yields. The structure of the OMET system make it possible for it to use less water as compared to the non-OMET system. The plastic underlying the growing medium on the OMET system makes it impossible for water to pass through via seepage and/or drainage. Water loss through drainage is completely eliminated in the OMET system unlike the non-OMET system that allows water loss through drainage because it lacks the underlying plastic. The high evaporation rate in the OMET system is eliminated by the top plastic covering the growing medium (Ferrari, 2013).

In contrary, the non-OMET system has no top sheet that prevents evaporation, hence the plants grown under the non-OMET system will require frequent irrigation to maintain the level of water required daily due to high loss of water through drainage and high evapotranspiration (ET). This simply means that the OMET system significantly conserves water and nutrients than the non-OMET system (Figure 3.10). There are other smart climate farming techniques that have been developed with the aim of water conservation including mulching, hydroponics, aquaponics, and others. Hydroponic systems, just as the OMET system use less water, as much as 10 times less water than traditional field watering method including the non-OMET system, because water in a hydroponic system is captured and reused, rather than allowed to run off and drain to the environment (DAFF, 2010).

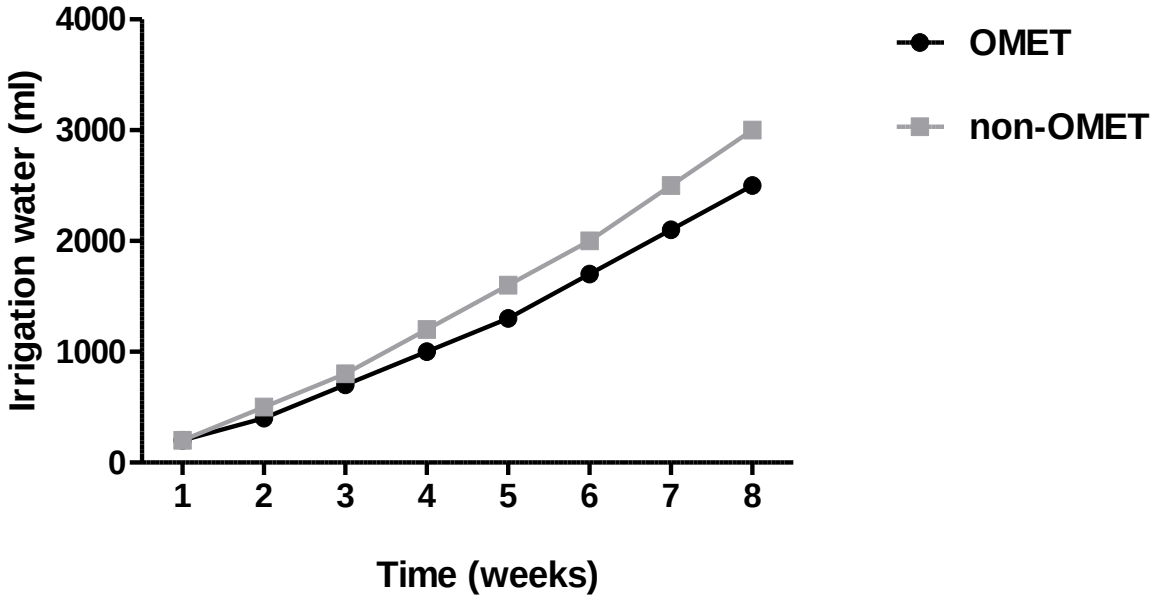

Figure 3.10: Cumulative amount of water utilised weekly/plant over a period of 8 weeks under OMET and non-OMET system

3.4 Conclusion and recommendations

The OMET system significantly ($p \leq 0.05$) enhanced the growth and yield attributes of three Amaranth species: *A. caudatus*, *A. cruentus* and *Amaranthus* Spp. The OMET system proved to be a water and nutrient conserving growing technique that can be adopted in low input farming. Compared to non-OMET system, the OMET system successfully prolonged the time of flowering by a week in 2 of the three Amaranth

species (*Amaranthus* Spp*. and A. caudatus*), resulting into vegetative growth and ultimately high yield. In this study, the growth (stem diameter and plant height) and at harvest (fresh biomass and root length) parameters of the Amaranth species increased significantly over time. The OMET system is a brilliant low technological farming technique that can probably overcome the future estimates of water shortage and soil nutrient loss. The OMET system has proved to enhance the ratio of the water use efficiency while simultaneously enhancing the growth and yield of Amaranth species.

There is a necessity to expand research on how other climate smart farming techniques affect the Amaranth species. The research gap on the development of innovative, low technological farming strategies that are sustainable and environmentally friendly but promote high yields and can be adopted by small scale farmers at low input farming areas. There's a need for diffusion of scientific knowledge regarding the benefits of the indigenous vegetables including the Amaranth species. This technique is suitable for small scale farmers for specialization and commercialization.

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CHAPTER 4: THE INFLUENCE OF OMET SYSTEM ON THE NUTRITIONAL COMPOSITION OF THREE AMARANTH SPECIES

Abstract: Amaranth species are indigenous vegetables with the potential to combat the crisis of malnutrition faced by Africa, South Africa in particular, due to their high nutritional level. In the current study, the aim was to investigate the effects of OMET system on the nutritional compositions of three Amaranth species: *A. caudatus*, *A. cruentus* and *Amaranthus* Spp. The nutritional attributes include protein, minerals and amino acids. The OMET systemimproved the % protein content of *A. caudatus*, *A. cruentus* and *Amaranthus* Spp. by 2.7, 4.5 and 2.4% respectively. All the mineral composition, both macro and micro elements of OMET grown Amaranth species were higher than the concentration measured in the same species grown under non-OMET system. Calcium (130 mg/kg DW) and phosphorus (34.7 mg/kg DW) were measured high in *A. cruentus* whereas magnesium was higher in *Amaranthus* Spp. (82 mg/kg DW). Potassium was also higher in *Amaranthus* Spp. (276 mg/kg DW). The micro elements copper (1.04 mg/kg DW), selenium (8.13 mg/kg DW) and zinc (1.66 mg/kg DW) were found higher in *A. cruentus* grown under OMEt system. Whilst manganese (3.14 mg/kg DW) and iron (8.13 mg/kg DW) were founf higher in *Amaranthus* Spp. grown under OMET system. The amino acid concentration measured in Amaranth species grown under OMET system and non-OMET system were not all significantly different. OMET system significantly ($p \leq 0.05$) influenced the concentration of essential amino acids compared to non-OMET system. On the other hand, non-OMET system was found to enhance the concentration of non-essential amino acids compared to the OMET system, although they were significantly different at $p \leq 0.05$. It is evident that the OMET system has significantly ($p \leq 0.05$) enhanced the nutritional attributes of the three Amaranth species compared to the non-OMET system. There is a need for investigation of the effects of OMET system on other nutritional composition of Amaranth species and other indigenous leafy vegetables.

4.1 Introduction

The route to improve food security is primarily based on the consumption of balanced diets. According to Cena and Calder (2020), balanced diets comprise of adequate macro and micronutrients. These nutrients such as calcium, magnesium, potassium and phosphorus are predominant in fruits and vegetables. Their contents depend on various cultural practices during their production period such as irrigation, fertilisation, and planting dates. For example**,** the application of N fertilisers (140 kg N/ha) promotes the accumulation of free protein and those of essential amino acids in *A. cruentus* grain (Mlakar *et al*., 2012). In fact, valine concentration increased with N-increased application, while leucine showed a reduction with an increase in N concentration. The application of N at 0.16 and 0.24 g/kg was optimal for improving lysine concentration and reduction of methionine content in *A. cruentus* grain (Thanapornpoonpong *et al*., 2008). Foliar application of vermicomposting leachate, Kelpac® and Eckol® enhanced the accumulation of proteins and carbohydrate (Ngoroyemoto *et al*., 2019). This enhancement was associated with the plant growth regulating hormones such as cytokinins, polyamines, abscisic acid, indole acetic acid auxins and gibberellins which become either upregulated or downregulated in the presence of the biostimulant. Such regulations form part of the nutritional component biosynthesis (Ngoroyemoto *et al*., 2019).

Maseko *et al*. (2019) investigated the impact of irrigation regimes on nutritional composition of *A. cruentus*. Clearly, severe drought or water deficit enhances the accumulation of Ca and Mg. The increased soil moisture improved the accumulation of Na, K, and Zn (Maseko *et al*., 2019). According to Manyelo *et al*. (2020), the nutritional composition in *A. cruentus* leaves harvested at day 65 differed from those harvested at day 120. As a result, samples harvested on day 65 weredominated by threonine, lysine, and leucine, whilst late harvest (day 120) enhanced the accumulation of Ca, Mg and Na minerals (Manyelo and Sebola, 2020). On the other hand, harvesting date showed to implicate the nutritional composition in *A. cruentus* leaves. Grain harvested at full maturity stage was dominated by five tocopherol nutrients including β-tocotrienol, γ-tocotrienol, α-tocotrienol and δ-tocotrienol in comparison to that harvested prematurely (Manyelo *et al*., 2020). In fact, mineral composition such as Ca, P, Mg, K and Fe were highly concentrated in mature grain than in premature grain. However, some minerals including Na, Cu, Mn, and Zn were

highly dominant in premature over matured grains in *A. cruentus* (Manyelo *et al*., 2022). Nutritional composition including Fe, Zn, protein, sugar, and fibre contents in grain Amaranth species were unaffected by four leaf harvests (Dinssa *et al*., 2018). Therefore the present study was conducted to generate scientific information with regard to the effect of OMET on nutritional compositions of three Amaranth species; *A. cruentus, A. caudatus* and *Amaranthus* Spp. This study is likely to improve our understanding on the nutritional composition and/or concentration of the Amaranth species grown under OMET and non-OMET system. It also highlights the distribution and adaptation of these indigenous species to the OMET system which may improve their nutritional status for improved food and nutritional security.

4.2 Methodology and statistical analysis

4.2.1 Study site

The study was conducted at the location described in nsection 3.2.1.

4.2.2 Experimental design and treatments

The experimental design was similar to that described in section 3.2.2.

4.2.3 Procedure

4.2.3.1 Plant material

Leaves for the three Amaranth species; *A. caudatus*, *A. cruentus* and *Amaranthus* Spp., were obtained from the experiment that was conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province in RSA. The fresh leaves that were free from damage were harvested eight (8) weeks after transplanting. Thereafter, the leaves samples for each Amaranth species were packaged separately for each species in brown bags and oven dried at 40 ºC for 72 hours. After drying them, they were then grinded separately for each Amaranth species into fine powder using a coffee grinder and packed into sachets ready for extraction.

4.2.4 Data collection

4.2.4.1 Determination of protein

The micro Kjeldahl method described by the Association of Official Analytical Chemists (AOAC) (1990), was used. In each Amaranth species, two grams (2 g) for 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 hood. The digest material were transferred into a 100 ml volumetric flask and made up with distilled water. Ten millilitre (ml) portion of the digest was mixed with 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 distillate was collected and titrated as well. The sample was duplicated 3 times, and the average value was taken. The nitrogen 34 content was calculated and converted to percentage protein by using a protein conversion factor of 6.25. This was given as:

$$\% \text{ nitrogen} = (100 \times W \times N \times 14 \times Vf)\ T\ 100 \times Va$$

Where; *W*= Weight of the sample, *N*= Normality of the titrate (0.1N), *Vf*= Total volume of the digest = 100 ml, *T*= Titre value and *Va*= Aliquot volume distilled.

4.2.4.2 Determination of minerals

For analysis of elements, approximately 10 g 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, 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).

4.2.4.3 Determination of amino acids

Amino acid analysis was performed using dried leaves of three Amaranth species, according to Mpai *et al.* (2018). A volume of 100 g was vortexed with 6 NHCl 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.

Analysis: Amino acid separation and detection was performed using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector. 1 µl of sample/standard solution was injected into the mobile phase which conveys the derivatised amino acids onto a Waters UltraTag C18 column (2.1 x 50 mm x 1.7 µm) held at 60 °C. Elution of analytes off the column was performed by running a gradient. Analytes eluting off the column were detected by the PDA detector, with each amino acid coming off the column at a unique retention time. Instrument control and data acquisition was performed by MassLynx software which integrates the peaks at the defined retention times and plots calibration curves for each amino acid based on the peak response (peak area/internal standard peak area) against concentration.

