PRE- AND POST-HARVEST RESPONSE OF SELECTED INDIGENOUS LEAFY VEGETABLES TO WATER STRESS

INNOCENT MASEKO

Submitted in fulfillment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY IN HORTICULTURE

Discipline of Horticultural Science
School of Agricultural, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Pietermaritzburg
South Africa

July 2019

Student Declaration

Pre- and post-harvest response of selected indigenous leafy vegetables to water stress

I, **Innocent Maseko**, student number: **215082595** declare that:

i. The research reported in this thesis, except where otherwise indicated, is the result of my own endeavours in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg.

ii. This thesis has not been submitted for any degree or examination at any other university.

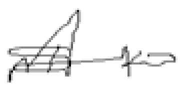
iii. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

iv. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

- Their words have been re-written, but the general information attributed to them has been referenced.
- Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

v. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed by Innocent Maseko on the 12th day of December 2018.



Innocent Maseko

Declaration Plagiarism 22/05/08 FHDR Approved

Declaration by Supervisors

We hereby declare that we acted as Supervisors of this PhD student:

Student full name: Innocent Maseko
Student number: 215082595
Thesis title: Pre- and post-harvest response of selected indigenous leafy vegetables to water stress

Regular consultation took place between the student and us throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Science Higher Degrees Office for examination by the University appointed examiners.

Supervisor: Prof. T. Mabhaudhi

Co-Supervisor: Dr. S. Tesfay

Co-Supervisor: Dr. B. Ncube

Declaration – Publications

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part of and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication).

Publication 1 (Published):

Maseko, I.; Mabhaudhi, T.; Tesfay, S.; Araya, H.T.; Fezzehazion, M.; Plooy, C.P.D. African Leafy Vegetables: A Review of Status, Production and Utilization in South Africa. *Sustainability* **2018**, *10*, 16.

Contributions: Manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 2 (Published):

I. Maseko, T. Mabhaudhi, S. Tesfay, B. Ncube, V.G.P. Chimonyo, H. T. Araya, M. Fezzehazion, C.P. Du Plooy. 2018. Moisture stress on physiology and yield of some indigenous leafy vegetables under field conditions. *in press*

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 3 (in preparation):

I. Maseko, T. Mabhaudhi, S. Tesfay, B. Ncube, V.G.P. Chimonyo, H. T. Araya, M. Fessehazion, C.P. Du Plooy. 2018. Productivity of selected African leafy vegetables to varying water regimes: Rain-shelter conditions.

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 4 (Published):

I. Maseko, T. Mabhaudhi, B. Ncube, S. Tesfay, V.G.P Chimonyo, H. T. Araya, M. Fessehazion, C.P. Du Plooy. 2018. Nutritional quality of selected African leafy vegetables cultivated under varying water regimes and different harvest. South African Journal of Botany, https://doi.org/10.1016/j.sajb.2019.06.016

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 5 (Published):

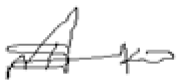
I. Maseko, T. Mabhaudhi, B. Ncube, S. Tesfay, H. T. Araya, M. Fessehazion, V.G.P Chimonyo, A.R. Ndhlala, C.P. Du Plooy, 2019. Postharvest drying maintains phenolic, flavonoid and gallotannin content of irrigated African leafy vegetables. Scientia Horticulturae. DOI: 10.1016/j.scienta.255: 70-76.

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 6 (in preparation):

Maseko, T. Mabhaudhi, B. Ncube, S. Tesfay, H. T. Araya, M. Fessehazion, V.G.P Chimonyo, C.P. Du Plooy, 2018. Influence of postharvest packaging, temperature and storage time on the phenolic composition and antioxidant properties of *Corchorus olitorius.*

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.



INNOCENT MASEKO

Declaration Publications FHDR 22/05/08 Approved

ACKNOWLEDGEMENTS

At the moment of writing these acknowledgements, I am taken back to O.R Tambo International Airport where I met Prof T. Mabhaudhi and we set the road for this PhD. There was no bursary or scholarship and there was no supervisor and Prof T. Mabhaudhi never intended to be one either. This study did not have specific research funds allocated to it; hence the success of this project was based on the people that Prof T. Mabhaudhi and I knew or had crossed path with. The completion of this study today marks an end of the discussion that started at the airport and became a by-product of this great unsung host who contributed in various ways in this study. To God be all the glory because He has always done exceedingly and abundantly, above all we have ever dreamt and imagined.

I acknowledge Agricultural Research Council in which the trials were conducted at, University of KwaZulu-Natal for awarding me the opportunity to study and Knilam Packaging (Pty) Ltd (Cape Town, South Africa) for providing the packaging papers free of charge. Prof T. Mabhaudhi; it has been a great privilege to meet such a calibre, who has played a major role in my academic life, first as an MSc mentor and as a Ph.D. supervisor. I hope there will be many possibilities for future cooperation. It was Prof T. Mabhaudhi who introduced me to my academic supervisor, Dr Samson Tesfay. "PhD is not a career and one needs to work hard to complete within stipulated time", this is Dr Tesfay's approach to student supervision. I am greatly indebted for his approach and consistently allowing this thesis to be my own work. Prof T. Mabhaudhi further introduced me to Dr F Melake, who became my research supervisor. I am greatly indebted to Dr F Melake for the technical support in setting trials, handing me over to Dr. H. T. Araya prior to his departure to USA and desiring the best out of me throughout the course of the study. My deepest gratitude goes to Dr. B. Ncube my co-supervisor who played a role in guidance, support, technical input during the course of the study. Further, Dr. B. Ncube introduced me to Dr Ashwell Ndhlala who is acknowledged for his input and guidance in biochemistry and agro processing activities.

I would also like to thank the experts who were involved in this research project. I am delighted to express my gratitude to Dr. C. P. Du Plooy for fitting me into the ARC projects and for his valuable inspiring guidance and dedication towards this research. Prof T. Mabhaudhi further introduced me to Dr V. Chimonyo who is acknowledged for assisting with scientific editing the chapters of the thesis and critical remarks on the manuscript.

I acknowledge the pleasure of working with Ndivhuwho Mpaphuli, for all the technical work, tireless and efficiency executed in this project. I am greatly indebted. I also appreciate my family for the struggles that we have fought together during the course of my studies. I would like to thank my loving wife, Musa Khabo for the support throughout the study as this has negatively impacted my relationship, holidays and visits to family and friends.

LIST OF ACRONYMS

ANT:	Antioxidant activity
ALVs:	African leafy vegetables
ANOVA:	Analysis of variance
ARC:	Agricultural Research Council
AWS:	Automatic weather station
CCI:	Chlorophyll Content Index
CF:	Chlorophyll Fluorescence
CLAMS:	Carotene-Linoleic Acid Model System
DMRT:	Duncan Multiple Range Test
ET_c:	Reference crop evapotranspiration
ETo:	Reference evapotranspiration
FAO:	Food and Agriculture Organisation
Folin C:	Folin Ciocalteu
FRAP:	Ferric-reducing power Assay
ISCW:	Institute of Soil, Climate and Water
LAN:	Lime (stone) ammonium nitrate
ORR:	Oxidation Rate Ratio
RCBD:	Randomised complete block design
VOPI:	Vegetable and Ornamental Plant Institute
WAT:	Weeks after transplanting

ABSTRACT

South Africa has wide diversity of African leafy vegetables (ALVs) rich in nutrients and adapted to marginal production. However, there is limited availability of ALVs in South Africa due to lack of cultivation owing to limited agronomic and postharvest management information. The increase in population growth, malnutrition and climate change necessitates production of more food using limited water resources. The aim of this study was to evaluate pre and postharvest response of *Amaranthus cruentus* (pigweed), *Vigna unguiculata* (cowpea), *Corchorus olitorius* (Jute mallow) and a reference crop *B. vulgaris* (Swiss chard) to varying irrigation regimes. The current study consisted of a literature review and five experiments (two agronomic studies and three post-harvest studies. In literature, the performance of ALVs is drawn in comparison to exotic counterparts grown under different conditions; yet agronomic and nutritional factors are only valid when crops are grown under the same condition. Hence in the four experiments of this study, Swiss chard was used as a reference crop grown under same locality. Swiss chard was chosen because it is an alien leafy vegetable that has been indigenised in sub-Saharan Africa and is highly nutritious (contains high levels of Fe, Zn and β-carotene).

Before conducting experiments there was need to identify potential gaps and research priorities for this study and even for future research. This was done by conducting a literature review study (Chapter 2) on the status of production and utilisation of ALVs in South Africa for the period 1994–2017. Results of the review indicated that there is a decline in consumption of ALVs partly as a result of limited availability and negative perception. In order to promote ALVs, further research on agronomy, post-harvest handling, storage and processing is required in South Africa.

Field and rain shelter experiments were conducted at Roodeplaat, Pretoria, over two summer seasons, 2015/2016 and 2016/2017 to evaluate growth, yield and water-use of selected leafy vegetables under varying water regimes. A randomised complete block design with three replicates was used. The treatments evaluated were: three irrigation regimes (30%, 60% and 100% of crop water requirement (ETc) on three ALVs – *Amaranthus cruentus*, *Corchorus olitorius* and *Vigna unguiculata* and a reference crop, *Beta vulgaris*. Seeds of *A. cruentus* and *C. olitorius* were obtained from the seed bank of the Agricultural Research Council (ARC) - Vegetable and Ornamental Plants (VOP), Roodeplaat, Pretoria seed bank. *Vigna unguiculata* (Bechuana white, a runner type) and Swiss chard (*B. vulgaris* L.) cultivar 'Ford Hook Giant' seeds were obtained from Hygrotech Seed Pty. Ltd., South Africa. Soil samples were taken from the field prior to land preparation and soil fertility analyses done at the Agricultural Research Council–Institute for Soil, Climate and Water (ARC–ISCW). Nitrogen, phosphate and potassium were applied according to the results and recommendations of the soil fertility analysis for both seasons. Seedlings of *A. cruentus*, *B. vulgaris* and *C. olitorius* were raised in commercial growing medium and covered with vermiculate to minimize water losses from above surface. *Vigna unguiculata* was sown directly. Seedlings were transplanted at four weeks after sowing. Irrigation scheduling was based on reference evapotranspiration (ET) and a crop factor for each crop. Data collection in field and rain shelter trials included plant height, leaf number, chlorophyll content index (CCI), chlorophyll fluorescence (CF) and yield.

In *A. cruentus*, drought stress (30% ETc) reduced yield consistently in both field and rain shelter trials. Plant height and chlorophyll content index (CCI) were significantly reduced by water stress under field conditions. For *C. olitorius*, drought stress (30% ETc) reduced yield under rain shelter conditions while all measured parameters were not affected under field conditions. In *V. unguiculata*, stem fresh mass increased with increase in water application from 30%-60 ETc with no further significant increase under field conditions while all measured parameters showed a similar trend under rain shelter although the results were not significant. In *B. vulgaris* leaf number, plant height, CCI, yield, Mg, Ca, Na, Zn, and Mn were reduced by water stress for rainshelter. Using 60% ETc proved to be suitable for production of *A. cruentus* and *B. vulgaris var. cicla* whereas 30% ETc would be recommended for *V. unguiculata*. For *V. unguiculata* and *C. olitorius* application of 30% ETc is recommended while application of 60% ETc can be used under to slightly improve yield. *Amaranthus cruentus* and *B. vulgaris* were comparable in their response to water regimes while *C. olitorius* and *V. unguiculata* performed better than *B. vulgaris* under water stress, an indication of an opportunity to use these vegetables under drought conditions.

The evaluation of nutritional quality of *A. cruentus*, *C. olitorius*, *V. unguiculata* and *B. vulgaris* was motivated by recommendations made in most agronomic studies based on biomass yield with no follow-up on nutritional value. Samples from each crop were collected from each of the three irrigation regimes (30%, 60% and 100%ETc) during each harvest (6, 8 and 10 weeks after transplanting for both seasons) and analysed for macro and micronutrients. Results from *A. cruentus* indicates that Ca and Mg were significantly higher under drought stress (30% ETc) while Na, K and Zn increased with water application up to 60% ETc with no further increase thereafter. Similarly, Ca and Mg were higher under drought stress and Zn under medium stress in *C. olitorius*. Calcium was high under drought stress condition in *B. vulgaris* while Na and Zn where high in medium stress; with a further increase in water application resulting in diminishing returns. Phosphate and potassium were high in medium stress condition in *V. unguiculata* while in water application up to 100% ETc the two elements showed diminishing returns. The high nutrients alternated between the most severe water stress (30% ETc) and medium stress (60 ETc) treatments across all crops in this study, an indication that although the crops can be grown under drought conditions, slight irrigation can lead to improved production. Leaf Fe, Zn, Mn, Mg and Ca increased with time of harvesting that increased from 6 to 8 weeks, with no further change in nutritional yield when crops were harvested at 10 weeks in *A. cruentus*, *V. unguiculata* and *B. vulgaris*. In *C. olitorius*, Fe, Zn, Mn, Mg and Na were high when harvested at 6 weeks compared to late harvesting (8 and 10 weeks).

The first postharvest study investigated the effect of three irrigation regimes (30%, 60% and 100% ETc) and three drying (sun, oven, shade) methods on phenolic, flavonoid and gallatannin content of the four vegetables. Fully irrigated *C. olitorius* and subjected to sun drying (100% ET_c x sun drying) had higher total phenolic content followed by medium stress subjected to shade drying (60% ET_c x shade drying). Furthermore, water stressed plants that were then shade- or sun-dried retained better gallotannin content than other treatment combinations. *Amaranthus cruentus* grown under drought then shade- or sun-dried (30% ET_c x shade and sun drying) retained better quality in all phenolic components measured.

In *V. unguiculata*, phenolic content was high in water-stressed plants subjected to sun-drying (30% ET_c x sun drying) while sun drying retained flavonoid and gallotannin than shade and oven drying. In *B. vulgaris*, well irrigated plants and shade- or oven-dried (100% ET_c x shade/oven drying) had better phenolic content. Shade dried leaves had better flavonoid while drought-stressed plants had better gallotannins content compared to other treatments in *B. vulgaris*. All three ALVs can be grown under drought stress and subjected to sun or shade drying to retain nutrient compared to *B. vulgaris*.

The second experiment on postharvest investigated the interaction of packaging (non-perforated and perforated), temperature [room storage, refrigerated storage (4℃), retail storage, 10℃] and storage duration (2, 4, 6, 8, 10 days) on *C. olitorius*. Plants rarely experience a single stress factor but are simultaneously exposed to multitude stress factors in their growing environment. The results showed that treatment combination of 4℃ with perforated packaging retains higher phenolic content followed by perforated packaging at 10℃ while lower phenolics were in treatment combinations that were stored at room temperature. Total phenolic content was higher at 2 days and 4 days storage in non-perforated packaging compared to all other treatments combinations. Furthermore, phenolic content decreased disproportionately with storage duration in non-perforated packaging treatment combinations, performing better than perforated in every storage duration. Flavonoid content and total phenolics decreased with increase in storage duration while better retaining these in any treatment combination of 4℃/10℃ compared to room temperature. Phenolic content was significantly higher from 2 to 4 days then declined from 6 through to 10 days at 4℃. At room temperature, phenolic contents decreased from 2 to 4 days storage durations but were higher at 6 and 8 days storage durations before dropping at 10 days. Antioxidant activity and overall acceptance was improved in any treatment combinations kept at 4 and 10℃ compared to room temperature for both types of packaging as storage duration increased. Antioxidant activity and overall acceptance degradation was reduced in treatment combination kept at 4 and 10℃ compared to room temperature for both types of packaging as storage duration increased. *Corchorus* stored at room temperature had a shelf life of 2 days, but 8 days at 4℃ and 10 days at 10℃ for both types of packaging.