4.2.5 Amino Acid Scoring Methods

4.2.5.1 Amino Acid Score (AAS)

Amino Acid Score is a numerical value showing how much the smallest limiting amino acid satisfies the scoring pattern. It refers to the ratio between the amino acid of the test protein and the amino acid of the reference protein, was calculated according to the Joint *et al*. (1973). The required amounts of 9 essential amino acids for human

health are called amino acids scoring patterns, as defined by the international organizations (FAO, WHO, UNU, 2005). It can be said that a protein with an amino acid score close to 100 is good quality protein. If an amino acid is less than the amino acids scoring pattern, it is called the limiting amino acid. The AAS equation is as follows:

AAS = Amino acid of test protein (mg/g)/Amino acid of protein in reference protein (mg/g) × 100%

4.2.5.2 Ratio Coefficient of Amino Acids (RC)

The ratio coefficient of amino acids 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. When RC = 1, it indicates that the amino acids in the test protein are consistent with the reference amino acid. If RC > 1, it indicates that the amino acid in the test protein has a relative surplus; conversely, if RC < 1, the amino acid in the test protein is relatively insufficient (Caire-Juvera *et al*., 2013). The RC formula is calculated as follows: RC = AAS/AAAS

Where AAAS is the Average Amino Acid Score for each type of amino acid

4.2.6 Statistical analysis

The study adopted a single factorial analysis (OMET) in each amaranth species. Mean separation for significant difference was achieved through a T-test at the significance level of 5% (P=0.05) using computer statistical software, Statistix 10.0.

4.3 Results and discussion

Comparison independently for each Amaranth species between OMET and non-OMET system

Table 4.1 Effects of OMET and non-OMET system on the elements and amino acid compositions of three Amaranth species

A. caudatus	**Micro elements**					**Macro elements**			
	Cu	**Mn**	**Fe**	**Se**	**Zn**	**Ca**	**Mg**	**K**	**P**
OMET	ns	*	ns	*	ns	ns	*	*	*
non-OMET	ns	*	ns	*	ns	ns	*	*	*
A. cruentus									
OMET	*	*	ns	*	ns	*	*	*	*
non-OMET	*	*	ns	*	ns	*	*	*	*
***Amaranthus* Spp.**									
OMET	ns	*	*	*	*	*	*	*	*
non-OMET	ns	*	*	*	*	*	*	*	*

* Indicates significance at p≤0.05; ns indicates no significance in column

Table 4.2 Effects of OMET and non-OMET system on the amino acid compositions of three Amaranth species

A. caudatus	**Essential amino acids**						**Non-essential amino acids**				
	Threonine	**Valine**	**Isoleucine**	**Lysine**	**Leucine**	**Phenylalanine**	**Arginine**	**Serine**	**Glycine**	**Aspartate acid**	**Glutamate acid**
OMET	*	*	*	*	*	*	*	*	*	*	*
non-OMET	*	*	*	*	*	*	*	*	*	*	*
A. cruentus											
OMET	*	*	*	*	*	*	*	*	*	*	*
non-OMET	*	*	*	*	*	*	*	*	*	*	*
***Amaranthus* Spp.**											
OMET	*	*	*	*	*	*	*	*	*	*	*
non-OMET	*	*	*	*	*	*	*	*	*	*	*

* Indicates significance p≤0.05; ns indicates no significance in column

4.3.1 Effects of OMET system on percentage (%) protein content in three Amaranth species

The % protein content of the three Amaranth species are shown in Table 4.3. There was a significant difference ($p \leq 0.05$) in protein content measured from each Amaranth species grown under OMET and non-OMET system. The highest protein content was noted in OMET grown *A. cruentus* (28.6%) than the protein content of the same species grown under the control. In contrast, the lowest protein content was noted in non-OMET grown *Amaranthus* Spp. (20.3%). The protein content of OMET grown *A. caudatus* (24.1%) and *Amaranthus* Spp. (22.7%) were also higher than those of the same species grown under non-OMET system (21.4 and 20.3% respectively). The two Amaranth species, *A. caudatus* and *A. cruentus* grown under OMET exhibited equal protein content (24.1%) (Table 4.3). The trend of protein content observed is identical to the trend observed for N. The highest N was shown in OMET grown *A. cruentus* (4.6%) whereas the lowest was shown in non-OMET grown *Amaranthus* Spp. (3.2%) (Table 4.3). Kwenin and Dzomeku (2011) reported 4.46% protein content in *A. cruentus*, both much lower than all the protein content of all the Amaranth species grown under OMET and non-OMET in this study, ranging between 20.3 and 28.6%. The protein content of *A. cruentus* (28.6%) is considerably higher than other vegetables consumed in SA. Hanif *et al*. (2006) measured protein content ranging from 0.9 to 2.1% in cauliflower, carrot, cabbage, lettuce, spinach.

OMET grown Amaranth species recorded higher protein content than the same Amaranth species grown under non-OMET system. There are numerous environmental and climatic factors that had an influence in the accumulation of protein in all the three Amaranth species. Wijewardana *et al*. (2019) revealed that the treatment with 100% ET (0.15 $m^3\ m^{-3}$), which achieved the highest yields, had the highest protein content, followed by soil moisture treatments 80% ET (0.14 $m^3\ m^{-3}$ and 60% ET (0.13 $m^3\ m^{-3}$), suggesting that maintaining a high level of soil moisture during the reproductive stage was beneficial to acquiring a higher protein content. In this study, OMET system compared to non-OMET system, was a moisture conserving growing technique which maintained a higher level of soil moisture content, which might have influenced highest accumulation of protein in OMET grown Amaranth species. Differences in species and growing medium (OMET and non-OMET system)

probably attributed to different protein contents. Amaranth vegetables grown under OMET system can be an important source of dietary protein.

Table 4.3: The effect of OMET system on total protein % found in three Amaranth species

Amaranth species		**Protein (%)**	**Nitrogen (%)**
A. caudatus	OMET	24.1a	3.9a
	non-OMET	21.4b	3.4b
P-value		0.042	0.037
A. cruentus	OMET	28.6a	4.6a
	non-OMET	24.1b	3.9b
P-value		0.048	0.041
***Amaranth* Spp.**	OMET	22.7a	3.6a
	non-OMET	20.3b	3.2b
P-value		0.040	0.036

Different letters in the same column= significance difference at p≤0.05 independently for each amaranth species

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4.3.2 Mineral compositions of three Amaranth species

Table 4.4 and 4.5 represents the concentrations of macro and micro-elements in three Amaranth species grown under the OMET and non-OMET growing system. All macro elements measured in both OMET and non-OMET grown Amaranth species were significantly different (p≤0.05), except for Ca measured in *A. caudatus*. In the present study, it has been revealed that the OMET grown *A. cruentus* had the highest Ca (130 mg/kg) and P (34.7 mg/kg) concentration, whilst the highest Mg (82.5 mg/kg) and K (276 mg/kg) concentrations were found in the OMET grown *Amaranthus* Spp. Although *A. cruentus* grown on the OMET system possessed the highest concentrations of Ca and P, the same species (*A. cruentus*) cultivated on a distinct treatment (non-OMET system) was found to contain the lowest concentrations of Ca

(88.3 mg/kg), Mg (56.6 mg/kg) and K (217 mg/kg) than all the other Amaranth species grown under both OMET and non-OMET. The same trend was observed in OMET grown *Amaranthus* Spp. that had the highest concentration of P. In contrary, the non-OMET grown *Amaranthus* Spp. had the lowest concentration of P (21.4 mg/kg) than the other species under both treatments. Except for the Ca concentration (104 mg/kg) of *A. caudatus* on both treatments, it is evident that the OMET system increasedthe concentration of the elements for all the Amaranth species.

Microelements such as Cu, Mn, Fe, Se, and Zn were quantified and recorded (Table 4.5). It is revealed that the OMET grown *A. cruentus* had the highest concentration of Cu (1.04 mg/kg), Se (8.13 mg/kg) and Zn (1.66 mg/kg), whereas the *Amaranthus* Spp. grown under OMET system was found to contain the highest concentration of the other two microelements, Mn (2.16 mg/kg) and Fe (3.41 mg/kg) than the rest of the species grown both under OMET and non-OMET system. OMET grown *A. caudatus* contained the second highest concentration of Fe (2.62 mg/kg) and Se (8.03 mg/kg). The highest Fe concentration of *Amaranthus* Spp. (3.41 mg/kg DW) was higher than the Fe concentration reported by Yahaya *et al.* (2012) in *A. caudatus* (1.06 mg/kg DW), Roselle (1.2 mg/kg DW) and Kenaf (1.17 mg/kg DW). In the study of Yahaya *et al*. (2012), Se concentrations for all the vegetables (*A. caudatus*, Roselle and Kenaf) were not determined. Yahaya *et al*. (2012) reported the Zn concentrations of *A. caudatus* (0.048 mg/kg DW), Roselle (0.043 mg/kg DW) and Kenaf (0.036 mg/kg DW) which are lower than the lowest Zn concentration of non-OMET grown A. cruentus (1.66 mg/kg DW) in the present study. The lowest concentrations of all the microelements in the present study were found in non-OMET grown Amaranth species. *Amaranthus* Spp. grown on non-OMET system had the lowest concentration of Cu (0.78 mg/kg), Fe (2.47 mg/kg), Se (6.36 mg/kg) and Zn (0.95 mg/kg) than the rest of the Amaranth species grown on both OMET and non-OMET system. In addition, the lowest concentration of Mn was found in non-OMET grown *A. cruentus* (1.17 mg/kg), higher than the concentrations reported by Yahaya *et al*. (2012) for *A. caudatus* (0.079 mg/kg DW), Roselle (0.104 mg/kg DW) and Kenaf (0.096 mg/kg DW). *A. caudatus* grown on both OMET and non-OMET system had no extreme (not highest or lowest) concentrations of the microelements as compared to other species. The OMET system enhanced the concentration of microelements compared to the non-OMET system.

Minerals are essential for plant growth, development, reproduction, and seed quality. Deficiencies in mineral uptake and transport due to abiotic stress such as drought especially at the reproductive stage result in yield loss and poor seed quality (Bellaloui *et al*., 2012). Lowered absorption of the minerals could be due to reduced transpiration flow, limited availability of energy for assimilation, and interference in the unloading mechanism (Farooq *et al*., 2009; Rouphael *et al*., 2012). OMET system has successfully increased the concentration of the mineral elements for Amaranth species than the non-OMET system. This could probably be due to its potential to eliminate leaching of nutrients, conserving and reserving them for uptake by the plant roots. The less to non-fluctuating temperatures of the OMET system growing medium is conducive for better nutrient absorption. The root exudates become useful for the plant since they are not leached. The non-OMET system on the other hand had little influence on the accumulation of mineral elements, probably due to its inability to conserve nutrients. The nutrients are leached during irrigation through deep drainage or seepage since there's no underlying plastic to inhibit drainage, the nutrients become out of reach for plant roots absorption. The mineral accumulation is also influenced by the degree of soil moisture stress. Both macro and micro-elements showed to be higher under OMET grown Amaranth species, subjected to highest soil moisture compared to non-OMET grown Amaranth species. It can be concluded that the optimum high moisture content positively enhances elemental composition.

Table 4.4: Macro element concentration of three Amaranth species grown under both OMET and non-OMET system

Macro elements (mg/kg DW)				
A. caudatus	**Ca**	**Mg**	**K**	**P**
OMET	104±0.043a	77.3±0.032a	251±0.041a	29.7±0.045a
non-OMET	104±0.043a	62.6±0.030b	244±0.038b	21.8±0.037b
A. cruentus				
OMET	130±0.038a	63.3±0.029a	254±0.046a	34.7±0.043a
non-OMET	88.3±0.033b	56.6±0.025b	217±0.033b	22.2±0.039b
***Amaranthus* Spp.**				
OMET	104±0.039a	82.5±0.038a	276±0.05a	30.3±0.041a
non-OMET	90.5±0.041b	66.2±0.032b	226±0.035b	21.4±0.036b

Values are expressed as means± standard error; different letters in the same column= significance difference at p≤0.05 per species between treatments

Table 4.5: Microelement concentration of three Amaranth species grown under both OMET and non-OMET system

Microelements (mg/kg DW)					
A. caudatus	**Cu**	**Mn**	**Fe**	**Se**	**Zn**
OMET	0.89±0.023a	1.73±0.046a	2.62±0.05a	8.03±0.049a	1.11±0.024a
non-OMET	0.84±0.021a	1.46±0.043b	2.5±0.050a	7.01±0.048b	1.09±0.036a
A. cruentus					
OMET	1.04±0.034a	1.78±0.048a	2.52±0.04a	8.13±0.050a	1.66±0.048a
non-OMET	0.89±0.023b	1.17±0.041b	2.51±0.04a	7.69±0.05b	1.28±0.045a
***Amaranthus* Spp.**					
OMET	0.93±0.025a	2.16±0.05a	3.41±0.39a	7.28±0.05a	1.27±0.047a
non-OMET	0.78±0.022a	1.64±0.04b	2.47±0.05b	6.36±0.048b	0.95±0.039b

DW=dry weight, values are expressed as means± standard error; different letters in the same column= significance difference at p≤0.05 per species between treatments

4.3.3 Amino acid composition

The total free amino acids in the three Amaranth species grown under OMET and non-OMET system were quantified and compared (Table 4.6). The results revealed that Amaranth species contained both essential and non-essential amino acids. There was a significant difference ($p \leq 0.05$) between amino acid concentration among the same OMET grown Amaranth species and non-OMET grow Amaranth species, except for the non-essential amino acid arginine in *A. caudatus*. On the other hand, although the non-essential amino acid concentration measured in the same species grown under both OMET and non-OMET system were significantly different ($p \leq 0.05$), the non-OMET improved the concentration of non-essential amino acids compared to OMET system.

Essential amino acids threonine, lysine, valine, isoleucine, leucine, and phenylalanine were detected in all Amaranth species grown under both OMET and non-OMET system. The levels of phenylalanine in OMET grown *A. cruentus* (2.54 mg/kg) (Table 4.6) were higher than the amount reported in in the leaves of *A. cruentus* (0.66 mg/kg) (Manyelo *et al*., 2020). The OMET grown *A. cruentus* also showed a slightly higher amount of leucine (2.21 mg/kg) than the amount reported for Cowpea cultivar VOP8 (2.19 mg/kg) and leaves of *A. cruentus* (1.55 mg/kg) at harvest (day 65 after planting) (Manyelo *et al*., 2020), although the leucine concentration in OMET grown *A. cruentus* was predominant, it was lower than the amount reported in the following Cowpea cultivars; VOP3 (2.46 mg/kg), VOP4 (2.44 mg/kg), VOP5 (2.35 mg/kg) and VOP7 (2.47 mg/kg) (Moloto *et al.*, 2020). The highest concentration of valine (1.45 mg/kg) detected in OMET grown *A. cruentus* showed to be lower than the amount reported by Moloto *et al*. (2020) in 7 Cowpea cultivars and the leaves of *A. cruentus* (1.51 mg/kg) (Manyelo *et al*., 2020). Moreover, the highest quantity of isoleucine (1.29 mg/kg), and threonine (1.15 mg/kg) detected in OMET grown *A. cruentus* showed to be both higher than the amount reported in leaves of *A. cruentus* [isoleucine (0.83 mg/kg) and threonine (0.85 mg/kg)] (Manyelo *et al*., 2020), but both lower than the amount reported by Moloto *et al*., (2020) in 7 Cowpea cultivars. The lysine concentration was 2.37 and 2.25 mg/kg in OMET grown *A. cruentus* and OMET grown *A. caudatus* respectively (Table 4.6), higher than the concentration reported in the leaves of *A. cruentus* (1.73 mg/kg) (Manyelo *et al*., 2020) and Cowpea cultivars, ranging between 1.39 and 2.04 mg/kg) (Moloto *et al.*, 2020).

Nonessential amino acids, serine, arginine, glycine, aspartate, and glutamate were detected in all Amaranth species grown under both OMET and non-OMET system. The glycine, aspartate and glutamate were identified as the predominant non-essential amino acids. The highest concentration of glycine in non-OMET grown *A. cruentus* (1.46 mg/kg) was much higher than the amount reported by Manyelo *et al*. (2020) in the leaves of *A. cruentus* (0.94 mg/kg), but all lower than the amount reported in Cowpea cultivars VOP3 (1.71 mg/kg), VOP4 (1.62 mg/kg), VOP5 (1.53 mg/kg) and VOP7 (1.50 mg/kg) (Moloto *et al.*, 2020). Aspartate content was the highest in non-OMET grown *A. cruentus* (2.02 mg/kg), lower than the amount reported in Cowpea cultivars, ranging between 3.46 and 5.96 mg/kg (Moloto *et al.*, 2020), and for the leaves of *A. cruentus* was not reported. The highest concentration glutamate (2.55 mg/kg) in non-OMET grown *A. cruentus* were lower than the concentrations reported in Cowpea cultivars, ranging from 3.07 mg/kg (VOP2) to 3.88 mg/kg (VOP5) (Moloto *et al.*, 2020). Serine was highest in non-OMET grown *A. cruentus* (1.16 mg/kg) (Table 4.5) which amount was higher than the amount reported in the leaves of *A. cruentus* (0.90 mg/kg) (Manyelo *et al*., 2020), but lower than the amount reported in Cowpea cultivar VOP4 (1.70 mg/kg). Moreover, the lowest concentration of arginine (0.53 mg/kg) was detected in non-OMET grown *A. cruentus* (Table 4.6), which amount was lower than the concentration reported in the leaves of *A. cruentus* (0.90 mg/kg) (Manyelo *et al*., 2020) and Cowpea cultivars, with the least amount being 1.80mg/kg obtained from VOP8 and the highest being 2.52 mg/kg from VOP4 (Moloto *et al*., 2020).

The essential and nonessential amino acids were determined and quantified, and it has been shown that they are highly available in the OMET grown *A. cruentus* than any other Amaranth species grown under both OMET and non-OMET system. The role played by accumulated amino acids in plants varies from acting as osmolyte, regulation of ion transport, modulating stomatal opening, and detoxification of heavy metals (Rai, 2002). Amino acids also affect synthesis and activity of some enzymes, gene expression, and redox homeostasis (Mhamdi *et al.*, 2010). Lysine, leucine, and phenylalanine were the three dominating essential amino acids whereas glycine, aspartate and glutamate were the dominating nonessential amino acids in all the three Amaranth species. Furthermore, both non-OMET grown *A. cruentus* and OMET grown *Amaranthus* Spp. shows to be the second potential sources of amino acids compared

to other Amaranth species with distinct treatments. Based on the amino acid analysis, it can be concluded that OMET grown *A. cruentus* can be regarded as a reliable source of essential amino acids (Table 4.6). Therefore, for the enhancement of a balanced diet, the daily consumption of *A. cruentus* needs to be encouraged. The trend or pattern in amino acid composition could relate to possible inherent differences between genotypes, species and/or treatment. Similar trends were observed regarding the concentrations of essential amino acids in *A. caudatus* and *A. cruentus* probably confirm that these cultivars respond almost the same to the same treatment as compared to *Amaranthus* Spp.

Although literature-based information on amino acids in commonly consumed vegetables is available, it is difficult to compare the findings due to the differences in analytical methods; the units and some of the results are presented on a dry rather than fresh weight basis or vice versa. Amino acids such as glycine, alanine, serine, threonine, lysine, aspartate, and glutamate, have greater influence on the flavour and taste (Wong *et al*., 2008). Furthermore, nonessential amino acids such as arginine, serine, glycine, aspartate, and glutamate possess numerous health benefits and the Amaranth species that are rich in these compounds can offer an ideal dietary supplement for vegetarians or to produce pharmaceutical or therapeutic products.

Table 4.6: Total essential and nonessential amino acids (mg/kg) found in three amaranth species grown both under OMET and non-OMET system

	Essential amino acids					
A. caudatus	**Threonine**	**Valine**	**Isoleucine**	**Lysine**	**Leucine**	**Phenylalanine**
OMET	0.92±0.024a	1.09±0.043a	0.97±0.031a	2.25±0.05a	1.66±0.044a	2.36±0.05a
non-OMET	0.66±0.025b	0.84±0.027b	0.76±0.026b	1.3±0.034b	1.25±0.038b	1.14±0.035b
A. cruentus						
OMET	1.15±0.034a	1.45±0.041a	1.29±0.027a	2.37±0.05a	2.21±0.042a	2.54±0.051a
non-OMET	0.93±0.036b	1.22±0.042b	1.12±0.039b	2.19±0.050b	1.92±0.051b	1.95±0.043b
Amaranthus Spp.						
OMET	0.88±0.021a	1.08±0.034a	0.98±0.028a	1.62±0.036a	1.66±0.041a	1.47±0.038a
non-OMET	0.43±0.021b	0.52±0.026b	0.46±0.022b	0.8±0.012b	0.74±0.024b	0.71±0.023b
Non-essential amino acids						
A. caudatus	**Arginine**	**Serine**	**Glycine**	**Aspartate**	**Glutamate**	
OMET	1.12±0.047a	0.97±0.034a	1.37±0.05a	1.2±0.041a	1.52±0.05a	

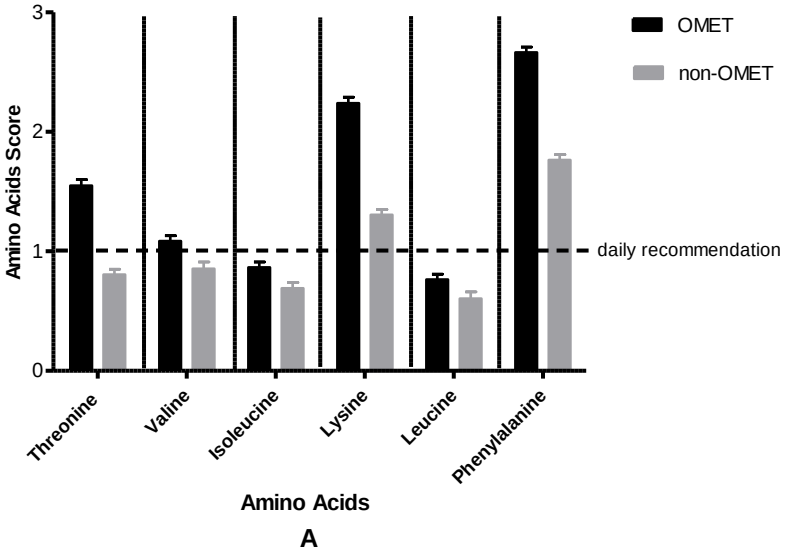
non-OMET	0.86±0.031a	0.7±0.030b	0.95±0.041b	0.96±0.042b	1.29±0.049b
A. cruentus					
OMET	1.37±0.048b	0.98±0.036b	1.33±0.044b	1.33±0.044b	1.75±0.05b
non-OMET	1.54±0.05a	1.16±0.045a	1.46±0.048a	2.02±0.05a	2.55±0.05a
***Amaranthus* Spp.**					
OMET	1.12±0.049a	0.91±0.042a	1.21±0.047a	1.53±0.049a	1.95±0.05a
non-OMET	0.53±0.028b	0.46±0.022b	0.59±0.029b	0.6±0.030b	0.73±0.032b

Values are expressed as means ± standard error; different letters in the same column= significance difference at p≤0.05 in column independently for each Amaranth species

4.3.4 Amino Acid Score (AAS) for essential amino acids measured in three Amaranth species

There was significant difference in Amino Acid Score for all the essential amino acids measured in OMET and non-OMET grown Amaranth species. Generally, the Amaranth species grown under OMET system scored the highest amino acid score (AAS) for each essential amino acid composition, except that the highest AAS for threonine, valine, isoleucine, and leucine were measured in *A. cruentus* grown under non-OMET system. The lowest AAS values for each essential amino acid were all found in *Amaranthus* Spp. grown under non-OMET system, followed by the non-OMET grown *A. caudatus* (Figure 4.1). Under OMET grown Amaranth species, the highest value of AAS for threonine (1.07), valine (1.16), isoleucine (0.94), lysine (2.3), leucine (0.84) and phenylalanine (2.82) were all found in *A. cruentus*, then in *A. caudatus* and *Amaranthus* Spp. The same trend for highest AAS of each essential amino acids is observed under non-OMET grown Amaranth species. The AAS for each essential amino acid composition found in OMET grown Amaranth species are greater than (>) 1 (Figure 4.1), meaning they are higher than the standard protein amino acid composition. In exception, the AAS values for isoleucine and leucine found in all three OMET grown Amaranth species are less than (<) 1, indicating that they are the limiting amino acids. In this study, leucine and isoleucine have shown to be the first and second limiting amino acids, respectively. The AAS values for threonine, valine, lysine, and phenylalanine found in *A. caudatus* grown under OMET are >1, therefore they are higher than the standard protein amino acid composition. In contrary, the AAS for isoleucine and leucine are <1, meaning they are the limiting amino acids. The AAS values for each amino acid found in *A. caudatus* grown under non-OMET system are <1, meaning they are the limiting amino acids, except lysine and phenylalanine with AAS values >1. Threonine, valine, lysine, and phenylalanine found in *A. cruentus* grown under OMET have the AAS that is >1 (refer to Figure 4.1), higher than the standard protein amino acid composition whereas isoleucine and leucine have the AAS <1, making them the limiting amino acids. The AAS values of each essential amino acid found in *A. cruentus* grown under non-OMET system were >1, except for the AAS of leucine which was <1. Threonine (1.01) and valine (1.03). The AAS values found in OMET grown *Amaranthus* Spp. are slightly >1, whereas the AAS values for isoleucine and leucine are <1 and for lysine and phenylalanine are >1, implying that they are the limiting amino acids and higher than the standard protein