TABLE OF CONTENTS

TABLE OF FIGURES AND TABLES

CHAPTER 1

GENERAL INTRODUCTION

1.1 Conceptualisation and study objectives

South Africa faces challenges of food and nutritional security at household levels due to nutrient deficiencies such as vitamin A, iron, zinc, and vitamins C (Oelofse and van Averbeke, 2012). Studies have shown that African leafy vegetables (ALVs) can contribute to addressing gaps in nutritional insecurities as they are considered to be healthy, affordable and nutrient dense. African leafy vegetables describe leafy vegetables that have been part of the food systems in African communities for generations (Van Rensburg et al., 2007). South Africa has a highly diverse pool of ALVs growing naturally and are available for consumption. The species utilised vary with indigenous knowledge, culture, species availability and economy. Wehmeyer and Rose (1983) identified more than 100 different species of plants that are used as ALVs in South Africa, out of which eight major groups are of importance (Van Rensburg et al., 2007). Their importance is based on ease of availability throughout the year, ease of collection, popularity, low production cost, distribution, taste, growth habitat, growing season and nutritional value. These include *Corchorus olitorius* (jute mallow), *Amaranthus cruentus* (pigweed), *Citrullus lanatus* (bitter melon), *Vigna unguiculata* (cowpea), *Cleome gynandra* (spider plant), *Cucurbita spp* (pumpkin), *S. nigrum complex* (night shade) and *Brassica rapa* subsp. *chinensis* (non-heading Chinese cabbage) (Van Rensburg et al., 2007; Oelofse and van Averbeke, 2012). These species have a wide genetic diversity in growth habit, leaf shape, leaf colour, leaf size and plant size (Van Rensburg et al., 2007). The large number of species for people to select from as well as a wider diversity of desirable traits can lead to successful commercilisation since farmers have a wider pool of species that are better adapted for their region within South Africa.

These vegetables are reported to be contributors of both micronutrients and bioactive compounds to diets (Afolayan and Jimoh, 2009). The nutrient levels found in ALVs are often comparable, and in some cases better than those from exotic vegetables such as cabbage; they are also compatible to use with starchy staples because they contain ascorbic acid, which enhance iron absorption (Nesamvuni et al., 2001). They are also good dietary sources of antioxidants such as flavonoids, tannins and other polyphenolic constituents (Manach et al., 2005). Phenolic compounds are secondary metabolites produced by plants to cope with the environment and exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio protective and vasodilatory effects (Amic et al., 2003). Some flavonoids and flavanols have been found to possess antioxidant and free radical scavenging properties that are much stronger than those of vitamins C and E (Zobolo et al., 2008).

Most ALVs are reported to be adapted to low input agriculture, tolerant to drought, pests and diseases (Oelofse and van Averbeke, 2012). However, most ALVs are not cultivated due to lack of adequate information on water use and postharvest handling practices. Those ALVs that are already in cultivation like *Brassica rapa* subsp. *chinensis* show variation in agronomic management practices, an aspect that indicate lack of proper production information (Oelofse and van Averbeke, 2012). Effective production of ALVs needs optimization of pre-harvest factors such as fertilizer, irrigation, and harvesting techniques (Oelofse and van Averbeke, 2012).

Nutritional value of plants has been reported to vary with soil fertility, environment temperature, plant type, plant age and the production techniques used (Chweya eand Nzava., 1997; Nnamani et al., 2009). Furthermore, mineral composition is reported to vary greatly due to seasonal variations (Yazzie et al., 1994) and on the analytical method used (Boukari et al. 2001). When plants are exposed to biotic (pests, disease) and abiotic stress (temperature, water stress) they respond by producing secondary metabolites (Nora et al., 2012). These include defence compounds such as polyphenols, alkaloids or healthy related compounds such as antioxidant (terpens, polyphenols) or organoleptic compounds such as polyphenols for bitterness, colour, firmness or terpenes for odour/colour (Nora et al., 2012). These compounds impact on quality of the produce. For ALVs to move from underutilised crops to commercial-level production, it is vital that production be based upon objective quality criteria. Abbot (1999) defined the term 'quality' as the degree of excellence of a product or its suitability for use and this encompasses seed properties (viability germination), physical properties (size, shape, colour, freshness), sensory properties (appearance, texture, taste, aroma) and nutritional properties (vitamin, mineral and other chemical constituents) (Groff et al., 1993; Govindasamy et al., 1997; Wolf, 2002; Gruda, 2005; Hussin et al., 2010). Mampholo et al. (2015) conducted a literature review on how to maintain overall quality of ALVs in southern Africa; considering that quality is produced in the field, there is need to conduct studies on pre- and post-harvest factors that impact quality. African leaf vegetables are still considered wild species and have never been considered for commercial production in South Africa compared to other countries in Southern Africa. Successful commercialisation of ALVs in South Africa should be accompanied by research on production factors such as irrigation and the impact they have on the quality of the product. Such information will lead to development of appealing and valuable products to the consumers.

There are few studies that have monitored changes in nutritional parameters in the same commodity from harvest through to storage (Rickman et al., 2007). The current study focuses on how pre- harvest factors such as irrigation regimes can impact harvest yield and post-harvest quality. Availability of such information will give an insight into how these factors can be manipulated in future within the ALVs' growing environments. Furthermore, previous studies have tried to make comparison on agronomic and nutritional factors of ALVs relative to their exotic counterparts grown under different conditions. In the current study ALVs were grown together with a reference crop, *Beta vulgaris* to make general comparisons. The main aim of the study was to conduct trials on selected African leafy vegetables (ALVs) in order to evaluate their growth, physiological and biochemical responses to varying water regimes. The trials were conducted iunder field and rain-shelter conditions and included both pre- (growth, yield) and post-harvest (mineral nutrients, phytochemicals) evaluations.

1.2 Objective

The main objective of the study was to evaluate pre-and post-harvest response of *Amaranthus cruentus*, *Corchorus olitorius*, *Vigna unguiculata* and reference crop *Beta vulgaris* to varying water stress levels in South Africa.

Specific objectives

1. The objective of the review was to investigate the factors that influence utilisation and production of the African leafy vegetables.
2. To evaluate the moisture stress on physiology and yield of some indigenous leafy vegetables under field conditions.
3. To evaluate the productivity and yield of *A. cruentus, C. olitorius, V. unguiculata* and a reference vegetable crop, *B. vulgaris* under varying water regimes under rain shelter conditions.
4. To evaluate the nutritional quality of *Amaranthus cruentus*, *Corchorus olitorius*, *Vigna unguiculata* and a reference vegetable crops–*Beta vulgaris* to varying water regimes

5. To determine the effect of irrigation and drying methods on the total phenolic, flavonoid and gallotannin content of *A. cruentus, C. olitorius, V. unguiculata* and a reference vegetable crop *B. vulgaris*.
6. To determine the effect of postharvest packaging, temperature and storage duration on the total phenolic content, flavonoid, antioxidant properties and marketability of *C.olitorius*.

1.3 Thesis structure

This thesis is written in a paper format with each chapter as manuscript in preparation or submitted for publication. The study consist of agronomic and postharvest experiments conducted at the Agricultural Research Council (ARC), Roodeplaat, Pretoria over two summer seasons, 2015/2016 and 2016/2017.

Chapter 2 Before the study commenced a review was conducted to document the state of utilisation and production of ALVs in South Africa in order to identify current and future research needs and reduce duplication of some work done so far. It addresses the first objective of the study. **(Published in Journal of Sustainability).**

Chapter 3 reports on how moisture stress affects physiology and yield of some indigenous leafy vegetables under field conditions. Leaf number, plant height, chlorophyll content index (CCI), water productivity and yield were measured. It addresses the second objective of the study. (***In press*, South African Journal of Botany).**

Chapter 4 address the third objective of the study and reports on how productivity is affected by varying water regimes in *V. unguiculata*, *C. olitorius*, *A. cruentus* and *B. vulgaris* under controlled environment (rain shelter). Leaf number, plant height, chlorophyll content index (CCI), chlorophyll fluorescence (CF), water productivity and yield were measured. **(Prepared for publication according to Journal of Agricultural Water Management).**

Chapter 5 is linked to chapter 4 and address how water regimes affect nutritional quality of *V. unguiculata, C. olitorius*, *A. cruentus* and *B. vulgaris*. It addresses the fourth objective of the study and phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), copper (Cu), manganese (Mn), Zinc (Zn) and potassium (K) were analysed. **(Published in South African Journal of Botany).**

Chapter 6 evaluates how postharvest drying maintains phenolic, flavonoid and gallotannin content of *V. unguiculata*, *C. olitorius*, *A. cruentus* and *B. vulgaris* grown from various water regimes. It addresses the fifth objective of the study**. (Published in Scientia Horticulturae).**

Chapter 7 reports on how postharvest packaging, temperature and storage time influences the phenolic composition and antioxidant properties of *Corchorus olitorius.* **(Prepared for publication according to Journal of the Science of Food and Agriculture).**

Chapter 8 is general discussion and highlights major findings, outcomes and recommendations of the study.

References

- Abbot J. 1999. Quality measurements of fruits and vegetables. *Postharvest Biology and Technology* 15(3): 207-225.
- Afolayan AJ, Jimoh FO. 2009. Nutritional quality of some wild leafy vegetables in South Africa. *International Journal of Food Science and Nutrition 60*:424-431.
- Amic D, Davidovic-Amic D, Beslo D., Trinajstic N. 2003. Structure-radical scavenging activity relationship of flavonoids. *Croatica Chemica Acta 76*: 55-61.
- Boukari I, Shier NW, Xinia E, Fernandez R, Frisch J, Watkins BA, Pawloski L, Fly AD. 2001. Calcium analysis of selected western African foods. *Journal of Food Composition and Analysis* 14: 37-42.
- Chweya JA, Nzava AM. 1997. Cat's whiskers. *Cleome gynandra* L. promoting the conservation and use of underutilized and neglected crops. Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute.
- Govindasamy R, Italia J, Liptak C. 1997. Quality of agricultural produce: Consumer preferences and perceptions. New Jersey: New Jersey Agricultural Experiment Station.
- Groff AJ, Hreidor CR, Toensmeyer UC. 1993. Analysis of the Delaware market for organically grown products. *JFDR* 24, 18-125.
- Gruda N. 2005. Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. *Critical Reviews in Plant Sciences* 24: 227-247.
- Hussin SR, Yee WF, Bojei J. 2010. Essential quality attributes in fresh produce purchase by Malaysian consumers *Journal of Agribusiness Marketing*. 3: 1-19
- Mampholo BM, Sivakumar D, Thompson AK. 2015. Maintaining overall quality of fresh traditional leafy vegetables of Southern African during the postharvest chain, *Food Reviews International* 32: 400-416
- Manach C, Mazur A, Scalbert A. 2005. Polyphenols and prevention of cardiovascular diseases. *Current Opinion in Lipidology 16*: 77–84.
- Nesamvuni C, Steyn NP, Potgieter MJ. 2001. Nutritional value of wild, leafy vegetables consumed by the Vhavhenda. *South African Journal of Science 97*: 51-54.
- Nnamani CV, Oselebe HO, Agbatutu A. 2009. Assessment of Nutritional Values of Three Underutilized Indigenous Leafy Vegetables of Ebonyi State, Nigeria. *African Journal of Biotechnology* 8(9): 2321- 2321.
- Nora L, Dalmazo GO, Nora FR, Rombaldi CV. 2012. Controlled water stress to improve

fruit and vegetable postharvest quality. In: Ismail Md Mofizur R Hiroshi H, eds. Water Stress. Rijeka: InTech Open Science. 59-72.

- Oelofse A, van Averbeke W. 2012. *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Water Research Commission: Pretoria, South Africa.
- Rickman JC, Barret DM, Brulin CM. 2007. Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part I Vitamin C and B and Phenolic compounds. *Journal of the Science of Food and Agriculture* 87: 930-944
- Van Rensburg WSJ, van Averbeke W, Slabbert R, Faber M, van Jaarsveld P, Van Heerden I, Oelofse A. 2007. African leafy vegetables in South Africa. *Water South Africa* 33: 317-326.
- Wehmeyer AS, Rose EF. 1983. Important indigenous plants used in the Transkei as food supplements. *Bothalia* 14 (3/4): 613- 615.
- Wolf MM. 2002. An analysis of the impact of price on consumer interest in organic grapes: A profile of organic purchases. A paper presented at the American Agricultural Economics Association Annual Meeting, Long Beach, California, July 28-31. Retrieved:20/04/2018 from http://ageconsearch.umn.edu/bitstream/19663/1/ sp02wo02.pdf
- Yazzie D, VanderJagt DJ, Pastuszyn A, Okolo A, Glew H. 1994**.** The amino acid and mineral content of baobab (*Adansonia digitata* L.) leaves. *Journal of Food Composition and Analysis* 7: 189-193.
- Zobolo AM, Mkabela QN, Mtetwa DK. 2008**.** Enhancing the status of indigenous vegetables through the use of kraal manure substitutes and intercropping. *African Journal of Indigenous Knowledge Systems 7*: 211–222.

CHAPTER 2

Review

African Leafy Vegetables: A Review of Status, Production and Utilization in South Africa

Innocent Maseko [1,*], Tafadzwanashe Mabhaudhi [2], Samson Tesfay [1], Hintsa Tesfamicael Araya [3], Melake Fezzehazion [3] and Christian Phillipus Du Plooy [3]

[1] Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa; Tesfay@ukzn.ac.za

[2] Crop Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa; Mabhaudhi@ukzn.ac.za

[3] Agricultural Research Council, Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria 0001, South Africa; ArayaH@arc.agric.za (H.T.A.); fmelake@gmail.com (M.F.); iduplooy@arc.agric.za (C.P.D.P.)

* Correspondence: 215082595@stu.ukzn.ac.za; Tel: +27-(0)-33-260-6108

Abstract: African leafy vegetables (ALVs) are mostly gathered from the wild, with few selected species being cultivated, usually as part of a mixed cropping system in home gardens or smallholder plots. They have important advantages over exotic vegetable species, because of their adaptability to marginal agricultural production areas and their ability to provide dietary diversity in poor rural communities. Despite their significance in food and nutrition security, there is limited availability or access to these crops leading to underutilisation. The objective of this review was to document the state of utilisation and production of ALVs in South Africa. A qualitative systematic approach review of online sources, peer reviewed papers published in journals, books and other publications was conducted. There is lack of suitable production systems, innovative processing, and value-adding techniques that promote utilisation of ALVs. Furthermore, there is a perception that ALVs are food for the poor among the youth and urban folks, while, among the affluent, they are highly regarded as being nutritious. To promote ALVs from household consumption and commercialisation, further research on agronomy, post-harvest handling, storage and processing is required in South Africa.

Keywords: drying; nutritional value; packaging; promotion; water use

(Published in Journal of Sustainability 2018, 10, 16; doi:10.3390/su10010016)

2.1 Introduction

African leafy vegetables (ALVs) are defined as plant species which are either genuinely native to a particular region, or which were introduced to that region for long enough period to have evolved through natural processes or farmer selection [1]. There are many names by which indigenous leafy vegetables are known by different authors including wild vegetables [2], African leafy vegetables [1], and traditional leafy vegetables [3,4]. In South Africa, they are called *imfino* in isiZulu and isiXhosa, *morogo* in Sesotho and *miroho* in tshiVhenda [5]. South Africa faces challenges of food insecurity at household levels due to nutrient deficiencies such as vitamin A, iron, zinc, and vitamin C [6]. Studies have shown that ALVs can contribute to addressing gaps in nutrition through offering healthy and affordable nutrient dense alternatives. Some ALVs are rich in compounds such as vitamins, minerals, anti-oxidants and even anti-cancer factors needed to maintain health and fight off infections [7]. This would be particularly beneficial for poor rural communities who cannot afford to purchase vegetables.

Most smallholder communities live in marginal areas where crops struggle to survive and face challenges of water scarcity. African leafy vegetables offer alternatives to such communities because they are tolerant to abiotic stresses such as drought and heat stress [8]. According to the Department of Agriculture, Fisheries and Forestry [9], ALVs are tolerant to drought, pests and diseases. They are also adapted to low input agriculture than exotic vegetables such as Swiss chard [5,10]. Thus, ALVs are a potential food source for poor people living in marginal areas and practising low input agriculture. Despite their abundance, they remain underutilised due to various constraints, including perception, processing, distribution and marketing, as well as nutritional information [11].

South Africa also faces challenges of water scarcity [12] and population growth [8]. Inclusion of ALVs in cropping systems can contribute to climate change adaptation, the environment, and employment creation in poor rural communities [13]. It is therefore worthwhile to identify the policy, socio-economic and institutional conditions that hinder/promote utilisation and production of ALVs. Availability of such information will give specific direction and guidance in research, production and marketing of ALVs. The objective of the study was to analyse factors that have influence in the use and production of ALVs to identify research needs. This study is expected to contribute to a broader scientific knowledge of important constraints and drivers in promoting ALVs. The goal of this paper is to critically review the status of utilisation and production of ALVs with a view to identifying research gaps that will facilitate scaling up their production in South Africa. The following questions are explored:

(1) What is the status of production and utilisation of ALVs in South Africa?
(2) What can be done to promote production and utilisation of ALVs in South Africa?
(3) What are the potential gaps and research priorities for future research of ALVs in South Africa?

Methods Used for Literature Search

A qualitative systematic approach was adopted for the current review. The search included online sources, peer reviewed papers published in journals, books and other publications such as popular articles. Published literature from universities, national research institutions, in the form of student theses, conference proceedings, working papers, and project reports was also considered. A comprehensive search was conducted using various search engines such as Google, MSN, Scopus etc., using the following terms: "indigenous leafy vegetable" or "African leafy vegetables" or "production or promoting ALVs" or "nutritional value of ALVs"; the search was limited to South Africa and the period 1994–2017.

Approximately 480 articles were retrieved of which ~10% were peer reviewed. Through an analysis of the content of returned entries, papers were screened based on relevance to South Africa. The records were furthered filtered to ~300 and classified according to topics such as biodiversity (20%), nutrient content (38%), production and utilisation (32%), marketing (2%) and postharvest handling and processing (8%). The entries were further classified in terms of category of research, as surveys, field trials or laboratory experiments. Most studies from returned entries were as follows: based on household surveys (55.0%), literature reviews (10.0%), field trials (15.0%), and laboratory experiments (25.0%). The observation for such a variation of returned entries within the topics selected can be attributed to the magnitude of research attention given to each category by the research community in South Africa. For example, there is little information of ALVs available on marketing, postharvest, field trial etc. resulting in less returned entries in comparison to other areas in this paper. Putting entries into categories gave each entry an equal opportunity to be screened or filtered. Within the mentioned categories above, the papers were filtered based on relevance to the subject under study. This also considered geographical locations of the entries to represent all areas where possible and variation in crop species covered. In cases where many entries reported on the same issue, the most suitable rated entry was selected to reduce repetition. This reduced entries to ~167. These articles were further screened for research methodology, whether the study involved actual data, literature review, appropriate sampling technique, or data analysis or statistical techniques. An overall rating of suitability of articles was assigned as poor or satisfactory. Only articles with ratings of satisfactory were selected for review. From this, 74 articles were considered relevant and included in the review.

2.2. Current Status of Utilisation and Production of Leafy Vegetables

2.2.1. Diversity of ALVs

South Africa has more than 100 different species of ALVs that have been identified; however, few groups of leafy vegetable species are still utilised [1]. These include *C. olitorius* (jute mallow)*, Amaranthus cruentus* (pigweed), *Citrullus lanatus* (bitter melon), *Vigna unguiculata* (cowpea), *Cleome gynandra* (spider plant), *Cucurbita* spp. (pumpkin) and *Brassica rapa* subsp. *chinensis* (non-heading Chinese cabbage). The local names, distribution and ecology of major African leafy vegetables in South Africa have been documented [1,14].

Amaranth is one of the most common ALVs in South Africa. Amaranth belongs to the *Amaranthaceae* family and is an extremely variable, erect to spreading herb (Figure 1b). Different species of amaranth are available all over South Africa [1,6,14]. These include: *Amaranthus thunbergii*, *A. greazicans*, *A. spinosus*, *A. deflexus*, *A. hypochondriacus*, *A. viridus* and *A. hybridus* [1,6,14]. The various amaranth species are tolerant to adverse climatic conditions, but prolonged dry spells induce flowering and decrease leaf yield [1,6,14]. Amaranth is a C4 plant that grows optimally under warm conditions (day temperatures above 25 °C and night temperatures not lower than 15 °C) bright light and adequate availability of plant nutrients. Hence amaranth is mainly grown in summer. Amaranth is rarely cultivated in South Africa because as with many other African leafy vegetables people believe the plants will grow naturally [1,6,14].

Corchorus belongs to the Tiliaceae family and is an erect annual herb that varies from 20 cm to approximately 1.5 m in height (Figure 1.1a). The stems are angular with simple oblong to lanceolate leaves that have serrated margins and distinct hair-like teeth at the base. Different *Corchorus* species are available in South Africa, namely *C. asplenifolius*, *C. trilocularis, C. tridens* and *C. olitorius* [1,6,14]. *Corchorus* prefers warm, humid conditions and performs well in areas with high rainfall (600 to 2000 mm) and high temperature (30 °C during the day and 25 °C at night). In South Africa it grows in summer. Despite the abundance of the species and having a potential for development as a crop, *Corchorus* is still considered a wild species and has never been cultivated.

(**a**) (**b**)

Figure 1.1. (**a**) *Corchorus olitorius*; (**b**) *Amaranthus cruentus* growing under commercial production in a trial at Roodeplaat in 2012 summer season

Cleome (Figure 2.2b) belongs to the Capparaceae family and it is an erect herbaceous herb, branched and rather stout [1,6,14]. Different Cleome species exist such as *C. monophylla* and *C. hirta* with *Cleome gynandra* most widely used as a leafy vegetable in South African gardens [1,6,14]. Cleome does tolerate a degree of water stress, but prolonged water stress hastens flowering and senescence. It grows in summer and does not grow well when the temperature drops below 15 °C. Cleome is not formally cultivated in South Africa although it is among the group of African leafy vegetables that has good potential for development as a crop [1,6,14].

(**a**) (**b**)

Figure 1.2. (**a**) *Vigna unguiculate*; and (**b**) *Cleome gynandra* growing under commercial production in a trial at Roodeplaat in 2012 summer season.

Vigna unguiculata is a leaf and pulse crop that belongs to the Leguminosae family (Figure 1.2a). It is an annual or perennial herbaceous plant with tri-foliate leaves [1,6,14]. Different varieties exist, varying from prostate indeterminate types to erect, determinate, low-branching types. The varieties mainly used as a leafy vegetable are the spreading, prostrate types. Various subspecies of *Vigna unguiculata* are found in the wild in the eastern parts of the KwaZulu-Natal, Mpumalanga and Limpopo Provinces. These subspecies include: *Vigna unguiculata* subsp. *dekindtiana* var. *dekindtiana, V. unguiculate* subsp. *dekindtiana* var. *huillensis, V. unguiculata* subsp. *rotracta, V. unguiculata* subsp. *stenophylla, V. unguiculate* subsp. *tenuis* var. *ovata,* and *V. unguiculata* subsp. *unguiculata*, with *Vigna unguiculata* subsp. *unguiculata* the most commonly found [1,6,14]. *Vigna unguiculata* is widely cultivated in summer for its seeds and as a fodder crop. Its use as a leafy vegetable has not received much attention [1,6,14].

Brassica rapa L. subsp. *chinensis* (Figure 3). belongs to the Brassicaceae family, an annual, flowering, leafy vegetable, in which the leaves form a rosette [1,6]. It originates in China and found its way from Asia into Africa as a result of trade between the two continents [1,6]. Vhembe District in Limpopo province is the centre of origin of the cultivation of *Brassica rapa* L. subsp. *chinensis* in South Africa, where an informal seed multiplication and distribution system is being maintained by selected producers. It is primarily grown during the dry winter months, making it reliant on irrigation for its water requirements. Different landraces have been reported in South Africa which have been given local names such as *dabadaba* and *lidzhainthi* being most commonly grown, followed by *tshikete* and *mutshaina wa u navha* [6]. Its cultivation by South African smallholders has been rapidly spreading from Vhembe District to many parts of the Limpopo, Mpumalanga and Gauteng provinces.

Figure 1.3. *Brassica rapa* L. subsp. *chinensis* growing under commercial production in the trials at Roodeplaat in 2013 winter season

Other ALVs species available in South Africa include *Cucurbita* family. *Cucurbitaceae* (pumpkin and relatives) are almost all vine like, annual, herbaceous plants. The most popular cucurbit species are *Citrullus lanatus* (Figure 4b)*,* *Cucumis melo*, and *Cucurbita pepo*, *C. maxima* and *C. moschata* [1,6,14]. *Cucurbita maxima* (Figure 1.4a) and *C. pepo* are drought tolerant and require relatively little water, but they respond positively to irrigation when conditions are very dry.They are the most heat tolerant type of pumpkin and they are also fairly drought tolerant. They are grown in summer.

(**a**) (**b**)

Figure 1.4. (**a**) *Cucurbita spp*; (**b**) *Citrullus lanatus* growing during summer season at Roodeplaat in 2012 season

2.2.2. Utilisation

Previous studies have tried to quantify the frequency of utilisation of ALVs in South Africa [2,15–17]. Their use has remained low despite their nutritive value and potential economic use. Van Rensburg et al. [10] reported that the utilization of indigenous leafy vegetables is declining in favour of exotic vegetables. Even at present the utilisation is still variable [1]. This is because they are not cultivated but mostly gathered from cultivated fields, fallowed land and the veldt [14,18]. Women are the major custodians in the gathering of wild vegetables [1,19]. The low levels of utilisation are also attributed to perception that they are food for the poor and indicate hard times among the youth and urban folks [20–23]. The loss of indigenous knowledge also causes low utilisation [21]. This supports the view that the youth do not have enough knowledge of the wild species to collect in wild; with the tendency of mixing wild vegetables with poisonous species [14].

2.2.3. Production

The occurrence and extent of cultivation of leafy vegetables in South Africa has been presented in Table 1.1. *Amaranthus cruentus, Cleome gynandra* and *Corchorus olitorius* as shown in Table 1 are still considered wild species and thus have never been considered for large-scale commercial production [24]. *Vigna unguiculata* is widely produced mainly for grain and as a fodder crop (Table 1.1). Its production as a leafy vegetable for human consumption is not widespread and has received limited research attention [24]. *Citrullus lanatus* and *Cucurbita* species are often grown as an intercrop with maize covering the soil surface which helps to control weeds [20,25,26] and for their fruits (Table 1). Some of the ALVs indicated in Table 1.1 such as *Brassica rapa* subsp. *chinensis* are already cultivated but the wide diversity in agronomic practices used indicate the absence of sound agronomic guidelines for these crops [27].

Table 1.1. African leafy vegetables commonly harvested from the wild or obtained through cultivation in South Africa

African Leafy Vegetable	Harvested from Wild	Cultivated	Growth Season	References
Abelmoschus esculentus Moench.		✓	Summer	[6,8,14]
Amaranthus spp.	✓		Summer	[1,6,8,14]
Bidens spinosa L.	✓		Summer	[6,8,14]
Brassica rapa L. subsp. *chinensis*		✓	Winter	[1,6,8,14]
Chenopodium album L.	✓		Summer	[6,8,14]
Citrillus lanatus		✓	Summer	[6,8,14]
Cleome gynandra L.	✓		Summer	[1,6,8,14]
Corchorus olitorius L.	✓		Summer	[1,6,8,14]
Cucumis melo L.		✓	Summer	[1,6,8,14]
Cucurbita spp.		✓	Summer	[1,6,8,14]
Galinsoga parviflora Cav.	✓		Summer	[6,8,14]
Momordica balsamina L.	✓		Summer	[6,8,14]
Portulaca oleracea L.	✓		Summer	[6,8,14]
Solanum retroflexum Dun.		✓	Winter	[1,6,8,14]
*Vigna unguiculata (*L.*)* Walp.		✓	Summer	[1,6,8,14]

Harvesting of ALVs without cultivation is unsustainable in that people have no control over availability as shown in Table 1. Others argue that these ALVs are only needed in small quantities and the naturally occurring amounts should be adequate. However, if the increase in promotion and consumption of these species is not matched with propagation or cultivation, this could lead to an unsustainable increase in harvesting from the wild or extinction of species in South Africa [14,28]. An alternative to this utilisation approach is the integration of African leafy vegetables in cropping systems [29]. Therefore, there is need to conduct more agronomic studies to generate basic production guidelines for these crops that will enable to match supply with demand. These agronomic studies will explore the planting dates appropriate for farmers to get better prices. Studies on various harvesting methods should be conducted alongside nutritional studies to ascertain the best time or different stages of harvesting.

Some agronomic studies aiming to develop optimal cultivation practices for improved yield in South Africa have indicated the possibility of improved production. Agronomic considerations such as nitrogen [8,27,30,31] and manure application [8,32] have been reported to improve production. However, further studies still need to be conducted on the effects of manure and nitrogen on the quality of ALVs in terms of bioactive compounds and quality parameters. It is after such studies have been conducted that some of the nitrogen rates can be adapted by farmers. Similar reports have been made on improved production due to agronomic factors such as planting date [27,33] irrigation management [34,35] and plant density [24,36]. Promoting cultivation of ALVs would increase their availability and accessibility to consumers and possibly generate household income for rural households [37]. There is still a need to investigate the relationship between water use, crop production and quality in terms of macro and micronutrients.

2.2.4. Marketing of Leafy Vegetables in South Africa

The marketing of leafy vegetables in South Africa is still low and limited to dried products [20,38]. Their marketing and distribution is mainly through street vendors [38,39]. Despite their perceived quality, ALVs are rarely found in supermarkets and upmarket groceries in South Africa. The rare presence of stocking of ALVs in supermarkets has greatly contributed to their reduced consumption. This is due to decreased availability and their low status among some South African communities. At the time of this research, there was no coordination of leafy vegetable production and marketing. Those who are already consuming these vegetables have not increased their demand for same, due to lack of improved presentation and availability from steady and reliable sources. The opening up of market outlets for ALVs in supermarkets and groceries can be achieved through training of farmers in modern production techniques, quality control and standardization of selling units, and then linking the farmers to the markets. According to Matenge et al. [22], marketing messages such as "old-fashioned but new" or "traditional but more convenient" might reach both younger and older consumer markets. For successful promotion of these crops there should be vertical integration that must be achieved through institutional linkages between the producers and the supply outlets. Linking up of the various market actors will lead to increased supply as well as increased efficiency in the value chains.