amino acid composition respectively. As it was mentioned previously, the AAS values of each essential amino acid found in *Amaranthus* Spp. cultivated on non-OMET system are the lowest, <1 (Figure 4.1), implying that they are the limiting amino acids. The variation is obviously brought about by the different growing condition (medium) and species.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Threonine
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Isoleucine
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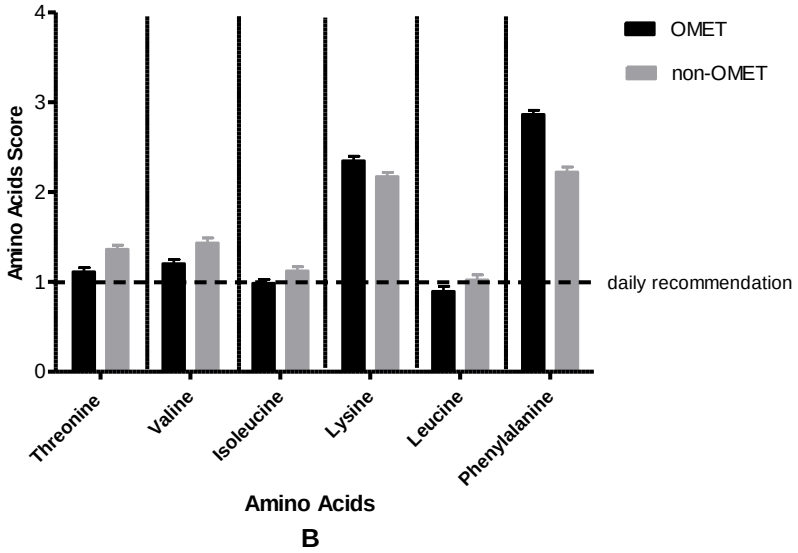
Amino Acids Score
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daily recommendation
Threonine
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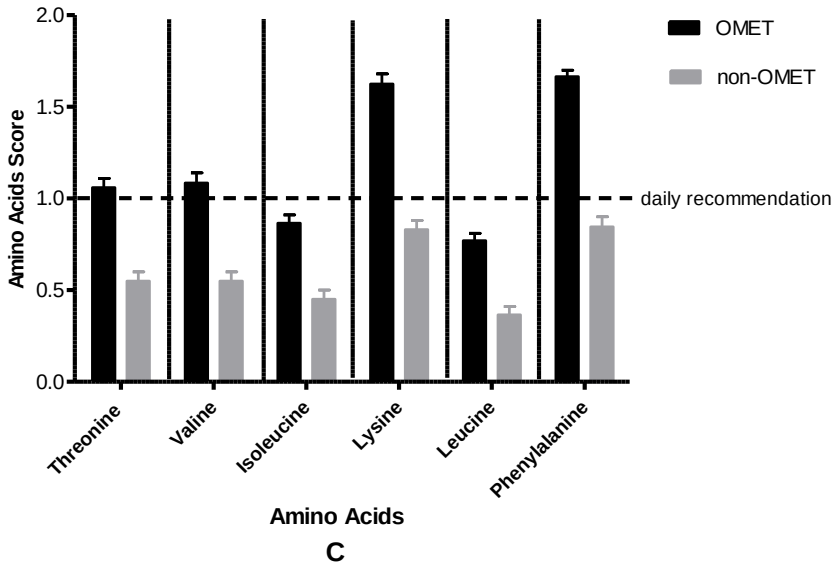
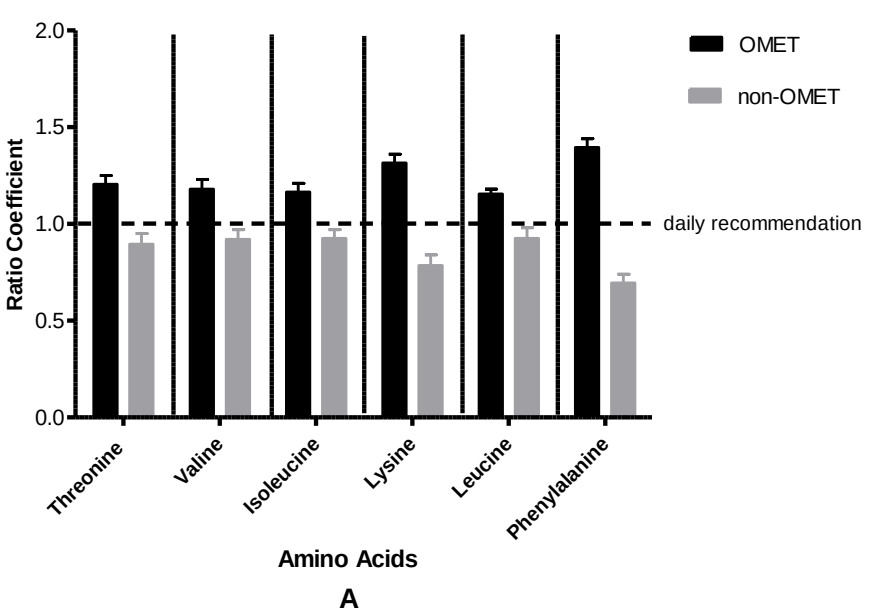


Figure 4.1: Amino Acids Score (AAS) for essential amino acids found in A- *A. caudatus*, B- *A. cruentus* and C- *Amaranthus* Spp. grown under OMET and non-OMET system

4.3.5 Ratio Coefficient (RC) of amino acids measured in three Amaranth species

In general, the effects of OMET and non-OMET system on the ratio coefficient of different amino acids composition are presented in Figure 4.2. OMET system influenced by increasing the RC of all the essential amino acids compared to non-OMET, except for threonine, valine, isoleucine and leucine found in *A. cruentus*. The highest RC of each essential amino acid composition was found in *Amaranthus* Spp. and *A. caudatus* grown under OMET system. The lowest RC values for all essential amino acid composition was found in non-OMET grown *Amaranthus* Spp., however, the RC value for valine under OMET grown *A. cruentus* was the lowest of them all (Figure 4.2).

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 (Björck *et al*., 1984; Shewry and Hey, 2015). As it is evident in Figure 4.2, the RC values for each essential amino acid under OMET grown *A. caudatus* and *Amaranthus* Spp. are >1, ranging from 1.12 to1.39, indicating that their contents were excessive. Conversely, the RC values for each essential amino acid composition under non-OMET grown *A. caudatus* and *Amaranthus* Spp. were <1, ranging from 0.61 to 0.88, implicating that their contents were scare in these two Amaranth species grown under non-OMET system and limited the nutritional value of proteins. The RC values for threonine, valine, isoleucine, and leucine found in *A. cruentus* were higher (>1, implying that it had surplus) under non-OMET system, than in OMET system, whereas lysine and phenylalanine found in *A. cruentus* had the highest RC values (>1, implying that their contents were excessive and limited nutritional value of proteins) under OMET than under non-OMET system (Figure 4.2). Threonine (0.9), isoleucine (0.93) and leucine (0.93) found in *A. cruentus* under OMET system and threonine (1.1), valine (1.09), isoleucine (1.07), lysine (0.96) and leucine (1.09) fond in *A. cruentus* under non-OMET system had the RC values that were closer to the standard amino acid (~1) (refer to Figure 4.2).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Ratio Coefficient
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Threonine
Valine
Isoleucine
Lysine
Leucine
Phenylalanine
Amino Acids

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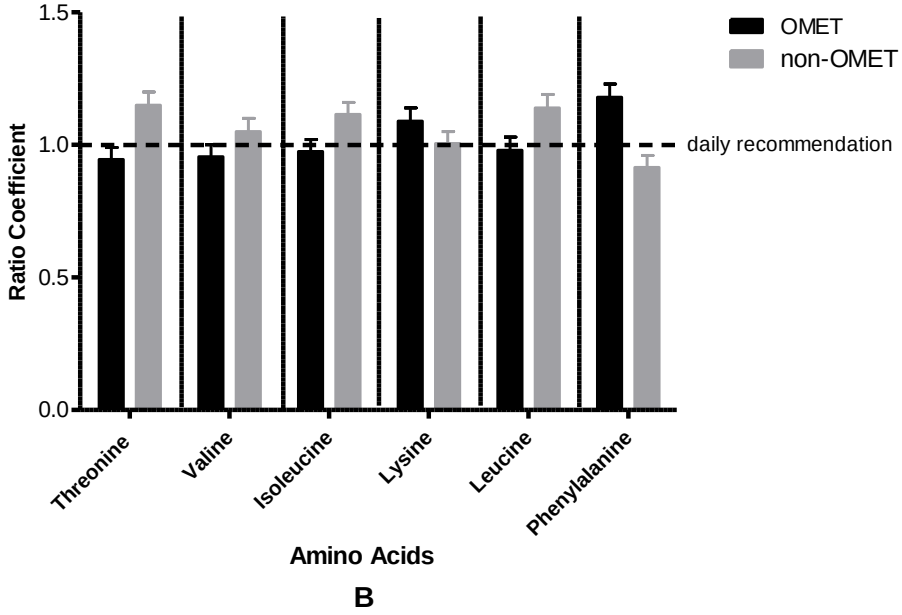
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daily recommendation
Threonine
Valine
Isoleucine
Lysine
Leucine
Phenylalanine
Amino Acids

B

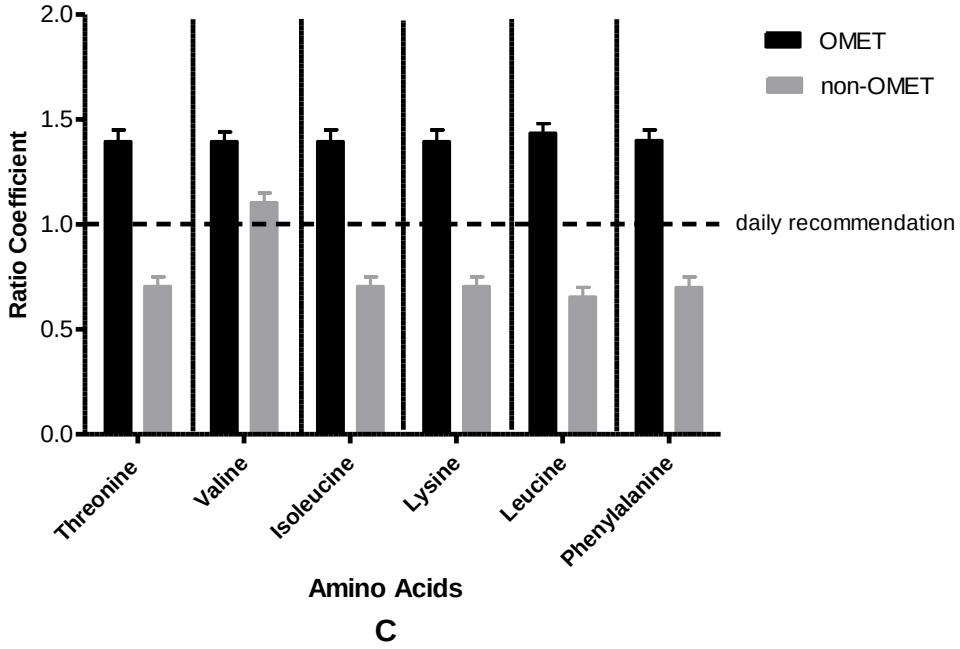

Figure 4.2 Ratio Coefficient for essential amino acids measured in **A**- *A. caudatus*, **B**- *A. cruentus* and **C**- *Amaranthus* Spp. grown under OMET and non-OMET

4.7 Conclusion and recommendation

The nutritional compositions of three Amaranth species were all significantly influenced by the OMET system. The protein content of OMET grown Amaranth species were significantly higher compared to the non-OMET grown Amaranth species. On the other hand, it was clear that the concentrations of the elements in three Amaranth species are affected by the treatments (OMET and non-OMET system) and variation of species. Macro elements were higher in Amaranth species grown under OMET system compared to the same species grown under non-OMET system. Micro elements including Cu, Fe and Zn were not all significantly different, whereas the rest of the micro elements determined were significantly different ($p \leq 0.05$). OMET grown *A. cruentus* was regarded as a reliable source of essential amino acids. Also, the *A. cruentus* grown under non-OMET system also showed high contents of non-essential amino acids Ala, Glu, Arg, Ser and Asp. Therefore, OMET grown *A. cruentus*, which was regarded as a useful source of non-essential amino acids, could be used more in the pharmaceutical industry as medicine.

The AAS and RC of amino acids were calculated, and it was observed that the RC values for essential amino acids found in OMET grown Amaranth species were higher and >1 except for threonine, valine, isoleucine, and leucine of *A. cruentus*. Those amino acids with RC values >1 showed to be excessive. Whereas the essential amino acids with RC value <1, indicating deficit content. The essential amino acids threonine, valine, lysine, and phenylalanine found in OMET grown Amaranth species had the AAS >1, implying that they are higher than the standard protein amino acid composition. On the other hand, isoleucine, and leucine in OMET grown Amaranth species had the AAS <1, implying that they are the limiting amino acids.

The findings of the study suggest maintaining optimum soil moisture, soil temperature and nutrient conservation (OMET system) is very significant for the accumulation of improved protein content, amino acids, and mineral content. It is important to investigate other growing techniques that will improve the yield and quality of indigenous vegetables. Furthermore, investigations in Amaranth species for the development of nutraceutical products will be essential. Inclusion of the Amaranth species in the food database is a good source of income for the government, Department of Agriculture, Forestry and Fishery.