2.2.5. The Role of the Private and Public Sectors in Promoting ALVs

Research of ALVs in South Africa has been ignored for a long time by policy makers and researchers although it is currently attracting interest [9]. The Agricultural Research Council (ARC) Vegetables and Ornamental Plants (VOPI) is one of the major role players involved in research and training of indigenous vegetables in South Africa. Indigenous crops research is since 1994 an existing research focus area for ARC-VOPI [18]. It has created awareness within the scientific community through publications, presentations, posters, workshops and conference attendance. The ARC-VOPI in collaboration with the International Institute of Plant Genetic Resources Institute, (IPGRI) has made efforts in promoting wild vegetables [18]. There are also current efforts being done by ARC-VOPI to promote different ALVs through compilation of important literature on the production, subsequent research collaboration with universities and farmer engagements. However, long term partnership and funding by government and the private sector is a key driving force behind the increased production of ALVs in South Africa.

Water Research Commission (WRC) has also been a major role player in promoting ALVs through research funding. Some of the funded WRC scoping studies have tried to document water use efficiency of selected ALVs, and then use these with nutritional values to estimate nutritional water productivity [35,40]. This is necessary as it will give insight on how increasing ALV production and diversity can be linked to addressing nutritional outcomes. Most of the water use efficiency data used in these studies were benchmark estimates and from various sources [35,40]. This is because there is limited published data on most ALVs and there is no literature on water use of some of the ALVs such as in South Africa. Despite its efforts in scaling up research, WRC should direct mostly of its research in agronomy to determine potential yield and water use efficiency to accurately calculate nutritional water productivity in South Africa.

The Medical Research Council as a role player has focused on the use and nutritional value of ALVs among rural households among other projects. The South African Department of Agriculture, Forestry and Fisheries (DAFF) is a key role player at policy level in promoting the value of ALVs [9]. At present, the current food security policy guiding research, production and marketing of agricultural produce is quite broad and lacks specific direction for the promotion of ALVs. At the time of this research, there was notably no formal or commercial seed production which is a prerequisite for sustaining the production trend. Discussions with colleagues from ARC-VOPI breeding department cited that there are no registered varieties of ALVs at present under the Department of Agriculture. According to Venter et al. [18], efforts should be made to ensure government agencies are supportive of ALV initiatives in current and future projects. Extension service in South Africa is not well facilitated to work properly and, on the other, even if it was, there would be a need for some basic training since training college curricula rarely cover ALVs. This is because Agricultural education in both commercial and communal areas is aimed at cash crop production [29].

All South African universities are role players in promoting use of ALVs. Universities have been partners on nutritional and consumption studies, thus helping to strengthen the capacity in the scientific community on ALVs [18]. From the discussion arising from Symposium on the Water Use and Nutritional Value of Indigenous and Traditional South African Underutilised Food Crops for Improved Livelihoods conducted in Pretoria in 2014, one of the challenges is research funding. Lack of funding in some South African universities towards research of ALVs results in fewer field studies conducted and in the case where they are conducted, it is in small plots or backyard fields leading to poor results. Another challenge is that researchers are focusing on their areas of interest or interesting studies with few dealing with basic agronomic studies that require extensive field work.

Promoting home-grown or small-scale food production is explored as a feasible contributor to food and nutrition security for the rural poor in South Africa [41]. Improved research funding, combined with public education and dissemination of information is required. Since the target is promoting home-grown or small-scale food production there is constant need for community feedback sessions, including interaction with farmers and scientists. According to van Rensburg et al. [1], the active promotion, use and conservation of ALVs will ensure that the status of these crops is enhanced, specifically their contribution towards sustainable nutrition as well as sustainable production in South Africa.

2.3. Nutritional Value

South Africa faces Vitamin A and iron deficiencies, while utilisation of ALVs is documented to alleviate malnutrition. In such cases one would expect an increased uptake of such species. However, there is a decreased tendency in the utilization of ALVs due to limited knowledge of the nutritional content [21]. African leafy vegetables are increasingly recognized as possible contributors of both micronutrients and bioactive compounds to the diets [42]. They contain nutrients such as calcium, iron and vitamins A and C, fibre and proteins [14]. They are a valuable source of nutrition in rural areas and they contribute substantially to protein, mineral and vitamin intake together with fibre; they also add diversity to the diet. African leafy vegetables should therefore be included in the diet to overcome various nutritional problems such as iron and vitamin A deficiencies [14]. The minerals and vitamins found in ALVs exceed the levels found in exotic vegetables such as cabbage; they are also compatible to use with starchy staples because they contain ascorbic acid, which enhance iron absorption [2].

Studies on the antioxidant properties of these vegetables also revealed that they are good dietary sources of antioxidants such as flavonoids, tannins and other polyphenolic constituents [43]. Phenolic compounds are secondary metabolites in plants which exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio protective and vasodilatory effects [44]. Many phenolics, such as flavonoids, have antioxidant properties that are much stronger than those of vitamins C and E. Flavanols and flavonoids have been found to possess antioxidant and free radical scavenging activity in vegetables [45]. One way to promote nutritional uptake of ALVs in South Africa is childhood exposure and education on ALVs at primary school level by incorporating these products into school feeding programmes [22].

Our literature research has indicated that few studies have been conducted on the nutritional composition of wild vegetables in South Africa [3,21,42,46]. However, most of these studies have been based on the collection of plant samples from the wild. Hence, variations in soil and climatic conditions might have influenced the chemical composition of the crop species. Studies comparing the superior nutrient composition of wild vegetables to conventional vegetables such as cabbage (*Brassica oleracea* var. *capitata*) and Swiss chard [2,3,47,48] are documented in South Africa. In some cases, these studies have been conducted in separate soils or samples purchased from the market to conduct tests, hence there is need to conduct studies in the same field environment to reach meaningful comparison. More controlled experiments on aspects such as effect of soil type, effect of fertiliser amount and type, and age of harvesting on the nutritional composition of ALVs still needs urgent attention. The amounts of nutrients reported for the same species from different studies vary widely [13]. Possible cause to such is variation in the age of plant material used and variations in protocols of analysing the bio compounds from one lab to the other [14]. Therefore, there is need to conduct studies with standardised assays or protocols to make comparisons and to consider the age of plant materials used.

2.4. Drought Tolerance and Resilience

African leafy vegetables could make a positive contribution to world food production because they adapt easily to harsh or difficult environments [49]. The input required for growing them is lower compared with other crops, and they are highly resistant to pathogens thus requiring fewer chemicals and pesticides [49]. They are considered low input crops, which are more tolerant to abiotic and biotic stresses as compared to exotic vegetables [50,51]. The notion that ALVs grow in the wild or adverse environments could mean they have various strategies/mechanisms to deal with drought stress. Drought stress is defined as the moderate loss of water which results in stomatal closure and limitation of gas exchange [52]. A plant may escape, avoid, and/or tolerate stress. Drought tolerance has been defined as the plant's capacity to maintain metabolism under water stress [53]. Drought avoidance involves crop responses such as stomatal regulation, including enhanced capture of soil moisture through an extensive and prolific root system [54,55]. Studies conducted elsewhere have shown that cowpea [56] and Amaranth [57] are tolerant to adverse climatic conditions. Few studies conducted in South Africa have also shown that leafy vegetables are drought tolerant. Neluheni et al. [58] showed that reasonable yield in Amaranth can still be obtained even at lower moisture availability. Slabbert et al. [59] in screening for drought tolerance showed that the six major indigenous leafy vegetable could maintain higher relative water content and leaf area compared to *B. vulgaris.* var. *cicla.*

Studies have shown that not all African leafy vegetables are tolerant to water stress. *Brassica rapa* subsp. *chinensis* has been shown to require adequate availability of soil water for optimum growth [34]. Neluheni et al. [58] reported that stress tolerant in amaranth depends on the specie with *A. graezizans* being more tolerant than *A. cruentus*. This concurs with previous researchers elsewhere who reported that drought tolerance in amaranth depends on the species [26,60]. Farmers can still choose species that are drought tolerant. Therefore, ALVs can act as a substitute for other cultivated crops to alleviate nutrient deficiencies by increasing nutrient supplies [37]. Therefore, the need to breed for drought resistant varieties and to conduct irrigation trials throughout the year to ensure continuous availability remains to be established in South Africa.

2.5. Water Use of ALVs

South Africa is a water stressed country [12] and irrigated agriculture takes place under water scarcity. According to Annandale et al. [61], in the next two to three decades, water availability is likely to drop below benchmark of 1000 m^3 person $year^{-1}$. One way to deal with inadequate availability of water is to utilise crops that are tolerant to water stress [6]. African leafy vegetables can be exploited to contribute towards food and nutrition security without upsetting the existing burden on water shortages [6]. The promotion of production of ALVs in South Africa include addressing the notion of “more crop per drop”, thus the production of more food per millimetre of water used. This is necessary despite ALVs being drought tolerant, with low water requirement, poor water management could upset the existing water burden once these crops are commercilised. Therefore, to optimise the amount of water, water use efficiency (WUE) and water productivity should be known with considerable precision. WUE is defined as mass of dry matter produced per unit volume of water evapotranspired expressed in kg m^{-3}. Studies conducted on water use efficiency indicate that black nightshade (*Solanum nigrum*) and cleome (*Cleome gynandra*) among other crops have low water use and high water use efficiency compared to Swiss chard [41]. Water use efficiencies obtained in South Africa substantially differs with those published internationally [40]. Therefore, there is need to conduct more studies as little local research has been published on water use efficiency of ALVs in South Africa.

Crop water productivity is the amount of water required per unit total biomass or specified biomass produced expressed in kg m^{-3} [62]. A study conducted to determine the water requirements of selected ALVs in South Africa showed that adequate amount of water is needed to produce marketable yield [35]. Highest water productivity was obtained at deficit irrigation which indicates that production of ALVs is possible in water scarce areas. However, deficit irrigation compromised the leaf quality as observed by Beletse et al. [35]. This study was conducted under a rain shelter and in one locality, hence need to conduct more field trials in different regions of South Africa.

Furthermore, in promoting production in South Africa, researchers need to shift from emphasizing production per unit area towards maximizing nutritional content per volume of water consumed, the nutritional water productivity (NWP) as defined by Renault and Wallender [63]. According to Mabhaudhi et al. [64] South African benchmarked values of macronutrient water productivities indicates that indigenous leafy vegetables such as Amaranth and pumpkin leaves are efficient in terms of water consumed per protein produced. Dark green vegetables are efficient protein synthesizers and high efficient iron accumulators [40]. The sets of nutritional water productivity (NWP) values were calculated using the equation of Renault and Wallender [63]. It should be noted that the values were calculated using the same trials hence the influence of the environment on water productivity and nutrient content questions the reliability of the results [40]. There is limited published information on nutritional water productivity (NWP) in South Africa [40]. Therefore, there is need to conduct systematic research in the determination of yields, water use efficiencies and nutritional water productivity under a range of production environments in South Africa.

2.6. Post-Harvest Handling and Storage of ALVs

The main constraint to increased production, marketing and consumption of ALVs is the high perishability in the fresh form [42]. Another major constraint is that they are seasonal and produced mainly in summer [19]. A study by Modi et al. [21] in South Africa at Ezigeni, KwaZulu–Natal observed that the availability of wild vegetables suddenly declined in May and became scarce between July and August and only increased as the season progressed from August to October. Therefore, there is a need to develop and promote appropriate processing techniques to minimize post-harvest losses and ensure regular supplies of leafy vegetables from the production areas to consumers in peri-urban and urban centres.

2.6.1. Cooling and Storage

Post-harvest losses of leafy vegetables are generally caused by poor handling and storage conditions after harvest. Cooling extends shelf life by reducing the rate of physiological change (i.e., rate of respiration and transpiration) and retarding the growth of spoilage microorganisms. In most cases, if these vegetables are not sold within 24 h after harvest, the likelihood of deterioration is imminent. Some farmers have tried to sprinkle water and leave them in the open overnight. However, problems of disease development and thus microbiological contamination still hamper their efforts.

Temperature is the most important environmental factor that influences the deterioration of harvested commodities [65]. Higher temperatures accelerate physiological deterioration and quality loss. Elsewhere, Nyaura et al. [66] reported that ascorbic acid declined by 88% in vegetable amaranth when kept at room temperature after four days of storage while the lowest loss was observed at 5 °C (55% loss) after 23 days of storage. Based on this study, it is suggested that shelf life extension and nutrient preservation of vegetable amaranth can be achieved through storage at temperatures of 5 °C. A study conducted in South Africa on Baby Spinach (*Spinacia oleracea*), a member of the Amaranthaceae family showed that storage period and temperature have different effects on Mg, Fe, Zn, phenolics, antioxidant activity, flavonoids carotenoids, and vitamin C [67]. Baby spinach leaves stored at 4 °C maintained good quality for 4–6 days as compared with those stored at 22 °C such as at a retail store [67]. There is limited information on storage temperature on ALVs. Information on various ranges of storage temperatures for small holders farming and commercialisation of leafy vegetables in South Africa needs urgent research attention.

2.6.2. Packaging

According to Matenge et al. [22], there is need to improve the image of ALVs to improve acceptability, preference and consumption by younger consumers, thereby presenting food product developers and marketers with the opportunity to make more acceptable ALV products available. Proper packing is essential to protect ALVs against spoilage and microorganism decay, preserve their quality and provide convenience of handling [68]. At present ALVs are simply uprooted or cut at the stems, sometimes washed, then tied into bunches and presented in the market. African leafy vegetables would fetch better prices if there were innovative ways of presenting them in the markets because packaging would attract the attention of consumers. This conforms to the findings of Mampholo et al. [68] that appearance of the product plays a major role in influencing consumer acceptance. Voster et al. [19] reported that some farmers packaged dried leafy vegetables products to increase shelf life in South Africa.

The knowledge of appropriate packaging for ALVs is still limited. Recent studies conducted in South Africa on *A. cruentus* and *S. retroflexum* [69] and on *Brassicas chinensis* [68] indicates that modified packaging can reduce postharvest losses and retain the overall quality and bioactive compounds on the retailer's shelf during marketing. These studies have focused on modified packaging and research still need to be conducted on various low-cost packaging techniques for small holder farmers in South Africa. Furthermore, the effect of pre-harvest or agronomic practices on postharvest and shelf life still needs urgent research needs. At the time of this research there was no pre-cut, branding or packaging of fresh ALVs in the South African formal market. Packaging and instructions on how to prepare the ALVs would assist potential customers who do not know how to cook them.

2.6.3. Drying

Drying is a way of processing leafy vegetables to make them available during periods of short supply [42]. Drying is a post harvesting process that can promote availability of leafy vegetables to farmers especially those who cannot afford packaging. Drying reduces microbiological activity through reduced moisture content in food. There are several methods of drying leafy vegetables that have been reported elsewhere which include sun drying, solar drying, vacuum drying, oven drying, and dehydrofreezing [70].

In South Africa, drying is the major method of processing leafy vegetables to make them available during periods of scarcity [19]. Whilst drying solves the problem of perishability, it does not satisfy the needs of a large population of consumers; particularly urban dwellers who prefer freshly harvested vegetables [42]. Voster et al. [19] reported that there are two main methods of preserving indigenous leafy vegetables in South Africa. These include sun drying of fresh leaves and sun drying of blanched leaves. Both these methods transform the leafy vegetable into dry products that have long shelf lives [19]. Van Averbake et al. [27] reported that electrification of the rural areas has introduced the new preservation technology, of freezing of leaves. Various drying methods have been reported elsewhere to affect quality parameters such as texture, flavour, colour, and bio-compounds of leafy vegetables. However, there are limited published data on the effect of various drying methods on the quality of indigenous leafy vegetables in South Africa. Such information is necessary to establish suitable drying methods for cultivated leafy vegetables within South Africa. There is a need to develop and promote locally appropriate processing techniques and ensure regular supplies of leafy vegetables from the production areas to consumers in peri-urban and urban centres.

2.6.4. Cooking

Cooking induces significant changes in chemical composition affecting concentration and nutrient bioavailability [71]. Various cooking methods are used based on convenience and taste preference rather than nutrient retention. Some cooking methods may oxidize antioxidants [72] and affect the vegetable nutrient retention. It is therefore important to choose a cooking method that leads to optimal nutrient retention and bioavailability [73]. Cooking for a longer time leads to a higher loss of most of the nutrients especially if cooking water is discarded since most nutrients leach into it [71,73]. The choice and age of plant harvested also influences the quality of the leafy vegetable.

Voster et al. [74] reported that young growing and tender leaves are used in the preparation of vegetables dishes in South Africa. Petioles and in some cases young tender stems are also included but old, hard stems are discarded [74]. The leafy vegetables dishes are prepared from a single species or from a combination of different species [6]. Cooking methods vary through boiling, to steaming [6]. The recipes used to prepare the vegetables tend to be similar among people belonging to a particular cultural group, limiting culinary diversity [19]. At the time of this research there was less published data retrieved on the recommended cooking methods and diversified recipes for various ALVs in South Africa.

Smith and Eyzaguirre [42] reported that ALVs are indispensable ingredients in soups or sauces that accompany carbohydrates or staples. The crucial component of the leafy vegetable promotion strategy will be through recipe developments to show ways of preparing these food ingredients. The recipes should encourage the use of the ALVs in preparing foods other than accompanying sauces to ensure that the vegetables are used daily, thus increasing the opportunities for their consumption. To promote these crops, the developed vegetable products can be consumed as snacks or accompany a beverage thus broadening the consumption habits. Value addition through product development will help address the issue of perishability and fluctuating supply of the vegetables on the market. There is need for research in the development of diversified recipes that are nutrient-dense and for alternative uses of these indigenous vegetables. Awareness creation, coupled with the development of brochures on how to prepare ALVs—as well as informing the potential consumers of where to find them—will help to extend demand even to those who do not know much about these vegetables. The demonstrations of proper cooking methods will result in increased utilization in ALVs species, some of which have an unpleasant taste (e.g., African night shade)—a factor which has been detrimental to acceptance by some people.

2.7. Conclusions

In South Africa, there is a decline in consumption of ALVs partly because of low availability and poor perception. Low availability is because production continues to be in small scales and they are considered wild species hence have never been commercialised. They are obtained by means of collection rather than cultivation hence they face threats of over-exploitation. Promotion of conservation and collection of genetic resources and germplasm exchange need urgent attention. There is need to develop support policies for seed systems for both the public and private sectors. Although neglected and underutilised in South Africa, ALVs offer unique opportunities to diversify farming systems, ensure food security and alleviate poverty, while increasing income and improving human health. Some of the challenges hindering promotion of ALVs include lack of sound agronomic information due to limited research, shortage of seeds as currently there are no registered varieties for most of ALVs and lack of value-adding technologies. For leafy vegetables to move from underutilised crops to commercial-level production there is a need to generate production information as has been done on major crops. Public education in production, conservation and marketing through workshops and seminars is also key to their promotion. Increased research on production, nutrition, processing and marketing still requires attention. Promotion of ALVs needs engaging of policy-makers who will incorporate it into government policies and programmes. Furthermore, policy makers can influence curriculum development at schools and universities to integrate ALVs into the educational curriculum. There is need to develop joint programmes among government, private sectors and NGOs to promote ALVs. ALVs are part of the region's cultural heritage and are rich in nutrients, e.g., vitamin A and iron. Therefore, there is need to promote the cultivation and utilisation of ALVs by farmers, especially women and other vulnerable groups. Successful promotion should result in ALVs forming part of the daily staple diet of South Africans.

Acknowledgments: The authors acknowledge funding from the South African Department of Rural Development.

Author Contributions: Innocent Maseko, Tafadzwanashe Mabhaudhi and Samson Tesfay did the initial conceptualisation of the paper. Innocent Maseko then led the write-up of the paper and all authors then reviewed and approved of the paper prior to publication.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Van Rensburg, W.S.J.; Van Averbeke, W.; Slabbert, R.; Faber, M.; Van Jaarsveld, P.; Van Heerden, I.; Wenhold, F.; Oelofse, A. African leafy vegetables in South Africa. *Water SA* **2007**, *33*, 317–326.
2. Nesamvuni, C.; Steyn, N.P.; Potgieter, M.J. Nutritional value of wild, leafy vegetables consumed by the Vhavhenda. *S. Afr. J. Sci.* **2001**, *97*, 51–54.
3. Odhav, B.; Beekrum, S.; Akula, U.; Baijnath, H. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. *J. Food Comp. Anal.* **2007**, *20*, 430–435.
4. Vorster, I.H.J.; Stevens, J.B.; Steyn, G.J. Production systems of traditional leafy vegetables, Challenges for research and extension. *S. Afr. J. Agric. Ext.* **2008**, *37*, 85–96.
5. Maunder, E.M.W.; Meaker, J.L. The current and potential contribution of home-grown vegetables to diets in South Africa. *Water SA* **2007**, *33*, 401–406.
6. Oelofse, A.; van Averbeke, W. *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Water Research Commission: Pretoria, South Africa, **2012**.
7. Abukutsa-Onyango, M.O. Unexploited Potential of Indigenous African Vegetables in Western Kenya. Department of Botany and Horticulture. *Maseno J. Educ. Arts Sci.* **2003**, *4*, 103–122.
8. Van Averbeke, W.; Chabalala, M.P.; Okorogbona, A.O.M.; Rumania, T.D.; Azeez, J.O.; Slabbert, M.M. Plant nutrient requirements of African leafy vegetables. In *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa, **2012**.
9. Department of Agriculture, Fisheries and Forestry, Strategic Plan for the Department of Agriculture *Consolidting the Partnership for Poverty Eradication, Accelerated Growth and Wealth Creation*; Directorate Agricultural Information Services: Pretoria, South Africa, **2004**.
10. Van Rensburg, J.W.S.; Venter, S.L.; Netshiluvhu, T.R.; Van Den Heever, E.; Vorster, H.J.; De Ronde, J.A. The role of indigenous leafy vegetables in combating hunger and malnutrition. *S. Afr. J. Bot.* **2004**, *70*, 52–59.
11. Shiundu, K.M.; Oniang'o, R. Marketing African leafy vegetables, challenges and opportunities in the Kenyan context. *Afr. J. Food Agric. Nutr. Dev.* **2007**, *17*, 4–12.

12. Mabhaudhi, T.; Modi, A.T.; Beletse, Y.G. Response of taro (*Colocasia. esculenta* L. Schott) landraces to varying water regimes under a rainshelter. *Agric. Water Manag.* **2013**, *121*, 102–112.
13. Mabhaudhi, T.; O'Reilly, P.; Walker, S. The role of underutilised crops in Southern African farming systems, a scoping study. *Sustainability* **2016**, *8*, 302.
14. Mavengahama, S. The Contribution of Indigenous Vegetables to Food Security and Nutrition within Selected Sites in South Africa. Ph.D. Thesis, Stellenbosch University, Cape Town, South Africa, **2013**.
15. Van Wyk, B.E. *Food Plants of the World, Identification, Culinary Uses and Nutritional Value*, 1st ed.; Briza Publication: Pretoria, South Africa, **2005**.
16. Steyn, N.P.; Olivier, J.; Winter, P.; Burger, S.; Nesamvuni, C. A survey of wild, green leafy vegetables and their potential in combating micronutrient deficiencies in rural populations. *S. Afr. J. Sci.* **2001**, *97*, 276–278.
17. Shackleton, C.M. The prevalence of use and value of wild edible herbs in South Africa. *S. Afr. J. Sci.* **2003**, *99*, 23–25.
18. Venter, S.L.; van Rensburg, W.S.J.; Vorster, H.J.; van den Heever, E.; van Zijl, J.J.B. Promotion of African Leafy Vegetables within the Agricultural Research Council—Vegetable and Ornamental Plant Institute, The impact of the project. *Afr. J. Food Agric. Nutr. Dev.* **2007**, *7*, 1–7.
19. Vorster, H.J.; Van Rensburg, W.J.; Venter, S.L.; Van Zijl, J.J.B. (Re)-creating awareness of traditional leafy vegetables in communities. In Proceedings of the Regional Workshop on African Leafy Vegetables for Improved Nutrition, IPGRI, Nairobi, Kenya, 6–9 December **2005**.
20. Vorster, H.J.; van Rensburg, W.S.J.; Van Zijl, J.J.B.; Van den Heever, E. *Germplasm Management of African Leafy Vegetables for the Nutritional and Food Security Needs of Vulnerable Groups in South Africa*; ARC-VOPI: Pretoria, South Africa, **2002**.
21. Modi, M.; Modi, A.T.; Hendriks, S. Potential role for wild vegetables in household food security, A preliminary case study in KwaZulu-Natal, South Africa. *Afr. J. Food. Agric. Nutr. Dev.* **2006**, *6*, doi:10.4314/ajfand.v6i1.19167.
22. Matenge, S.T.P.; van der Merwe, D.; De Beer, H.; Bosman, M.J.C.; Kruger, A. Consumers' beliefs on indigenous and traditional foods and acceptance of products made with cow pea leaves. *Afr. J. Agric. Res.* **2012**, *7*, 2243–2254.
23. Taleni, V.; Goduka, N. Perceptions and Use of Indigenous Leafy Vegetables (ILVs) for Nutritional Value: A Case Study in Mantusini Community, Eastern Cape Province, South

Africa. In Proceedings of the International Conference on Food and Agricultural Sciences, Melakaelaka, Malaysia, 5 October **2013**.

24. Maseko, I. Effect of Agronomic Management on Growth and Yield of Selected Leafy Vegetables. Master’s Thesis, University of South Africa, Pretoria, South Africa, 2014.
25. Silwana, T. The Performance of Maize/Bean and Maize/Pump-Kin Intercrops under Different Planting Combinations and Weeding in Transkei, South Africa. Master’s Thesis, University of Fort Hare, Alice, South Africa, **2000**.
26. Schippers, R.R. African Indigenous Vegetables. In *An Overview of the Cultivated Species*; Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation: Chatham, UK, **2000**.
27. Van Averbeke, W.; Juma, K.A.; Tshikalange, T.E. The commodity systems of *Brassica rapa* L. subsp. *chinensis* and *Solanum retroflexum* Dun. In Vhembe, Limpopo Province, South Africa. *Water SA* **2007**, *33*, 349–353.
28. Lewu, F.B.; Adeboola, P.O.; Afolayan, A.J. Commercial harvesting of *Pelargonium sidoides* in the Eastern Cape, South Africa, Striking a balance between resource conservation and rural livelihoods. *J. Arid Environ.* **2007**, *70*, 380–388.
29. Mavengahama, S.; McLachlan, M.; de Clercq, W. The role of wild vegetable species in household food security in maize based subsistence cropping systems. *Food Sec.* **2013**, *5*, 103–122, doi:10.1007/s12571-013-0243-2.
30. Van Averbeke, W.; Juma, K.A.; Tshikalange, T.E. Yield response of African leafy vegetables to nitrogen, phosphorus and potassium: The case of (*Brassica rapa* L. subsp. Chinensis) and (*Solanum. retroflexum* Dun.) *Water SA* **2007**, *33*, 355–362.
31. Maseko, I.; Beletse, Y.G.; Nogemane, N.; du Plooy, C.P.; Musimwa, T.R.; Mabhaudhi, T. Productivity of non-heading Chinese cabbage (*Brassica rapa* subsp. *chinensis*) under different agronomic management factors. *S. Afr. J. Plant Soil* **2017**, doi:10.1080/02571862.2017.1295324.
32. Mhlontlo, S.; Muchaonyarwa, P.; Menken, P.N.S. Effects of sheep kraal manure on growth, dry matter yield and leaf nutritional composition of a local *Amaranthus* accession in the central region of Eastern Cape Province, South Africa. *Water SA* **2007**, *33*, 363–368.
33. Motsa, M.M.; Slabbert, M.M.; van Averbeke, W. Germination of leafy vegetables. In *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa, **2012**.

34. Van Averbeke, W.; Netshithuthuni, C. Effect of irrigation scheduling on leaf yield of non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*). *S. Afr. J. Plant Soil* **2010**, *27*, 322–327.
35. Beletse, Y.G.; du Plooy, C.P.; van Rensburg, J.W.S. Water requirement of eight indigenous vegetables. In *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa, **2012**.
36. Mulandana, N.S.; Mamadi, N.E.; Du Plooy, C.P.; Beletse, Y.G. Effect of spacing and transplanting time on amaranths yield. *Afr. Crop Sci. Conf. Proc.* **2009**, *9*, 243–246.
37. Tesfay, Z.S.; Mathe, S.; Modi, A.T.; Mabhaudhi, T. A Comparative Study on Antioxidant Potential of Selected African and Exotic Leafy Vegetables. *Hortscience* **2016**, *51*, 1529–1536.
38. Manyelo, K.W.; van Averbeke, W.; Hebinck, P. Smallholder irrigators and fresh produce street traders in Thohoyandou, Limpopo province, South Africa. In *Rural Development and the Construction of New Markets*; Hebinck, P., van der Ploeg, J.-D., Schneider, S., Eds.; Routledge: London, UK, **2015**; pp. 131–148.
39. Weinberger, K.; Pichop, G.N. Marketing of African vegetables along urban and peri urban supply chain in sub Saharan Africa. In *African Indigenous Vegetables in Urban Agriculture*; Schackelton, C.M., Pasquiini, M.W., Drescher, A.W., Eds.; Earthscan: London, UK, **2009;** p. 298.
40. Wenhold, F.; Annandale, J.; Faber, M.; Hart, T. *Water Use and Nutrient Content of Crop and Animal Food Products for Improved Household Food Security*; *A Scoping Study*; Water Research Commission: Pretoria, South Africa, 2012.
41. Faber, M.; Phungula, M.A.S.; Venter, S.L.; Dhanat, M.A.; Benade, A.J.S. Home gardens focusing on the production of yellow and dark green leafy vegetables increase the serum retinol concentrations of 2–5-year-old children in South Africa. *Am. J. Clin. Nutr.* **2002**, *76*, 1048–1054.
42. Smith, I.F.; Eyzaguirre, P. African leafy vegetables: Their role in the World Health Organization's global fruit and vegetables initiative. *Plant Food* **2007**, *7*, 3–5.
43. Afolayan, A.J.; Jimoh, F.O. Nutritional quality of some wild leafy vegetables in South Africa. *Int. J. Food Sci. Nutr.* **2009**, *60*, 424–431.
44. Manach, C.; Mazur, A.; Scalbert, A. Polyphenols and prevention of cardiovascular diseases. *Curr. Opin. Lipidol.* **2005**, *16*, 77–84.
45. Amic, D.; Davidovic-Amic, D.; Beslo, D.; Trinajstic, N. Structure-radical scavenging

activity relationship of flavonoids. *Croat. Chem. Acta* **2003**, *76*, 55–61.