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Chapter 5: Effect of OMET system on phytochemical composition in Amaranthus species

Abstract: Amaranth species are vegetables with a potential significantly reduce malnutrition. The OMET system is a non-drainable growing technique that is organic and prevent water and nutrients loss through drainage and/or seepage. The aim of the study was to investigate the effects of the OMET system on targeted and untargeted metabolites profile in *A. caudatus*, *A. cruentus* and *Amaranthus* Spp. using the liquid chromatography-mass spectrometry (LC-MS). Satistical analysis for significance difference was done at a confidence interval of 95% (p=0.05). The targeted metabolites included total phenolic acids, flavonols, tannins, carotenoids and chlorophyl. The non-OMET system has shown in this study to enhance the concentration of the three targeted metabolites including total phenolic acids, flavonols and tannins measured in the three Amaranth species compared to OMET system. In contrary, the OMET system significantly enhanced the concentration of plant pigments; carotenoids and chlorophyll measured in the three Amaranth species. Principal component analysis (PCA) of the metabolites clearly showed that the species were grouped according to the treatment. The use of unsupervised PCA gave two main clusters; *Amaranthus* Spp. on OMET system showed distinctive metabolome profile different from samples of *A. caudatus* and *A. cruentus* irrespective of their growth condition. The clustering between samples of *A. caudatus* and *A. cruentus* grown on either OMET or non-OMET was unclear. A supervised OPLS-DA plot was generated, and results showed good model statistics with predictive ability (Q2 cum value: 62%) above 50%. Two clear clusters were observed to separate *Amaranthus* Spp. and *A. caudatus* grown on OMET, and *A. cruentus* grown on OMET and the other three species grown on non-OMET. The OPLS-DA model was generated to demonstrate metabolites biomarker responsible to the OMET cluster (*mz*: 467.32) and non-OMET cluster (*mz*: 421.28) irrespective of the studied cultivar. The study revealed that the OMET system significantly affect metabolites of the *Amaranthus* Spp., with the same/slight difference influence as non-OMET in *A. caudatus* and *A. cruentus*. Further studies on its quantification are recommended and necessary.

5.1 Introduction

Diet-related diseases including type-2 diabetes (Stroke), cardiovascular disease, and cancer, are the major non-communicable diseases contributing to mortality in South Africa (Stats S.A., 2021). In fact, these non-communicable diseases are responsible for 41 million people deaths per year worldwide (Stats S.A., 2021). The incidences of non-communicable diseases have been more predominant in populations who often consume ready-made- high fat and sugary foods (Stats S.A., 2021). Yet, research reports have proved the efficacy for consumption of indigenous plant-based-diet on improving immunity and diet related disease mitigation (Tchuenchieu and Kesa, 2020). Indigenous food crops including vegetables, fruits and grains are usually regarded as underutilised, forgotten, or orphaned (Tchuenchieu and Kesa, 2020). However, their inclusion in diets contributes to food diversification which is a key foundation for healthy human immunity (Akinola *et al*., 2020). The value to indigenous food crops within a society contributes not only to diets, but it also constitutes to green environment climate and biodiversity which further creates sustainable production (Akinola *et al*., 2020).

Indigenous food crops are often adapted to a wide range of marginal growth conditions which improvestheir accessibility during crucial period of non-production such as drought. Furthermore, indigenous food crops are a rich source of phytochemicals. Phytochemicals are defined as ‘non-nutritive secondary metabolites which contain preventative and therapeutic actions against degenerative non-communicable diseases’ (Manach *et al*., 2009). Their role is based on improving immunity which ultimately improves defence against an ‘offender’ (Manach *et al*., 2009). Moreover, different pre- and post-harvest conditions, and cultivar variation can be useful tools to release multifaceted phytochemical profiles and enhance their bioavailability, bio-accessibility, and bioactivity (Kocira *et al*., 2020; Mpai and Sivakumar, 2020). Indigenous vegetables and fruits are consumed as fresh or processed and known to be among the most important sources of phytochemicals for the human diet (Oz and Kafkas, 2017). Phytochemical compositions depends on the species type, plant organ/part, cultivation practices and cooking methods (Akin-Idowu *et al*., 2017; Karamać *et al*., 2019;Managa *et al*., 2020; Manyelo *et al*., 2020).

For instance, Mateos-Maces *et al*. (2020) reported phytochemical compositions in different *Amaranthus* species such *A. cruentus* to be dominated by total phenolic

compounds equipped with phenolic acids (hydoxycinnamic and hydroxybenzoic acid) and flavonoids. A different study found traces of alkaloids, flavonoids, saponins, tannins, phenols, hydrocyanic acid in *A. hybridus* leaves (Akubugwo *et al*., 2007). Furthermore, studies aimed at identification of phytochemicals such as vitamin C, and different phenolic compounds (ferulic, chlorogenic, caffeic, gallic, chlorogenic, vanillic, p-hydroxybenzoic, p-coumaric syringic acids; rutin; phloridzin; myricetin; quercetin; naringenin; phloretin; galangin and apigenin) were conducted in a diverse *Amaranthus* species including *A. tricolor, A. acanthochiton, A. deflexus A. viridis* and *A. hybridus* (Santiago-Saenez *et al*., 2018). In addition, the phytochemical composition in the red coloured Amaranth species was found to outperform the green coloured species (Santiago-Saenez *et al*., 2018). Given the extensive work on phytochemicals studies in amaranth species, there is still a lack of information relating to the effect of the OMET growth technique on phytochemical compositions in amaranth species of South Africa, and thus the motive of the present study.

5.2 Methodology and analytical procedure

5.2.1 Study site

The study was conducted at the location descirbed in section 4.2.1.

5.2.2 Statistical analysis

The study adopted a single factorial analysis (OMET) in each amaranth species. Mean separation for significant differences was achieved through a T-test at the significance level of 5% (P=0.05) using computer statistical software, Statistix 10.0.

5.2.3 Procedure

5.2.3.2 Plant materials

Leaves from the three Amaranth species; *A. caudatus*, *A. cruentus* and *Amaranthus* Spp., were obtained from the experiment that was conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province in South Africa. Fresh leaves that were free from damage were harvested eight (8) weeks after transplanting. Thereafter, the leaf samples for each Amaranth species were packaged separately for each species in brown bags and oven

dried at 40 ºC for 72 hours. After drying them, they were then ground separately for each Amaranth species into fine powder using a coffee grinder and packed into sachets and were therefore ready for extraction.

5.2.3.3 Extraction of polar biochemical assays and metabolites in three Amaranth species

Oven dried leaf samples of three Amaranth species were prepared according to the method described by Mpai *et al*. (2018) with minor modifications. A volume of 2000 mg was mixed with 2 ml acidified methanol containing 80 % methanol, 19.5 % distilled water and 0.05 % hydro-chloric acid (HCl) in a thermostatic shaking water bath at 70 °C for 30 min−1. Subsequently, the mixture was centrifuged at 10 000 rpm for 15 min^{-1} at −4 °C, and the supernatant filtered through Whatman® no 1 filter paper. The pooled filtrates were dried under N_2 gas flow at 35 °C, thereafter, re-suspended with 1.5 ml of extraction solution and filtered through a hydrophobic PTFE syringe filter (0.22 µm pore size) prior to untargeted and targeted metabolites analysis.

5.2.3.5 Extraction of carotenoid and chlorophyll

The estimation of β-carotene and chlorophyll was performed using oven dried leaves of Amaranth species (2 g) and the procedure adopted was similar to that described by Mpai *et al*. (2020) with some modifications. β-carotene and chlorophyll were extracted using 1.5 ml of acetone-hexane mixture (4:6 volume/volume, v/v) which was again used as a blank. After vigorous shaking and centrifugation at 10000 rpm for 10 min, 250 µl of the supernatant was transferred into a 96 well micro plate and the absorbance was read at 453, 555 and 663 nm wavelength.

5.2.4 Data collection

5.2.4.1 Determination of total phenols

The total phenolic content of the Amaranth leaves of the three species was 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 10% Folin-Ciocalteu reagent (Sigma) in each test tube. Sodium carbonate (Na2CO3) (Sigma) (1.25 ml) was added and the mixtures were incubated 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.

5.2.4.2 Determination of total flavonoids content

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 acid (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 quercetin equivalence/ gram of extract (mg CA/g extract).

5.2.4.3 Determination of total tannins

The Folin-Ciocalteu method (Tambe and Bhambar, 2014) was used to determine the tannin content of the three Amaranth species leaves. Briefly, 100 µl of 10 mg/ml of the

concoctions and aqueous plant extracts was added to a clean test tube containing 7.5 ml of distilled water. Then 35% of Folin-Ciocalteu reagent (Sigma) was added to the mixture and vortexed. Ten millilitres of a 35% solution of sodium carbonate (Na_2CO_3) were added to the mixture. The mixture in the tube was transferred to a 10 ml volumetric flask and the volume of the mixture was made up to 10 ml with distilled water. The mixture was shaken and kept at room temperature for 30 min in the dark. Catechin acid (Sigma) was used as a standard and reference standard solutions (1.0-0.625 mg/ml) were prepared. The absorbance for the solutions was measured against a blank that was prepared in the same manner as the test solutions. A UV/VIS spectrophotometer was used to measure the absorbance at 725 nm. Tannin content was expressed as milligram gallic acid equivalence/ gram of extract (mg CA/g extract). The experiment was conducted in triplicates and independently repeated three times.

5.2.4.5 Determination of the total carotenoids

The estimation of β-carotene was performed using the dried samples of the Amaranth species and the procedure adopted was similar to that described by Tinyane *et al*. (2013). β-carotene was extracted using 1.5 ml of acetone-hexane mixture (4:6 volume/volume, v/v) which was again used as a blank. After vigorous shaking and centrifugation at 10000 rpm for 10 min, 250 µl of the supernatant was transferred into a 96 well micro plate and the absorbance was read at 453, 505 and 665 nm wavelength. The β-carotene was determined using the following calculation: β-carotene = (0.216 A663) – (0.304 A505 + 0.452 A453) and was expressed as µg of β-carotene per g of the dry sample (DW).

5.2.4.6 Determination of total chlorophyll

The chlorophyll a (Chl a), b (Chl b) and total chlorophyll (Chl a+b) were determined according to Managa *et al*. (2020) without modifications using leaf samples (0.2 g) ground in 2 ml of acetone and hexane 4:6 (v/v) and extracted for 2 hours. Afterwards, the sample mixture was centrifuged for 10 minutes at 4 ºC (9558× g). Thereafter, the resulting supernatant was decanted, and a portion of the solution was measured at 470, 646 and 662 nm (Biochrom Anthos Zenyth 200 Microplate Reader; SMM Instruments, Biochrom Ltd., Johannesburg, South Africa). The Chl a and Chl b

contents were determined according to equations: Chl a+b= (11.75. A470) – [(2.32. A646) + (0,452. A662)]. The content of Chl a+Chl b gives the total chlorophyll content, and it was expressed in mg per 100 g on a fresh weight basis (Mampholo *et al*., 2013).

5.2.4.7 UPLC-MS/MS analysis

The metabolites of the extracts were analysed using RP High Strength Silica (HSS) T3 C18 column (100 mm 2.1 mm containing 1.7 mm diameter particles, Waters), using a Waters Acquity UPLC system.40 The mass spectra were acquired by full scan MS in both positive and negative ionization modes on an exactive high resolution orbitrap-type MS (Thermo Fisher, Bremen, Germany) (Salem *et al*., 2017).

5.2.5 Statistical Analysis

A randomized complete block design (RCBD) was adopted with 3 replicates for each species. A single factorial type of experiment was conducted, which include only the OMET system vs Amaranth species. T-test was used to analyse the difference between different effects of OMET and non-OMET system at a significance level of 5%. Interaction between each species of the three Amaranth species and OMET system was investigated in this study.

5.3 Results and discussion

Comparison is done independently for each Amaranth species between OMET and non-OMET system

Table 5.1: Effects of OMET system on antioxidant capacity of three Amaranth species

A. caudatus	**Total phenols**	**Total flavonoids**	**Total tannins**	**Carotenoids**
OMET	*	*	**	*
non-OMET	*	*	**	*
A. cruentus				
OMET	*	*	**	ns
non-OMET	*	*	**	ns
***Amaranthus* Spp.**				
OMET	*	*	**	*
non-OMET	*	*	**	*

** and * in the same column= significant difference at p≤0.01 and p≤0.05, respectively; ns= not significant

Table 5.2: Effects of OMET system on chlorophyll composition of three Amaranth species

A. caudatus	**Chl a (mg/kg)**	**Chl b (mg/kg)**	**Chl a+b (mg/kg)**
OMET	ns	ns	ns
non-OMET	ns	ns	ns

A. cruentus			
OMET	ns	ns	ns
non-OMET	ns	ns	ns
***Amaranthus* Spp.**			
OMET	*	*	*
non-OMET	*	*	*

* in the same column= significant difference at p≤0.05; ns= not significant

5.3.1 Effects of OMET system on total phenolic compounds in three Amaranth species

The total phenolic compounds obtained from the present study are shown in Figure 5.1. There was significant difference (p≤0.05) in phenolic contents found in Amaranth species grown under OMET and non-OMET respectively. There was a significant increase of phenolic content in Amaranth species grown under non-OMET system. Those that were cultivated under OMET system showed phenolic contents lower that the non-OMET grown Amaranth species.