46. Zobolo, A.M.; Mkabela, Q.N.; Mtetwa, D.K. Enhancing the status of indigenous vegetables through the use of kraal manure substitutes and intercropping. *Indilinga Afr. J. Indig. Knowl. Syst.* **2008**, *7*, 211–222.
47. Van der Walt, A.M.; Loots, D.T.; Ibrahim, M.I.M.; Bezuidenhout, C.C. Minerals, trace elements and antioxidant phytochemicals in wild African dark-green leafy vegetables (morogo). *S. Afr. J. Sci.* **2009**, *105*, 444–448.
48. Ndlovu, J.; Afolayan, A.J. Nutritional analysis of the South African wild vegetable *Corchorus olitorius* L. *Asian J. Plant Sci.* **2008**, *7*, 615–618.
49. Van Der Walt, A.M.; Mossanda, A.M.; Jivan, K.S.A.; Swart, S.D.; Bezuidenhout, C.C. Indigenous African food plants: Vehicles of diseases or source of protection. *IAJIKS Indilinga* **2005**, *4*, 279–279.
50. Okeno, J.A.; Chebet, D.K.; Mathenge, P.W. Status of indigenous vegetables utilization in Kenya. *Acta Hort.* **2003**, *621*, 95–100.
51. Adebooye, O.C.; Opabode, J.T. Status of conservation of the leafy vegetables and fruits of Africa. *Afr. J. Biotechnol.* **2004**, *3*, 700–705.
52. Jaleel, C.A.; Manivannan, P.; Wahid, A.; Farooq, M.; Aljaburi, H.J.; Somasundaram, R.; Panneerselvam, R. Drought Stress in Plants, A review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.* **2009**, *11*, 100–105.
53. Blum, A. Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* **2005**, *56*, 1159–1168.
54. Turner, N.C.; Wright, G.C.; Siddique, K.H.M. Adaptation of grain legumes (pulses) to water-limited environments. *Adv. Agron.* **2001**, *71*, 123–231.
55. Kavar, T.; Maras, M.; Kidric, M.; Sustar-Vozlic, J.; Meglic, V. Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress *Mol. Breed.* **2007**, *21*, 81–87.
56. Singh, B.B.; Ajeigbe, H.A.; Tarawali, S.; Fernandez-Rivera, S.; Abubakar, M. Improving the production and utilization of cowpea as food and fodder. *Field Crops Res.* **2003**, *84*, 169–177.
57. Grubben, G.J.H. *Amaranthus cruentus* L. In *PROTA 2, Vegetables/Légumes*; Grubben, G.J.H., Denton, O.A., Eds.; PROTA: Wageningen, The Netherlands, 2004; pp. 71–72.
58. Neluheni, K.; Du Plooy, C.P.; Mayaba, N. Yield response of leafy amaranths to different irrigation regimes. *Afr. Crop Sci. Conf. Proc.* **2007**, *8*, 1619–1623.

59. Slabbert, M.M.; Sosibo, M.S.; van Averbeke, W. The response of six African leafy vegetables to drought and heat stress. In *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; *WRC TT535/12*; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa, **2012**.

60. Palada, M.C.; Chang, L.C. Suggested Cultural Practices for Vegetable Amaranth. In *International Cooperates Guide*; AVRDC Pub #03-552; AVRDC-The World Vegetable Center: Shanhua, Taiwan**, 2003**; p. 4.

61. Annandale, J.G.; Stirzaker, R.J.; Singels, A.; Van Der Laan, M.; Laker, M.C. Irrigation schedule research, South African experiences and future prospects. W*ater SA* **2011**, *37*, 751–763.

62. Steduto, P.; Hsiao, T.C.; Fereres, E. On the conservation behaviour of biomass productivity. *Irrig. Sci.* **2007**, *25*, 189–207.

63. Renault, D.; Wallender, W.W. Nutriotional water productivity and diets. *Agric. Water Manag.* **2000**, *45*, 275–296.

64. Mabhaudhi, T.; Chibarabada, T.; Modi, A. Water-Food-Nutrition-Health Nexus, Linking Water to Improving Food, Nutrition and Health in Sub-Saharan Africa. *Int. J. Environ. Res. Public Health* **2016**, *13*, 107.

65. Kader, A.A. The return on investment in postharvest technology for assuring quality and safety of horticultural crops. *J. Agric. Invest.* **2006**, *4*, 45–52.

66. Nyaura, J.A.; Sila, D.N.; Owino, W.O. Postharvest stability of vegetable amaranth (*Amaranthus dubius*) combined low temperature and modified atmospheric packaging. *Food Sci. Qual. Manag.* **2014**, *30*, 66–72.

67. Mudau, A.R.; Nkomo, M.M.; Soundy, P.; Araya, H.T.; Ngezimana, W.; Mudau, F.N. Influence of postharvest storage temperature and duration on quality of baby spinach. *HortTechnology* **2015**, *25*, 665–670.

68. Mampholo, M.B.; Sivakumar, D.; Beukes, M.; Van Rensberg, W.J. Effect of modified atmosphere packaging on the quality and bioactive compounds of Chinese cabbage (*Brasicca rapa* L. ssp. *chinensis*). *J. Sci. Food Agric.* **2013**, *93*, 2005–2015.

69. Mampholo, M.B.; Sivakumar, D.; Van Rensberg, W.J. Variation in bioactive compounds and quality parameters in different modified atmosphere packaging during postharvest storage of Traditional Leafy Vegetables (*Amaranthus cruentus* L. and *Solanum retroflexum*). *J. Food Qual.* **2015**, *38*, 1745–4557.

70. Fellows, P.J. *Food Processing Technology, Principles and Practice*, 3rd ed.; CRC Press: Boca Raton, FL, USA; Woodhead Publishing Limited: Oxford, UK, **2009**.

71. Yuan, G.; Sun, B.O.; Jing, Y.; Wang, Q. Effects of different cooking methods on health promoting compounds of broccoli. *J. Zhejiang Univ. Sci. B* **2009**, *10*, 580–588.
72. Shahnaz, A.; Khan, KM.; Sheikh, M.A.; Shahid, M. Effect of peeling and cooking on nutrients in vegetables. *Pak. J. Nutr.* **2003**, *2*, 189–191.
73. Funke, O.M. Evaluation of nutrient contents of amaranth leaves prepared using different cooking methods. *Food Sci. Nutr.* **2011**, *2*, 249–252.
74. Vorster, H.J.; van Rensburg, W.S.J.; Stevens, J.B.; Steyn, G.J. The role of traditional leafy vegetables in the food security of rural households in South Africa. *Acta Hort.* **2009**, *806*, 23–28.

CHAPTER 3

Moisture stress on physiology and yield of some indigenous leafy vegetables under field conditions

Innocent Maseko[1], Bhekumthetho Ncube[3*], Tafadzwanashe Mabhaudhi[2], Samson Tesfay[1], Vimbayi G.P. Chimonyo[2], Hintsa T. Araya[3], Melake Fessehazion[3], Christian P. Du Plooy[3]

[1] *Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa.*

[2] *Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209, South Africa.*

[3]*Agricultural Research Council – Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria, 0001, South Africa*

***Correspondence**

Dr B Ncube, Agricultural Research Council – Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria, 0001, South Africa. E-mail address: bbkncube@yahoo.com. *Tel: +27 12 808 8000*

Abstract

South Africa is rich with a diverse range of leafy vegetables that are rich in nutrients. African leafy vegetables (ALVs) are limited in terms of their commercial production due to lack of production information such as water requirements and yield. The effect of water stress on growth, physiology and yield of ALVs were evaluated under field conditions at the Agricultural Research Council (ARC), Roodeplaat, Pretoria, over two seasons, 2015/2016 and 2016/2017. A randomised complete block design was used with: three water levels [30%, 60% and 100% of crop water requirement (ETc)] and four ALVs (*Amaranthus cruentus* L., *Corchorus olitorius* L, and *Vigna unguiculata* (L.) Walp and *Beta vulgaris* L.), replicated three times. In *A. cruentus*, moisture stress (30% ETc) reduced plant height, chlorophyll content index (CCI) as well as yield. In *B. vulgaris* leaf number, plant height and yield were reduced by water stress. In both *A. cruentus* and *B. vulgaris*, yield increased with increase in water application from 30% ETc to 60% ETc and remained the same at 100% ETc. For *C. olitorius* and *V. unguiculata*,

CCI, plant height and yield were not affected by water stress although stem fresh mass was reduced by water stress in *V. unguiculata*. Using 60% ETc appears ideal for production of *A. cruentus* and *Beta vulgaris*, whereas 30% ETc is recommended for *V. unguiculata* and *C. olitorius*. Results of *A. cruentus* and *Beta vulgaris* were comparable under similar conditions. *V. unguiculata* and *C. olitorius* performed better than *Beta vulgaris* indicating an opportunity to improve productivity under drought conditions.

Keywords: Leafy vegetables; moisture stress; physiology; production; yield

Abbreviations

ALV	African leafy vegetables
ARC-VOP	Agricultural Research Council-Vegetable and Ornamental Plants
CCI	Chlorophyll content index
ARC-ISCW	Agricultural Research Council-Institute for Soil Climate and Water
RCBD	Randomised complete block design
AWS	Automatic weather station
WAT	Weeks after transplanting
DMRT	Duncan's multiple range test

3.1. Introduction

South Africa is a hyper-arid to semi-arid country (Bennie and Hensley, 2001). Agricultural moisture limitation remains one of the major impending factors to crop production and a threat to food security (Mabhaudhi et al., 2013, Chimonyo et al., 2018). According to Annandale et al. (2011) in not so far future, water availability for individual use will drop rapidly. Growing drought tolerant crops is one of the ways of averting the challenges of inadequate water availability (Oelofse and van Averbeke, 2012). South Africa is endowed with diverse African leafy vegetables (ALVs) that are rich in nutrients and can grow in marginal production areas. According to Van Rensburg et al. (2007), *Amaranthus cruentus* (pig weed), *Corchorus olitorius* (Jews mallow) and *Vigna unguiculata* (cowpea) are among the major ALVs in South Africa. These crops contain significant levels of calcium, iron, zinc, vitamin B, vitamin A and β-carotene, nutrients of which are highly deficient in South African diets (Oelofse and van Averbeke, 2012). Despite their significance, these crops are less cultivated due to, in part, limited agronomic information such as water use which is crucial in devising water management strategies (Oelofse and van Averbeke, 2012). *Amaranthus cruentus* and *C. olitorius* are rarely cultivated but harvested from cultivated lands, fallow land and in the wild (Van Rensburg, 2007; Maseko et al., 2018). *Vigna unguiculata* is mainly produced for its grain and fodder with little attention as a leafy vegetable (Van Rensburg et al., 2007). The ability to adapt to marginal growing conditions makes indigenous vegetable crops more advantageous over exotic types and their contribution to dietary diversity and options makes them lucrative to resource poor, mostly rural communities (Maseko et al., 2018). However, in South Africa information on production, yield and quality of ALVs under varying water regimes that can be used to promote their production is very scant (Nyathi et al*.,* 2018b).

Studies conducted elsewhere reports *C. olitorius* as being tolerant to moisture and salinity stress (Ayodele and Fawusi, 1989; Chaudhuri and Choudhuri, 1997; Fawusi et al. 1984). In contrast, Fasinmirin and Olufayo (2009) reported that higher yield and water use efficiency (WUE) in *C. olitorius* could be possible when full irrigation is applied to the crop. Although cowpea is regarded as a drought tolerant crop, limited irrigation has been found to cause significant yield reduction (Watanabe et al., 1997). The literature has shown that Amaranthus is tolerant to adverse climatic conditions (Grubben, 2004). However, adopting results from complicated by the variations in plants response to variable climates, plant species, variety and levels of stress imposed to the plant among other factors. Few studies conducted in South Africa under controlled environments (rain shelter, green house) have shown a possibility of producing ALVs in water-limited areas although economic yield may be compromised (Beletse et al., 2012; Slabbert et al., 2012). Neluheni et al. (2007) highlights that significantly reasonable yield can still be attained in *Amaranthus* at low moisture levels under field conditions, although it was only a one season trial. Preliminary studies conducted in South Africa on nutritional water productivity of *Cleome, Beta vulgaris* and *Amaranthus*, reported a decrease in biomass yield and mineral content with increase in water stress (Nyathi et al*.,* 2016, 2018b). Furthermore, the performance of selected ALVs was comparable to that of *Beta vulgaris* produced under the same conditions (Nyathi et al*.,* 2018b). South Africa has a high diversity of ALVs that are available for consumption; therefore the notion in this study was that other ALVs species would perform comparably or better than *Beta vulgaris.* Therefore, there is need for further research on water management strategies for other ALVs such as *V. unguiculata*, *C. olitorius*, *A. cruentus* and commercialised *B. vulgaris* (used as a reference crop) under the same growing conditions. Such information would be useful to generate production guidelines for these crops. The aim of this study was to evaluate the physiological and yield parameters of *C. olitorius*, *V.unguiculata, A. cruentus*, and *B. vulgaris* grown under different moisture regimes in the field conditions.

3.2. Material and Method

3.2.1. Plant material

Amaranthus cruentus and *Corchorus olitorius* seeds were obtained from the seed bank at the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP) while those for *V. unguiculata* and Swiss chard (*B. vulgaris* L.) cultivar 'Ford Hook Giant' were sourced from Hygrotech Seed Pty. Ltd., South Africa). The seeds were used as is without any treatment.

3.2.2. Description of trial site

The trials were conducted in the 2015-2016 and 2016-2017 seasons at ARC-VOP (25°35' S; 28°21' E; 1164 m a.s.l), Pretoria, South Africa. The mean annual precipitation for the study site, for the past 18 years (2000-2018) was 635 mm. During the 2015/2016 and 2016/2017 seasons the total annual rainfall was about 274 mm and 515 mm respectively. The mean daily minimum and maximum temperatures at the study sites during summer (November – April) are 8°C and 34°C respectively. The soil type is classified as Hutton clay loam (red apedal, aprox. 25% clay, 6% silt, 69% sand and pH 6.6) from the South African soil taxonomic system. The soil physical and chemical characteristics within the top 30 cm are described in Table 3.1.

Table 3.1**.** Physical and chemical characteristics of the soil in the experimental field

Soil attribute	2015/16 summer season	2016/17 summer season
P (mg kg^{-1})	40.0	5.9
K (mg kg^{-1})	227.0	250.0
Ca (mg kg^{-1})	825.0	696.0
Mg (mg kg^{-1})	240.0	273.0
Na (mg kg^{-1})	34.0	17.8
Exchangeable cation Ca (%)	60.2	53.8
Exchangeable cation Mg (%)	29.2	35.1
Exchangeable cation K (%)	8.5	9.9
Exchangeable cation Na (%)	2.2	1.2
pH	7.0	6.9
Clay (%)	18.0	20.0
Silt (%)	6.0	4.0
Sand (%)	76.0	76.0
N-NO_3	6.2	6.4
N-NH_4	5.5	3.1

3.2.3. Experimental design and treatments

The experimental design used for the trial was a randomised complete block design (RCBD) with three replicates for both seasons. Moisture at three levels was investigated on four different ALV species. (*C. olitorius, V. unguiculata, A. cruentus*, and *B.vulgaris*). The moisture treatments were: 30%, 60% and 100% of crop water requirement (ETc). The plot sizes were 36 m^2 (12 m x 3 m) for each crop in both seasons. A plant population of 66666 plants ha^{-1} was used for each crop in each season (Maseko et al., 2015). Moisture was applied using the drip irrigation. Watering was based on the reference evapotranspiration (ET) and a crop factor and these ET_o values were obtained from automatic weather station (AWS); which calculates ET_o daily as per the O Penman–Monteith's approach (Allen et al., 1998, Mabhaudhi et al., 2013). Crop coefficient (K_c) values used were for spinach (Allen et al., 1998) where $Kc_{initial}$ = 0.7, Kc_{med} = 1 and K_{clate} = 0.95. With these K_c and ET_o values, crop water requirement (ETc) was then calculated as using the following formula:

$$ETc = ET_o * K_c$$

where, ET_c = crop water requirement

ET_o= reference evapotranspiration, and

K_c = crop factor.

To help the vegetables establish crop stands, all treatments were given the same amount of water during the first two weeks and thereafter the different moisture levels were administered. The total amount of water applied, inclusive of the initial watering, were 610 mm (100% ETc), 366 mm (60% ETc) and 183 mm (30% ETc) for 2015/16. During 2016/17 season it was 425 mm (100% ETc), 255 mm (60% ETc) and 127 mm for (30% ETc). Throughout the experimental period, soil moisture status was monitored using Theta probes.

3.2.4. Agronomic practices

Prior to land preparation and planting, soil samples were taken for nutrient analyses at the ARC-Institute for Sil Climate and Water (ARC-ISCW). Fertiliser application was then based on the soil analysis results for 2015/2016 and 2016/2017 seasons (Maseko et al., 2019). Potassium was deemed sufficient based on results of soil fertility analyses for both seasons. *Amaranthus cruentus*, *C. olitorius* and *B. vulgaris* seedlings were first raised in 200 cavity polystyrene trays using a commercial growing medium, Hygromix® (Hygrotech Seed Pty. Ltd., South Africa) and covered with vermiculate from above surface. The seedlings were then transplanted to the field four weeks after sowing. *V. unguiculata* seeds were directly sown using at a rate of one (1) seed per station due to the high germination percentage based on the results of the germination tests done. Pest, disease and weed control were applied as per best agronomic management practices in all trials.

3.3.5. Data collection

Climatic data were monitored through an automatic weather station (AWS) stationed within a 100 m of the field trials. A total of twelve (12) plants per plot were tagged for data collection. All measurements were taken on leaves that had at least 50% green leaf area. Plant height, leaf number and chlorophyll content index (CCI) were measured from four weeks after transplanting (WAT). Chlorophyll content index was were measured from the adaxial surface of the leaf using the CCM-200 *Plus* chlorophyll content meter (Opti-Sciences, Inc., USA). All data were collected mid-day prior to irrigation.

Harvesting started six (6) WAT and every two weeks thereafter. The sample size for yield was 1 m^2 for each replicate. In each harvest, *A. cruentus* and *C. olitorius* yields were measured through cutting the above ground mass of the plant leaving 0.2 m of plant height above ground level while three to four fresh marketable leaves including their tender stems towards the growing tip of each runner were picked for *V. unguiculata*. The harvested material was then separated into stems and leaves. Fresh marketable leaves were picked for *B. vulgaris*. Marketable leaves were defined as fresh green and tender leaves that were large enough to be marketable starting from the fifth true leaf. For accuracy of results, plant sample weights were measured within an hour of collection to minimise moisture loss. Dry matter content was obtained after oven drying at 50°C for 48 hours. Total dry and fresh mass yields of the consumable portion were used in the calculation of the crop water productivities. Crop water productivity was determined as follows:

Water productivity = Biomass / ETc

Where: Crop water productivity in kg m^{-3},

Biomass = FM (fresh matter) and DM (dry matter) yields above ground in (t ha^{-1}, and

ETc = Crop evapotranspiration/ water-use/ crop water requirement in m^3.

3.2.6. Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) using SPSS software for Windows (IBM SPSS, version 25.0, Chicago, IL, USA). Duncan's multiple range test (DMRT) ($P \leq 0.05$) was used to separate significantly different means.

3.3. Results and discussion

3.3.1. Meteorological conditions and soil water content

The weather data recorded during the study period (2015-16 and 2016-17) indicate variations in rainfall with insignificant differences in temperatures (minimum and maximum) (Table 3.2).

Table 3.2. Summary of monthly averages for meteorological variables in the experimental field at ARC, Roodeplaat, Pretoria, South Africa

Season 2016-17 **Month**	**[a]T_x (°C)**	**[b]T_n (°C)**	**Total radiation ($MJ\ m^{-2}\ day^{-1}$)**	**Wind speed ($m\ s^{-1}$)**	**Rain mm**	**ET_o**
November	29,4	15,49	24,7	0,83	175,51	148,48
December	30,14	17,39	24,31	0,87	67,57	155,51
January	29,36	17,24	23,02	0,89	131,83	146,04
February	28,74	17,37	2,06	0,96	140,98	23,15
Mean	**29.41**	**16.87**	**18.52**	**0.89**	**128.97**	**118.30**
Season 2015-16						
November	31,77	13,95	27,88	1,15	29,72	176,03
December	33,88	18,09	26,54	0,94	60,2	176,96
January	31,67	17,63	25,68	0,87	135,13	165,89
February	32,46	17,82	24,4	0,94	49,53	152,84
Mean	**32.44**	**16.87**	**26.13**	**0.98**	**68.65**	**167.93**

[a]Maximum temperature; [b]Minimum temperature; [c]FAO reference evapotranspiration; *Monthly total. Monthly averages and totals were calculated from hourly data.

A comparison of rainfall received in the two study seasons with the mean long-term rainfall (678 mm) for the study site indicate that total rainfall received in the 2015-16 (275 mm) was 59 % lower. In the following season (2016-17) the rainfall received was 31% (516 mm) lower than the long term average. In the 2015-16 season, the amount of water applied was higher than in the 2016-17 experiment. The first season had higher average temperature (32 °C) while during the second season the temperature averaged 29 °C. The soil moisture content of the three watering regimes are represented in Figure 3.1.

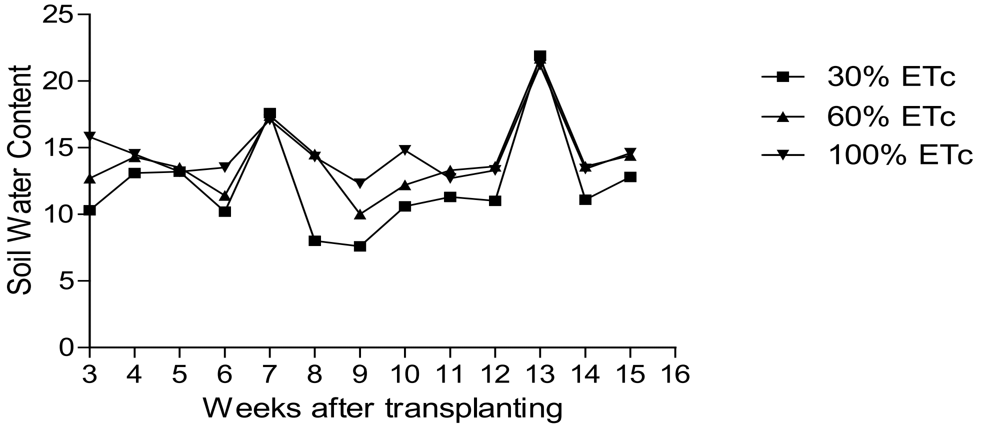


Figure 3.1. Volumetric soil water content observed from 3 WAT showing differences between 30%, 60% and 100% ETc irrigation regimes

The results revealed significant differences among the three treatments except in times where there was rainfall that altered the predefined soil moisture deficits.

3.3.2. Growth parameters

In *A. cruentus* plant height increased significantly ($P<0.05$) from 30% ETc (32cm) to 60% ETc (47cm), while a further increase in water application to 100% ETc (47cm) did not significantly increase plant height during the first season (Table 3.3).

Table 3.3. Effect of moisture stress on growth parameters of selected African leafy vegetables for two growing seasons

Crops	Parameters	Irrigation levels (ET$_c$)					
		2015/16 summer (Season 1)			2016/2017 summer (Season 2)		
		*30%	60%	100%	30%	60%	100%
A. cruentus	*Plant height	31.97^{a}	47.88^{b}	47.04^{b}	27.25^{a}	29.58^{a}	31.14^{a}
	Leaf number	49.03^{a}	55.12^{a}	52.35^{a}	34.23^{a}	34.97^{a}	35.23^{a}
C. olitorius	Plant height	52.75^{a}	51.52^{a}	55.68^{a}	27.85^{a}	30.26^{a}	37.85^{a}
	Leaf number	55.20^{a}	53.46^{a}	60.80^{a}	32.02^{a}	35.06^{a}	33.34^{a}
V.unguiculata	Plant height	43.64^{a}	46.91^{a}	51.31^{a}	23.55^{a}	24.90^{a}	25.50^{a}
	Leaf number	46.24^{a}	53.51^{a}	45.75^{a}	37.14^{a}	46.40^{a}	35.78^{a}
B.vulgaris	Plant height	19.46^{a}	$22.86^{a,b}$	29.16^{b}	18.91^{a}	20.10^{a}	19.35^{a}
	Leaf number	8.00^{a}	6.93^{a}	14.86^{b}	8.05^{a}	9.53^{a}	8.42^{a}

Means followed by the same letters within a row are not significantly different according to Duncan's multiple range tests at $P\leq0.05$. *Plant height-cm

Similar results where plant height decreased under low soil moisture were reported elsewhere in *A. tricolor* (Singh and Whitehead, 1992) and *A. hybridus* (Masarirambi et al., 2012). Plants deal with with moisture stress by reducing in plant size (Mitchell et al., 1998) as a drought avoidance strategy (Turner, 1986). Plant height increased during the second season, with an increase in water application although not significantly (P>0.05). Differences in results between the two seasons could be attributed to the differences in rainfall which could have led to variation in drought effect**.** In *A. cruentus* leaf number increased from 30% ETc to 60% ETc, then declined at 100% ETc in the first season, while leaf number was higher in well-watered condition of 100% ETc compared to lower water application during the second season. However, the differences observed in leaf number of *A. cruentus* were insignificant (P>0.05) for all seasons (Table 3.3). Although not significant, the observed trend suggests that increasing severity of water stress can lead to reduced number of leaves as has been observed by Yarnia *et al*. (2010) in amaranths.

In *C. olitorius*, the well-watered plants (100% ETc) had higher plant height than those grown under limited water supply for both seasons. However, the plant height differences recorded in *C. olitorius* were not significant (P>0.05) for both seasons (Table 3.3). Although statistically insignificant, limited moisture conditions had less leaf number in the first season than the well-watered treatments in *C. olitorius*. In the second season, however, the trend was such that leaf number increased with increase in water application from 30% ETc to 60% ETc, with a slight decline at 100% ETc. The reduced growth as a result of moisture stress has been previously reported in *C. olitorius* (Shiwachi et al., 2008).

Plant height in *V. unguiculata* increased with increase in water application although the differences were not significant (P>0.05) for both seasons (Table 3.3). There were no significant differences in leaf number in both seasons for *V. unguiculata* (Table 3.3). Despite this lack of statistical significance, a general characteristic increase in leaf number from 30% ETc to 60% ETc was observed with a further increase in moisture application to 100% ETc resulting in reduced leaf number. Although not significant, the trend indicates that severe stress level can lead to reduction in leaf number and plant height. Reduction in leaf production due to moisture stress has been reported in *V. unguiculata* (Abidoye 2004). Drought stress did not cause any major limitation to plant growth in the present study. These findings, however disagreed with those of Aderolu (2000) who reported a reduction in leaf number in cowpea under moisture stress. Variation in results may be attributed to variation in species used or climatic condition among other factors. Nkaa et al. (2014) reported that different cowpea variety performed differently under various stress conditions.

Plant height and leaf number of *B. vulgaris* was significantly (P<0.05) higher at 100% ETc (29cm) compared to lower water regimes (19cm in 30% ETc and 22cm in 60% ETc) in the first season of this study although this was not significantly affected in the subsequent season (Table 3.3). Reduction in plant height concurs with the findings from other researchers who reported reduced plant height in AVLs such as wild mustard and wild melon under moisture stress (Mbatha and Modi, 2010; Zulu and Modi, 2010). This suggests drought to be one of major factors that strongly influence crop growth (Slabbert et al., 2012). The reduced leaf number in moisture-deficient conditions may possibly have been a result of reduced leaf formation, a mechanism employed by plants to curtail transpiration by reducing the leaf surface area (Luvaha et al., 2008). During the second season of the study, leaf number and plant height increased from 30% ETc to 60% ETc, then declined at 100% ETc although these differences were not significant. Differences in the two seasons may be due to variation in rainfall since during the second season more rainfall could have reduced severity of water stress.

3.3.3. Chlorophyll Content Index (CCI)

In *A. cruentus*, chlorophyll content index significantly ($P<0.05$) increased from 30% ETc to 60% ETc, with no further significant increase at 100% ETc during the second season (Figure 2). During the first season; application of 100% ETc produced the highest CCI although statistics showed that it was similar to 30% ETc (Figure 2). Generally, the trend was similar for both seasons, with higher CCI in higher water application and lower CCI in lower water application. Mensha et al. (2006) reported decreased chlorophyll content in other crops like sesame subjected to water stress. According to Slabbert and Van den Heever (2007) chloroplasts are known to be severely affected by drought stress leading to a decline in photosynthetic rate. A decrease in photosynthetic activity may occur as a result of reduced chlorophyll concentration in moisture-stressed plants (Jafar et al., 2004; Mafakheri et al., 2010). Photosynthesis is a crucial process that supports crop growth and development and can be sensitive to moisture stress in many higher plant species (Maksymiec and Baszynski, 1996). In a study by Muthomi and Musyimi 92009), chlorophyll concentrations decreased with increased moisture deficit, a phenomenon that could be attributed to elevated oxidative stress. Dehydration of plant tissues under water deficit, can lead to elevated levels of oxidative stress and thus compromising the chloroplast structure and loss of chlorophyll. In other crops, chlorophyll content was shown to decrease in sunflower plants grown under limited moisture (Kiani et al. 2008). The high CCI recorded at 60% ETc in the current study adds to the market value of the vegetable crop as the market perceives the greenness of leafy vegetables as a good quality attribute (Maseko et al., 2015).