As observed in Figure 5.1, the highest value for phenolic compounds in non-OMET grown Amaranth species was measured in *A. cruentus* (262.45 mg GAE/100 g DW). The phenolic content of the non-OMET grown Amaranth species in this study ranged between 257.234 and 262.45 mg GAE/100 g DW. Less values for phenolic compounds were found under OMET grown Amaranth species, with the lowest phenolic content measured in *Amaranthus* Spp. (207.7 mg GAE/100 g DW). The values of total phenolic reported by Muriuki *et al*. (2014) for *A. cruentus* (3.59 mg GAE/100 g) and *A. hybridus* (2.77 mg GAE/100 g) were much lower than the phenolic composition measured in *A. cruentus* grown under non-OMET system (262.45 mg GAE/100 g DW) and those that were reported by Nana *et al*. (2012) who measured 8.30 mg GAE/100g in *A. hybridus*. Although the total phenolic reported by Nana *et al.* (2012) were higher than those reported by Muriuki *et al*. (2014), they were still much

lower than the lowest value measured in *Amaranthus* Spp. (207.7 mg GAE/100 g DW) grown under OMET system. The phenolic content of the OMET grown Amaranth species in this study ranged from 207.7 to 210.24 mg GAE/100 g DW.

Phytochemicals have several functions in plants. They act as cell wall support material (Neutelings, 2011), as colourful attractants for birds and insects helping seed dispersal and pollination (Stevenson *et al*., 2017). They are also important in plant defence against different environmental stress conditions such as 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 phenolic compounds in Amaranth species is probably due to reduced stimulation of phenolic biosynthesis by light, hence the experiment was conducted in the greenhouse. Moisture stress has a positive relationship with biosynthesis of phenolic compounds. In this study, non-OMET grown Amaranth species measured the highest phenolic compounds probably due less moisture available for plant uptake compared to OMET system. The accumulation of phenolics is increased in plants grown under non-OMET system, probably because they were subjected to abiotic stress/ non conducive growing conditions compared to OMET grown Amaranth species. It is evident that the synthesis of phenolic compounds and accumulation in Amaranth species could be directed by growing conditions.

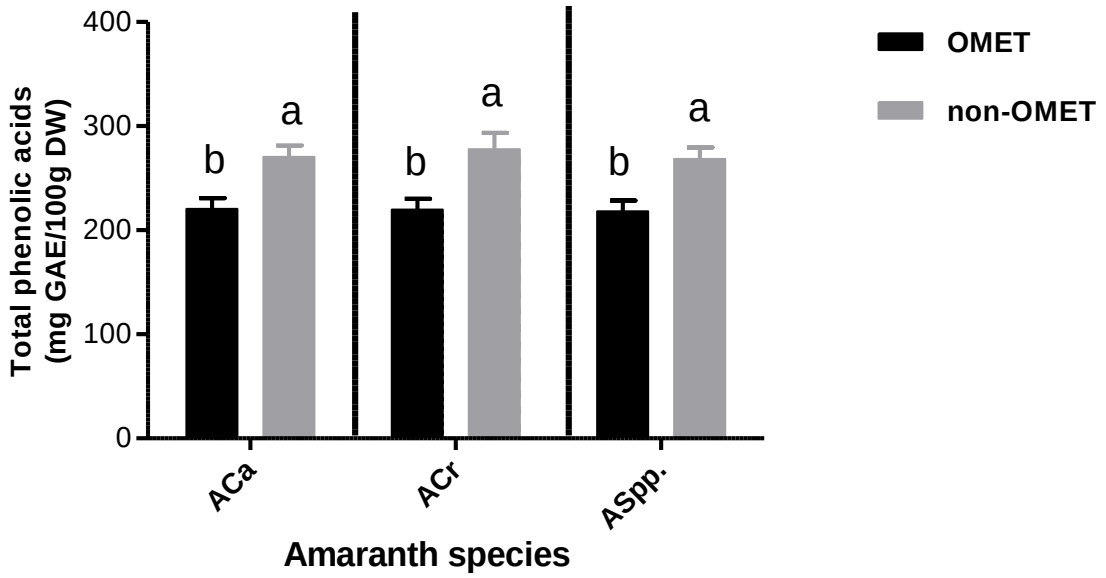


Figure 5.1: The effect of OMET system on total phenolic compounds three Amaranth species. Bars with different letters indicate significant difference (p≤0.05). ACa. = *A. caudatus;* ACr. = *A. cruentus,* ASpp. = *Amaranthus* Spp.

5.3.2 Effects of OMET system on total flavonoids in three Amaranth species

The flavonoid content of three Amaranth species grown under both OMET and non-OMET system are shown in Figure 5.2. The flavonoids content between OMET and non-OMET grown Amaranth species was significantly difference (p≤0.05. The highest flavonoid concentrations for each species were measured in Amaranth species grown under non-OMET system and compared to the OMET system. The highest flavonoid content was measured in non-OMET grown *A. cruentus* (473.28 mg CA/100 g DW). Conversely, the lowest flavonoids concentration was measured in *Amaranthus* Spp. (364.24 mg CA/100 g DW) grown under OMET system. The flavonoid concentration measured in non-OMET grown *A. caudatus* (449.34 mg CA/100 g DW) and *Amaranthus* Spp. (447.59 mg CA/100 g DW) had no significant difference. These potentially indicate that the OMET system has a negative relationship with flavonoid synthesis and accumulation. But, the non-OMET system appears to increase flavonoids in all three Amaranth species. This is probably due to the effects of abiotic factors such as light, temperature and moisture content.

Just like phenolics, light appears also to induce flavonols synthesis in the chloroplast and cytoplasm. Flavonoids as polyfunctional compounds in green plastids fulfil three major functions as: substrates (use polyphenolic and their catabolic products for other kinds of biosynthesis), energy sources (electron and proton transport, ion exchange and membrane potential, radical formation) and regulators (involvement in enzyme reactions as inhibitors or activators). During photosynthesis under light, flavonoids change the rate of electron transport and photophosphorylation, bringing about change of ATP/NADPH ratio (Khandaker *et al*., 2008).

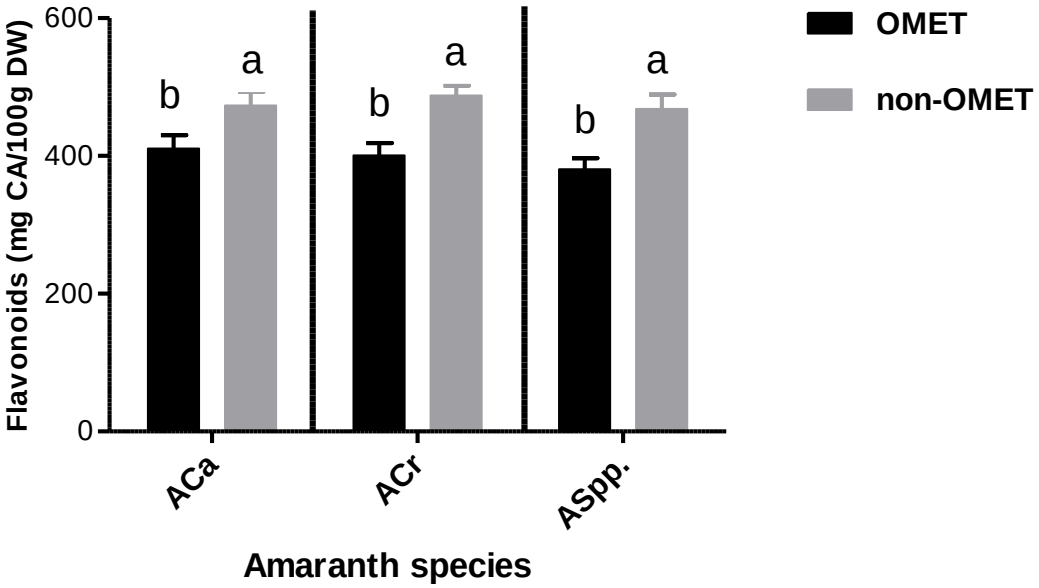


Figure 5.2: The effect of OMET system on total flavonoids available in three Amaranth species. Bars with different letters indicate significant difference (p≤0.05). ACa. = *A. caudatus;* ACr. = *A. cruentus,* ASpp. = *Amaranthus* Spp.

5.3.3 Effects of OMET system on total tannins in three Amaranth species

The concentration of total tannins measured in three Amaranth species is illustrated in Figure 5.3. There was a significance difference (p≤0.05) in total tannins measured between OMET and non-OMET grown Amaranth species. It is clearly shown that each of the non-OMET grown Amaranth species measured the highest tannins concentration as compared to the same Amaranth species grown under OMET system. Non-OMET grown *A. caudatus* contained the highest concentration (9.6 mg CA/100 g DW), followed by *A. cruentus* grown on non-OMET (8.78 mg CA/100 g DW).

The lowest content of tannins was measured in OMET grown *Amaranthus* Spp. (3.61 mg CA/100 g DW), followed by OMET grown *A. cruentus* (3.98 mg CA/100 g DW). Non-OMET system has shown to significantly (p≤0.05) improve the concentration of tannins in three Amaranth species than OMET system, that was probably due to variation in moisture content found in both treatments. The results were compared to findings of other authors on leafy vegetables. However, the comparison of the presented results with those reportedremains difficult because each study uses a different extraction method.

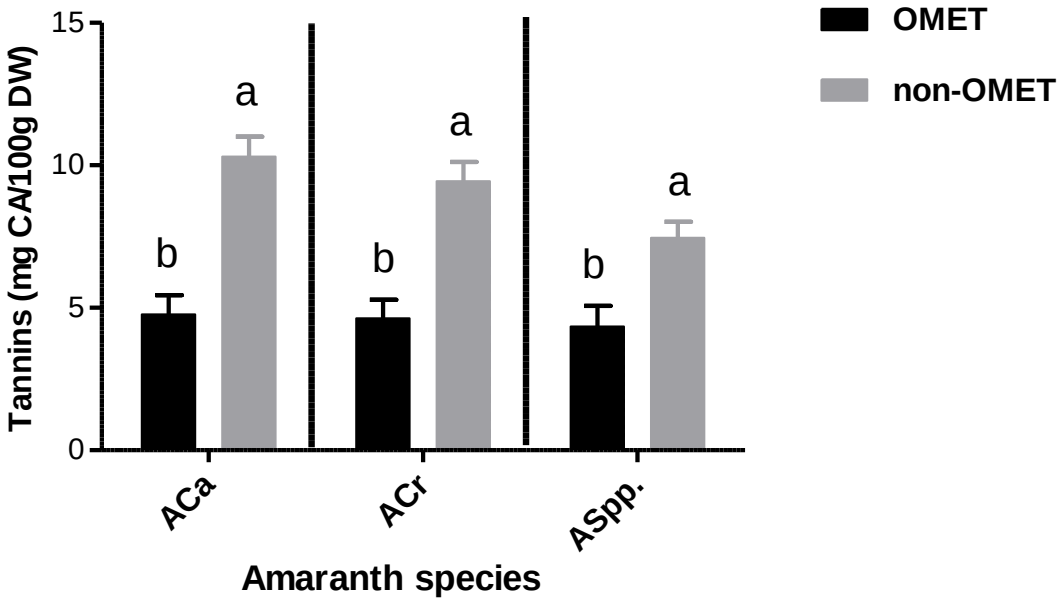


Figure 5.3: The effect of OMET system on total tannins (mg/kg) measured in three Amaranth species*]*. Bars with different letters indicate significant difference (p≤0.05). ACa. = *A. caudatus*; ACr. = *A. cruentus*, ASpp. = *Amaranthus* Spp.

5.3.4 Effects of OMET system on total carotenoid in three Amaranth species

Indigenous African leafy vegetables including Amaranth species, are rich in carotenoids (Neugart *et al*., 2017). Carotenoid-rich diets are correlated with a significant reduction in the risk for certain cancers, coronary heart disease, and several degenerative diseases (Mayne, 1996; Milani *et al*., 2017). Plants carotenoids play essential roles in functioning, survival, and expansion of plants (Uarrota *et al*., 2018).

Figure 5.4 illustrates the total carotenoid (mg/kg) of the three Amaranth species. There was a significant difference ($p \leq 0.05$) in total carotenoids measured in three Amaranth species grown under both OMET and non-OMET system. In the present study, the highest total carotenoid concentration was obtained in *A. caudatus* (52.96 mg/kg) grown under OMET system. Conversely, the lowest concentration of total carotenoid was observed in *Amaranthus* Spp. (33.84 mg/kg) grown under non-OMET system. The concentrations were slightly lower in *A. cruentus* (50.3 mg/kg) grown under non-OMET system and *A. cruentus* (48.28mg/kg) grown under OMET system respectively. There was a non-significant difference between the total carotenoid obtained from *A. cruentus* grown under non-OMET system and OMET system, OMET grown *Amaranthus* Spp. and non-OMET grown *A. caudatus*. Generally, OMET system has successfully enhanced the concentration of total carotenoid for *A. cruentus* and *Amaranthus* Spp. than the non-OMET system. *A*. *cruentus* grown under non-OMET system had the highest concentration of carotenoid than the *A. cruentus* grown under OMET system, although the difference was not significant.

The highest total carotenoid content obtained in *A. caudatus* (52.96 mg/kg) grown under OMET system was excessively higher than the results reported by Neugart *et al*. (2017) for *A. cruentus* (7.04 mg/kg), Cowpea [(918 ug/g DW) or (9.18 mg/kg)] and Spider plant (8.53 mg/kg). The total carotenoid reported for cowpea, spider plant and red amaranth (*A. cruentus*) by Neugart *et al*. (2017), were also excessively lower than the lowest total carotenoid content for *Amaranthus* Spp. (33.84 mg/kg) grown under non-OMET system in the present study. Although the total carotenoid for *A. cruentus* (7.04 mg/kg DW) reported by Neugart *et al*. (2017) was lower than the one reported in the present study for *Amaranthus* Spp. (33.84 mg/kg DW), it was higher than the total carotenoid for common kale (6.26 mg/kg DW), African nightshade (4.37 mg/kg DW) and Ethiopian kale (3.25 mg/kg DW) reported by Neugart *et al*. (2017).

This variation probably is caused by the variation in growing conditions and/or treatment (s). The accumulation of carotenoid is associated with plant physiological stress. The idea that plant stress stimulates synthesis of carotenoids in photosynthetic tissues is sensible because (1) many carotenoids are antioxidants and (2) some carotenoids play a central role in dissipation of absorbed energy in excess through the xanthophyll cycle (Mozzo *et al*., 2008; García-Plazaola *et al*., 2012).

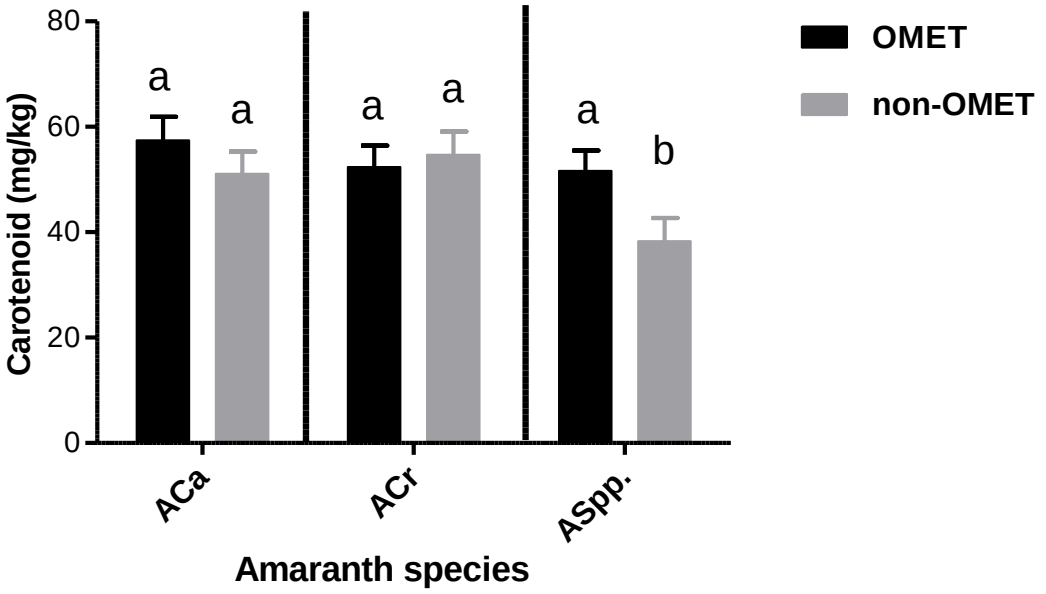


Figure 5.4: The effect of OMET system on total carotenoid (mg/kg) measured in three Amaranth species. Bars with different letters indicate significant difference (p≤0.05). ACa. = *A. caudatus*; ACr. = *A. cruentus*, ASpp. = *Amaranthus* Spp.

5.3.5 Effects of OMET system on total chlorophyll in three Amaranth species

Indigenous African leafy vegetables are a good source for chlorophyll. Chlorophyll, photosynthetic green pigments found mainly in plants, possess numerous health benefits (Muthusamy *et al*., 2020). Among these, chlorophyll a and chlorophyll b are the most important pigments and are found abundantly in almost all the organisms showing photosynthesis (da Silva Ferreira and Sant'Anna, 2017).

The highest chlorophyll (Chl a+b) was observed in OMET grown *A. caudatus* (48.085 mg/kg), followed by the non-OMET and OMET grown *A. caudatus* and *A. cruentus* respectively, which had the exact same concentration of Chl a+b (47.443 mg/kg). The lowest Chl a+b concentration was obtained in non-OMET grown Amaranthus Spp. (30.946 mg/kg). The results illustrate that the OMET system increased chlorophyll content in all the three amaranth species (Table 5.3).

Total chlorophyll content under OMET grown Amaranth species was higher compared to that of the same Amaranth species grown under non-OMET system. Drought stress is associated with major causes for crop loss and poor performance. Under such

stress, water deficit in plant tissues of the Amaranth species grown under non-OMET system develops, leading to a significant inhibition of photosynthesis. Several in vivo studies demonstrated that water deficit results in damages of the photosynthesis II (PSII) oxygen-evolving complex (Skotnica *et al*., 2000). Growing medium temperature and moisture content of OMET treatments was higher than that of non-OMET treatments. Chlorophyll is vital for photosynthesis as it helps to channel the energy of sunlight into chemical energy. With photosynthesis, chlorophyll absorbs energy and then transforms water and carbon dioxide into oxygen and carbohydrates.

Table 5.3: Total chlorophyll concentration (mg/kg) of *A. caudatus*, *A. cruentus* and *Amaranthus Spp*. grown under both OMET and non-OMET system.

A. caudatus	**Chl a (mg/kg)**	**Chl b (mg/kg)**	**Chl a+b (mg/kg)**
OMET	0.306±0.005a	47.779±0.004a	48.085±0.005a
non-OMET	0.301±0.0047a	47.142±0.003a	47.443±0.007a
A. cruentus			
OMET	0.301±0.0048a	47.142±0.008a	47.443±0.008a
non-OMET	0.296±0.0034a	46.306±0.003a	46.603±0.004a
***Amaranthus* Spp.**			
OMET	0.289±0.044a	45.253±0.048a	45.543±0.045a
non-OMET	0.197±0.036b	30.749±0.041b	30.946±0.039b

Chl a=Chlorophyll a; Chl b=Chlorophyll b; Chl a+b=Chlorophyll a+b; different letters in the same column= significance difference at p≤0.05

5.3.7 Chemometric approach to determine the effect of OMET system on untargeted metabolites profile in three Amaranth species

To summarise the obtained results from the HPLC-MS-QT of untargeted metabolites profiling, the chemometric analysis approach was applied to observe metabolite variations and similarities within samples treated with OMET and Non-OMET in three Amaranth species. With the use of unsupervised principal component analysis (PCA) (Fig 5.5A), two main clusters were observed and separated based on the Amaranth species. In fact, samples of *Amaranthus* Spp. grown on OMET system showed a holistic distinctive metabolome profile from samples of *A. caudatus* and *A. cruentus* irrespective of their growth condition (OMET system). However, there was no clear clustering between samples of *A. caudatus* and *A. cruentus* grown on either OMET or non-OMET. Therefore, a supervised OPLS-DA plot was generated, and results obtained therein showed good model statistics with predictive ability (Q2 cum value: 62%) that was above 50% (Fig 5.5B). Two clear clusters were now observed to separate 1: *Amaranthus* Spp. and *A. caudatus* grown on OMET, and 2: *A. cruentus* grown on OMET and the other three species grown on non-OMET. In Figure 5.6, the OPLS-DA model was generated to demonstrate metabolites biomarker responsible to the OMET cluster (*mz:* 467.32) and non-OMET cluster (*mz:* 421.28) irrespective of the studied cultivar. With a tentative identification metabolite with *mz:* 421.28 is associated with 'Apigenin 7-O-glucoside', while metabolite *mz:* 467.32 was still unknown (Table 5.4). The 'Apigenin 7-O-glucoside' is flavonoid compound which plays a role on improving the adaptation of crops to abiotic stress such as drought. Further studies on its quantification are necessary.

The results for HPLC-MS-QT of untargeted metabolites profiling through Pareto Scaling are illustrated in Figure 5.6. It is observable that the Pareto scaling has distributed the untargeted metabolites according to their similarities and distinctions. Among the metabolites, only few biomarkers are observed although some are unknown. Through tentative identification of metabolites biomarkers generated from the Pareto Scale, metabolite with *mz*: 389.31 is associated with 2-(2-fluorophenyl)-2-hydroxyacetic acid, *mz*: 217.004 with 1,2,3,4,5,6,7,8,9,10-decadeuterioanthracene and metabolites with *mz*: 474.3884, 474.389 and 421.2820 are unknown. In human

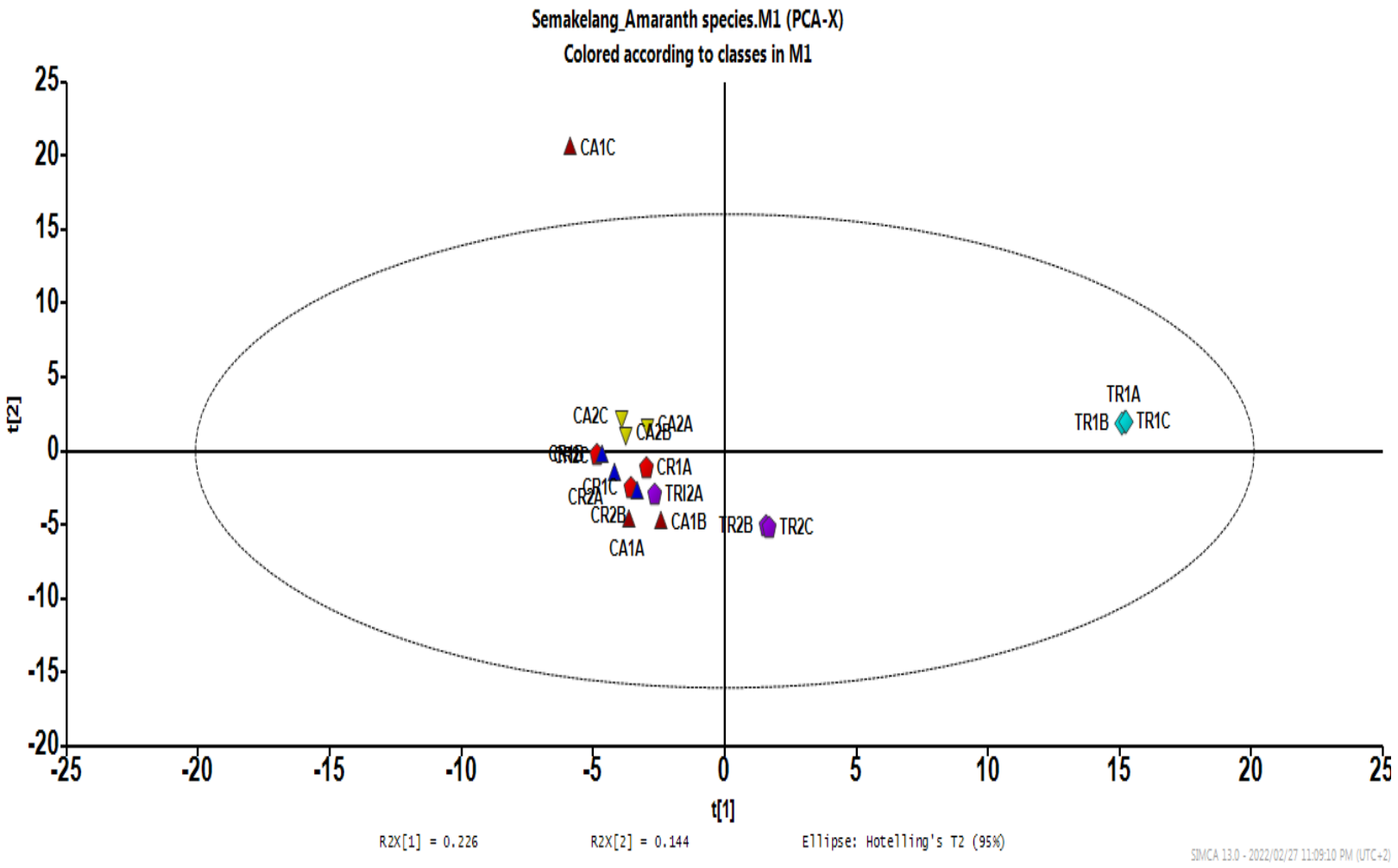
body, the 2-(2-fluorophenyl)-2-hydroxyacetic acid is responsible for illnesses such as skin irritation, eye irritation and respiratory irritation (specific target toxicity)

Table 5.4: Tentative identification of untargeted metabolites

Retention time (Min)	**Exact Mass (g/mol)**	**Mass generated ESI (-) TOF MS (g/mol)**	**Chemical Formula**	**Tentative structural assignment**
17.63	420.41	420.31	$C_{21}H_{24}O_9$	Apigenin 7-O-glucoside

TOF-MS= Time of Flight Mass Spectrometry

A


Semakelang_Amaranth species.M1 (PCA-X)
Colored according to classes in M1
CA1C
CA2C
CA2A
CA2B
CR1A
CR1C
CR2A
TRI2A
CR2B
CA1B
TR2B
TR2C
CA1A
TR1A
TR1B
TR1C
t[1]
t[2]
R2X[1] = 0.226
R2X[2] = 0.144
Ellipse: Hotelling's T2 (95%)
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B

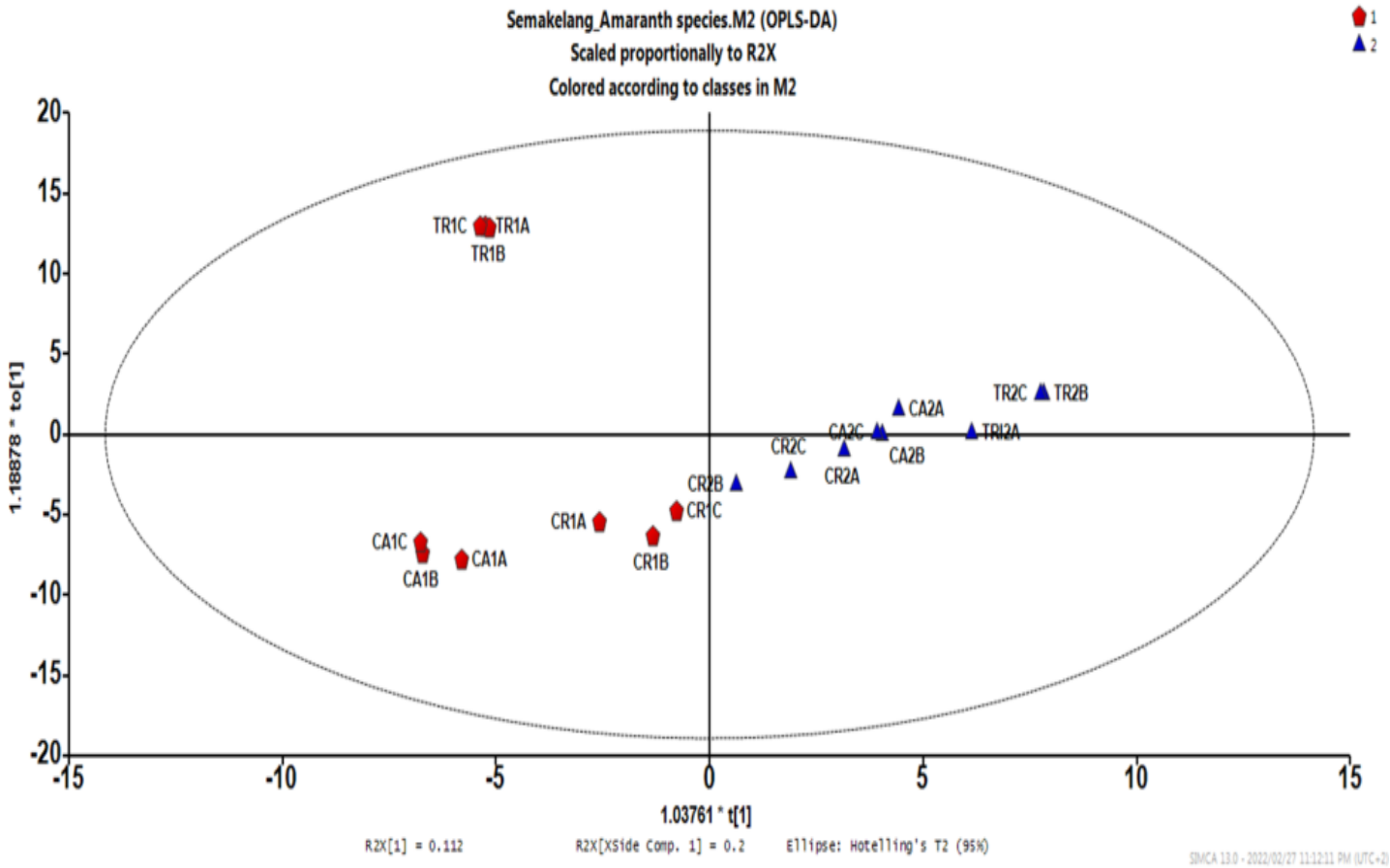

Figure 5.5: HPLC-MS-QT of untargeted metabolites profiling; A- unsupervised PCA and B- supervised PCA

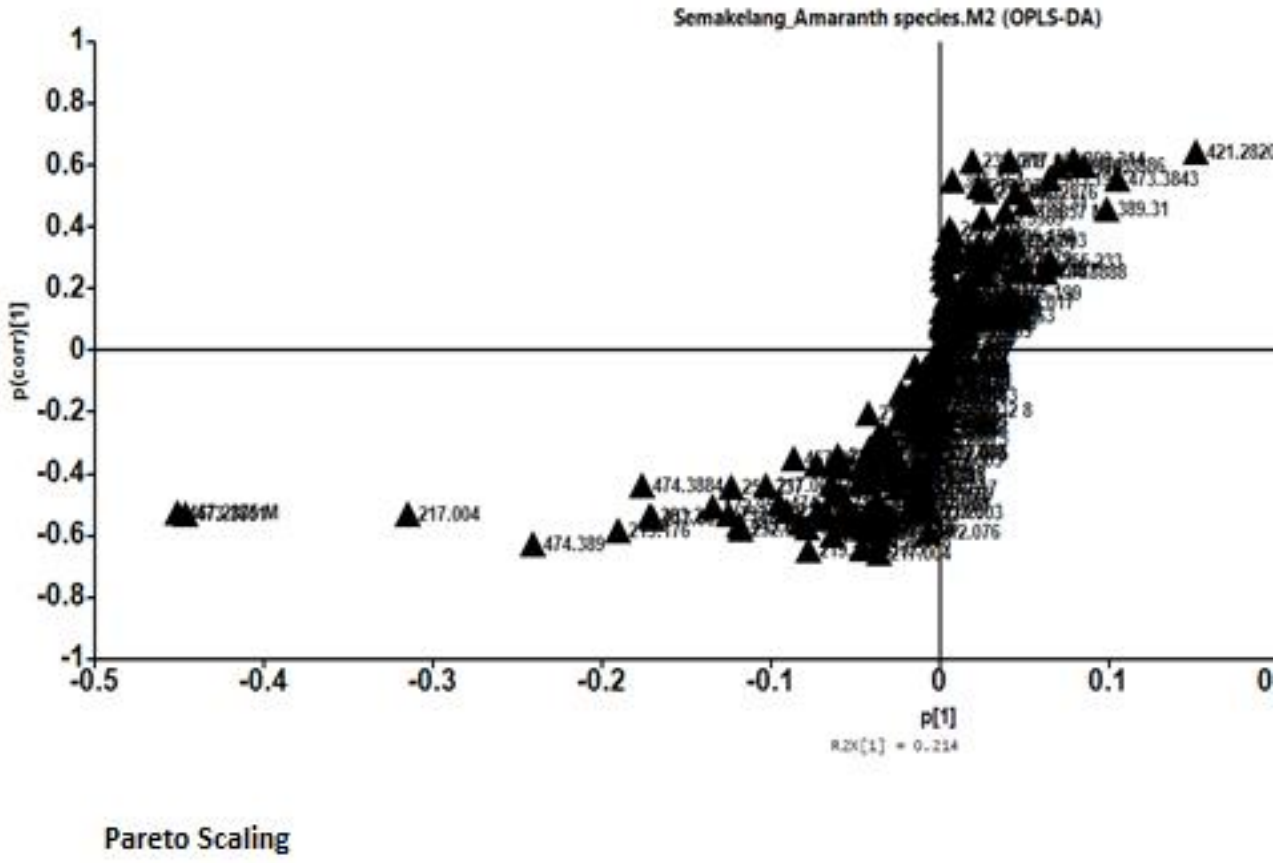

Figure 5.6: HPLC-MS-QT of untargeted metabolites profiling; Pareto scaling

Table 5.5: Tentative identification of untargeted metabolites profiling, Pareto scaling

Retention time (Min)	Exact Mass (g/mol)	Mass generated ESI (-) TOF MS (g/mol)	Chemical Formula	Tentative structural assignment
10.79	218.3	217.004	$C_{14}H_{10}$	1,2,3,4,5,6,7,8,9,10-decadeuterioanthracene
20.56	390.6	389.31	$C_8H_7FO_3$	2-(2-fluorophenyl)-2-hydroxyacetic acid
-	-	421.2820	-	Unknown
21.42	-	474.3884	-	Unknown
21.45	-	474.389	-	Unknown

TOF-MS= Time of Flight Mass Spectrometry

5.4 Conclusion and recommendations

This study illustrated the targeted and untargeted metabolites profile in three Amaranth species. The three Amaranth species have shown to be the rich sources of targeted metabolites (water-friendly metabolites) including phenolics, flavonoids and tannins, when cultivated under stressful growth conditions (non-OMET system) compared to OMET system. *A. cruentus* grown under non-OMET system was predominated by phenolic and flavonoids whereas *A. caudatus* was only predominated by tannins compared to different and/or same species grown under same and/or distinct treatment. On the other hand, carotenoids and chlorophyll measured the highest under OMET grown Amaranth species. Carotenoids predominated OMET grown Amaranth species (*A. caudatus* and *Amaranthus* Spp.) except for *A. cruentus*, which was higher in non-OMET system. Although there was no significant difference in chlorophyll composition between the same species grown under both OMET and non-OMET system, chlorophyll composition was predominant in all the OMET grown Amaranth species compared to non-OMET, except for *Amaranthus* Spp. Although the phenolics and flavonoids were found in higher concentrations non-OMET grown Amaranth

species, their health benefits depend on their bioavailability. Further studies on digestive stability, bio accessibility, bioavailability and subsequently bioactivity, both in vitro and in vivo, are strongly recommended to get a better understanding of nutritional values of cowpea leaves, an emerging food in the African market.

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CHAPTER 6: SYNTHESIS

Climate change is a pushing force for farmers to adopt new farming techniques and practices. The effects are unevenly distributed across the globe. Climate change indirectly impacts food and nutritional security, lower crop yields and nutritional quality due to drought, heat waves and flooding as well as increases in pests and plant diseases, leading to high rates of malnutrition and food insecurity. The good news is that there are tools in the form of science-based farming practices that buffer farmers from climate damage and help make their operations more resilient and sustainable for the long term, such as OMET system. In this study, the aim was to investigate the effects of the OMET system on the growth, yield, nutritional and phytochemical composition of Amaranth species. At the end of the study, analysis was done and the OMET system was found to enhance the growth attributes including stem diameter, root length, leaf length and plant height of the Amaranth species investigated in the study, especially *A. caudatus* and *A. cruentus*. The growing conditions under OMET system were conducive enough for to obtain high yield from the Amaranth species despite the fact that it utilizes less water compared to the non-OMET system. It was also observed that the time of flowering is also extended hence the plant is not under any physiological stress caused by extreme soil temperatures, weed competition, soil borne diseases, soil moisture and nutrients loss. Therefore, it can be concluded the use of OMET system will reduce the production cost while producing high and quality yield under sustainable use and conservation of resources.

The consistent efficiency and effectiveness of OMET system is observed via the enhanced and increased nutritional composition including protein content, mineral content and amino acid concentration. The highest protein content was found in OMET grown Amaranth species compared to the same species grown under non-OMET system. The same is observed in mineral elements content. OMET system has improved the essential and some of nonessential amino acids accumulation in grown Amaranth species than the non-OMET grown Amaranth species. All essential amino acids of OMET grown Amaranth species except for threonine, valine, isoleucine and leucine of *A. cruentus* had RC greater than (>) 1, indicating that they were excessive. Additionally, the RC values for non-OMET grown Amaranth species except for

threonine, valine, isoleucine and leucine of *A. cruentus* were less than (<1), implying that they were scarce.

Phytochemicals are bioactive non-nutrient plant compounds in plant foods. Their synthesis and accumulation are certainly influenced by several abiotic conditions. This study has clearly illustrated that the water friendly metabolites such as phenolic, flavonols and tannins are synthesized and accumulated in high concentration under plant stress conditions. The non-OMET system has fulfilled the high accumulation of these three metabolites in Amaranth species compared to the OMET system. In contrary, the OMET system increased the concentration of carotenoids and chlorophyll in all three Amaranth species compared to non-OMET system. The OMET system seeks to promote better growth and yield by providing conducive growing conditions (optimum moisture, nutrients availability etc.).