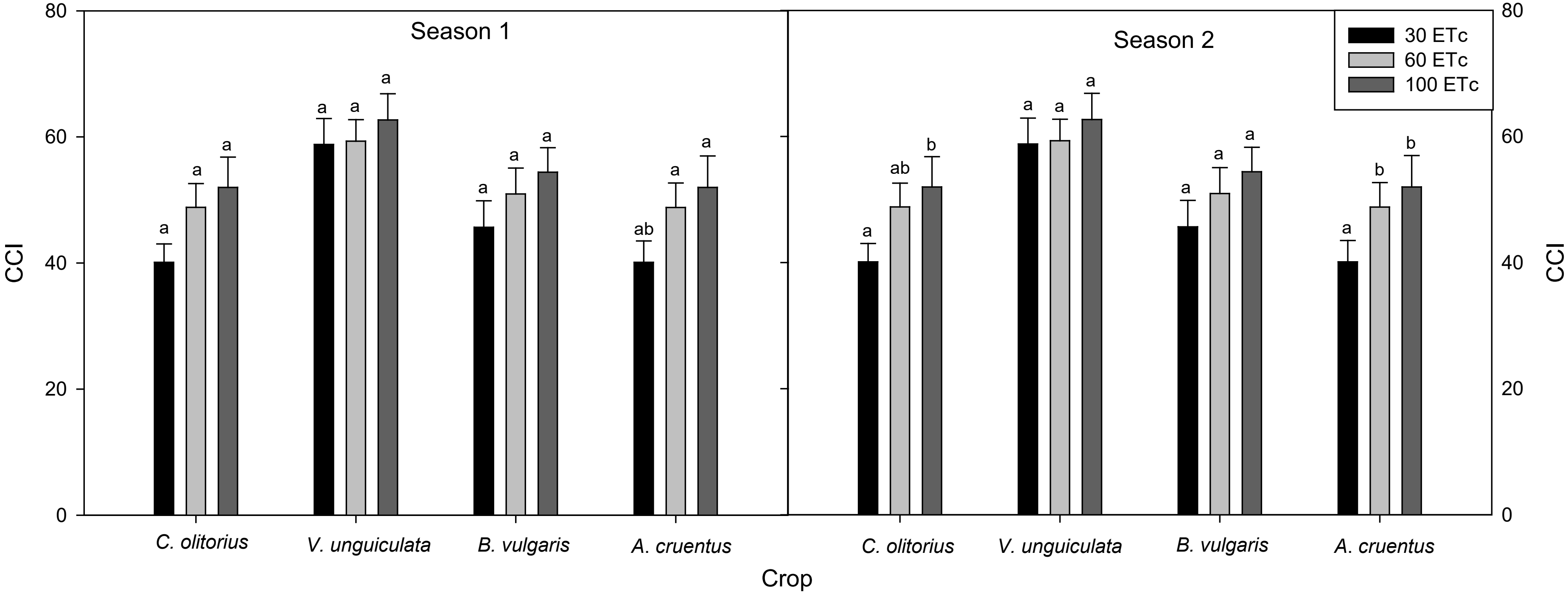


Figure 3.2. Effect of moisture stress on CCI of selected African leafy vegetables for two growing seasons

.

The results of CCI in *C. olitorius* are presented in Figure 3.2. Chlorophyll Content Index increased with increase in moisture content from 30% ETc to 60% ETc and then remained the same for both seasons. However, the only significant difference was observed during the second season. In crops such as okra and sunflower plants, reduced chlorophyll content as a result of moisture stress has been reported (Ashraf et al., 1994; Kiani et al., 2008). Severe drought stress has been reported to inhibit photosynthesis through altering the components and contents of the chlorophyll by damaging/distorting the photosynthetic apparatus (Iturbe Ormaetxe et al., 1998; Ommen et al., 1999). Stressed plants will have less chlorophyll content and reduced leaf area thereby compromising the market quality of the produce in terms of size and colour.

Chlorophyll content index of *V. unguiculata* and *B. vulgaris* was not significantly affected by water application in both seasons. Although not significant the trend was an increase in CCI with increase in moisture from 30% ETc to 60% ETc then remaining the same at 100% ETc in the first season. In the subsequent season, CCI increased proportional to the increase in water application. The trend suggests that increase in water stress can lead to reduced CCI. Decrease in photosynthetic activity due to decrease in chlorophyll concentration due to moisture stress in plants has also been recorded elsewhere (Jafar et al., 2004; Mafakheri et al., 2010). The lack of significant differences among different moisture regimes, suggests that chlorophyll contents in *C. olitorius* and *V. unguiculata* were not very sensitive to applied levels of moisture stress. Other researchers have reported various responses of CCI in plants, including a reduction in CCI in sunflower plants grown under limited moisture conditions (Kiani et al., 2008) and no significant effect on CCI of bambara groundnut landraces (Vurayai et al., 2011).

3.3.4. Yield parameters

Varying moisture regimes in this study significantly affected yield in *A. cruentus* during the first season (Table 3.4). Yield increased significantly from 30% ETc to 60% ETc and then declined significantly ($P<0.05$) at 100% ETc. Nyathi et al. (2016) reported similar results of yield reduction in water stressed conditions for crops such as *Amaranthus* and *Cleome*. Saleh et al. (2018) reported pod yield and other plant growth parameters to have increased proportional to the increase in mosture application from 60 to 80% of ETc, while further increase up to 100% of ETc did not improve yield in green pea.

Table 3.4. Effect of moisture stress on the yield of selected African leafy vegetables obtained from two growing seasons

Crop	Parameter (t. ha^{-1})	Irrigation levels					
		2015/16 summer (Season 1)			2016/2017 summer (Season 2)		
		30% ETc	60% ETc	100% ETc	30% ETc	60% ETc	100% ETc
A. cruentus	FM stem + leaves	3.15^{b}	5.63^{a}	3.26^{b}	7.09^{a}	8.77^{a}	9.39^{a}
	FM leaves	1.75^{b}	2.79^{a}	1.89^{b}	3.07^{a}	3.77^{a}	3.82^{a}
	FM stem	1.46^{b}	2.86^{a}	1.23^{b}	3.36^{a}	4.95^{a}	5.06^{a}
	DM leaves	0.57^{a}	0.70^{a}	0.58^{a}	0.61^{a}	0.73^{a}	0.72^{a}
	DM stem	0.47^{a}	0.65^{a}	0.47^{a}	0.69^{a}	0.85^{a}	0.81^{a}
C. olitorius	FM stem + leaves	3.32^{a}	2.77^{a}	2.64^{a}	7.13^{a}	6.17^{a}	7.50^{a}
	FM leaves	1.56^{a}	1.41^{a}	1.36^{a}	2.72^{a}	2.55^{a}	3.15^{a}
	FM stem	1.57^{a}	1.35^{a}	1.41^{a}	3.46^{a}	3.39^{a}	3.86^{a}
	DM leaves	0.54^{a}	0.53^{a}	0.46^{a}	0.68^{a}	0.88^{a}	0.92^{a}
	DM stem	0.39^{a}	0.37^{a}	0.34^{a}	0.69^{a}	0.75^{a}	0.77^{a}
V. unguiculata	FM stem + leaves	2.88^{a}	2.46^{a}	3.91^{a}	4.93^{a}	7.34^{a}	5.76^{a}
	FM leaves	2.17^{a}	1.83^{a}	2.82^{a}	2.28^{a}	3.60^{a}	2.91^{a}
	FM stem	0.78^{b}	0.75^{b}	1.25^{a}	2.56^{a}	3.42^{a}	2.93^{a}
	DM leaves	0.59^{a}	0.57^{a}	0.67^{a}	0.60^{a}	0.54^{a}	0.51^{a}
	DM stem	0.34^{a}	0.37^{a}	0.37^{a}	0.57^{a}	0.44^{a}	0.44^{a}
B. vulgaris	FM leaves	3.19^{a}	3.61^{a}	3.18^{a}	8.4^{a}	8.63^{a}	9.10^{a}
	DM leaves	0.54^{a}	0.54^{a}	0.58^{a}	0.97^{a}	1.09^{a}	1.28^{a}
	Leaf number	29^{b}	22^{b}	20^{b}	33^{b}	41^{ab}	46^{a}

*Means followed by the same letters within a row are not significantly different according to Duncan's multiple range tests at $P \leq 0.05$. FM=Fresh mass, DM = Dry mass

Further application of water up to 100% ETc reduced yield possibly because excessive water in the soil leads to the detrimental effects of oxygen deprivation in the roots (Saleh et al., 2018). The results also concur with that of Beletse et al. (2012) that *A. cruentus* grown in the less irrigated treatment produced the least average biomass yield on fresh and dry weight basis. The fresh mass yield obtained from the 30% ETc treatment was not of marketable quality. Therefore, irrigating *A. cruentus* at this level of water stress is not recommended, because both yield parameters were affected. Neluheni et al. (2007) also reported that *A. cruentus* is less tolerant compared to other species like *A. graezizans*. In the second season of this study no significant differences were observed among moisture treatments although generally yield increased with increase in water application (Table 3.4). Although *Amaranthus* can produce reasonable yield even at lower moisture availability as reported by Neluheni et al. (2007) variation in the two seasons maybe due to variations in rainfall. During the second season there was more rainfall which could have reduced the effect of irrigation. The results for both seasons indicate that for successful production of *A. cruentus,* a considerable amount of water is needed. Under severe stress conditions, farmers can utilise tolerant species as previous researchers have reported that drought tolerance in amaranth is species-dependent (Schippers, 2000; Palada and Chang, 2006). There were no significant differences in yield of *C. olitorius* as a result of moisture regimes for the two seasons (Table 3.4). Increasing moisture content from 30% ETc to 100 % ETc did not significantly increase biomass. The results contradict a report that *C. olitorius* is prone to moisture stress due to its shallow root depth (Fasinmirin, 2001). Plant responses to water stress depend on many factors, of which the amount of water loss, the rate of loss and the duration of the stressed condition play important roles.

Variation in results compared to other findings may be attributed to severity of stress imposed under field conditions. In times where there was rainfall, the predefined soil water deficits were altered/disturbed. *Cochorus olitorius* have the ability to quickly regain its growth vigour and viability following a moisture stress period if water supply is restored (Fasinmirin and Olufayo, 2009). Furthermore, the duration and severity of moisture stress as well as the growth stage of the plant shapes/determines the way it responds (Mabhaudhi, 2012).

In *V. unguiculata,* watering regimes significantly affected fresh stem yield during 2015/2016 season (Table 3.4). Full watering at 100% ETc resulted in significantly ($P<0.05$) higher stem fresh mass relative to 30% ETc and 60% ETc. Significant differences observed in this study corroborate with the report that water stress reduces yields in leguminous crops such as black beans and soybeans (Nielson and Nelson, 1998; Frederick et al., 2001).

Fresh and dry mass yields increased from 30% ETc to 60% ETc, then declined at 100% ETc in the second season of the study, although not significantly. Considering that application of 100% ETc produced the highest stem fresh mass without improving other measured parameters, it will be suitable to apply this amount of water when growing it for fodder rather than as a leafy vegetable.

Significant differences ($P<0.05$) were also recorded in leaf number (yield) of *B. vulgaris* due to water application in the second season (Table 4). Leaf number (yield) increased significantly by applying 30% ETc to 60% ETc, then remained the same at 100% ETc. Results concur with those of Saleh et al. (2018) who reported a corresponding increase in yield with the increase in moisture from 60 to 80% of ETc, while further increase up to 100% of ETc did not improve yield. Fresh mass of leaves and dry matter for both seasons increased with increase in application of water, however, the differences were not significant. Due to the fact that leaf vegetables are sold as a bunch (number of leaves) and not on a weight basis, 30% ETc yield may not be, in this case, not be a desirable option. Yield of *B. vulgaris* was consistent with growth results indicating that any stress that occurs at either of these developmental stages has a direct impact on vegetative growth, seedling establishment and final yield (Torrecillas and Alarcon, 2005).

3.3.5 Water productivity

Average fresh biomass yield in *A. cruentus* increased from 5.12 tha^{-1} (30% ETc) to 7.2 tha^{-1} (60% ETc) then declined to 6.30 t ha^{-1} (100 % ETc) (Table 3.5). Average crop water productivity for *A. cruentus* increased from 0.9 kgm^{-3} (30% ETc) to 1.02 kgm^{-3} 60% ETc then dropped to 0.69 kgm^{-3} (100 % ETc) although statistical analysis showed that the difference were negligible. The results indicate that 60% ETc irrigation treatment was more water productive than all other treatments in terms of fresh biomass yield (although statistically insignificant). Findings on water productivity response to limited water availability in *A. cruentus* were consistent with results of yield on fresh mass basis.

Corchorus olitorius average yield obtained from 30% ETc was higher compared to 60 and 100% ETc (Table 5). The highest water productivity was obtained in the driest irrigation treatment (30% ETc) which was statistical similar to other treatments. Higher yield and water productivity obtained in the low moisture treatment of 30% ETc indicates a possibility of production even under water stress conditions.

Table 3.5. Average total above ground fresh mass yield, irrigation water use and crop water productivity of selected indigenous leafy vegetables in two seasons (2015/2016 and 2016/2017)

Indigenous Leafy Vegetables	**Well-Watered (100 ETc)**			**Medium-Watered (60 ETc)**			**Deficit Irrigation (30 ETc)**		
	Average total above ground fresh yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($kg\ m^{-3}$)	Average total above ground fresh yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($k\ gm^{-3}$)	Average total above ground fresh yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($k\ gm^{-3}$)
A. cruentus	6.30	912	0.69^{a}	7.20	705	1.02^{a}	5.12	550	0.90^{a}
C. olitorius	5.07	912	0.55^{a}	4.47	705	0.63^{a}	5.22	550	0.94^{a}
V.unguiculata	3.90	912	0.52^{a}	4.90	705	0.70^{a}	4.80	550	0.87^{a}
B. vulgaris	6.14	912	0.67^{a}	6.12	705	0.86^{a}	5.70	550	1.04^{a}

In another separate study, a reduction in moisture application from 100 to 80 to 60% of ETc led to a progressive increase in WUE (Saleh et al., 2018). Deficit irrigation did not compromise leaf quality of *C. olitorius* indicating a possibility of production under rain fed conditions. Similarly, Nyathi et al. (2016) reported that results of water productivity of ALVs were comparable to those of *B. vulgaris*.

Average fresh biomass yield in *V. unguiculata* increased from 4.80 tha^{-1} (30% ETc), 4.90 tha^{-1} (60% ETc) then declined to 3.90 tha^{-1} (100 % ETc) (Table 5). Although not significant, average crop water productivity decreased with an increase in water application. The results obtained in this study are somewhat contrary to those found by Beletse et al. (2012) which recorded well irrigated treatments as having better yields in *V.unguiculata*. Variation in the results of the present study might be due to experimental conditions, which is field and rain shelter. Under rain shelter conditions the rainfall effect is minimized as the system is closed.

The average total yield of *B. vulgaris* increased with increase in water application (Table 3.5). Maximum crop water productivity was obtained in the 30% ETc, where deficit irrigation was applied although not signifiant. Water productivity decreased as applied irrigation water increased but higher fresh mass yield was obtained in the 100% ETc treatment. Results of the study showed that irrigating at 60 and 100% ETc gave higher yield compared to 30% ETc. Therefore, in *B. vulgaris* the less irrigated treatment of 30% ETc produced lower yield. This indicates little feasibility of producing the crop under limited water conditions compared to other ALVs.

The results of this study concur with the current claim that wild vegetables are better adapted to marginal areas compared to exotic vegetable species. Yield and growth in *V. unguiculata* and *C. olitorius* were not significantly affected by varying water application compared to *B. vulgaris.* At limited water level of 30% ETc. *V. unguiculata* and *C. olitorius* performed similar in terms of growth and yield compared to well-watered treatments**.** This suggests that production of these crops is still possible under limited water supply. Yield in *A. cruentus* and *B. vulgaris* had a similar trend, an increase from 30% ETc to 60% ETc, and then remained the same at 100% ETc. This means the optimum level was reached at 60% ETc. Considering that application above 60% ETc significantly reduced yield in *A. cruentus* there is a possibility that the optimum level may still be below 60% ETc. This is very economical in producing these crops.

Limited water supply did not compromise yield quality and quantity of *V. unguiculata* and *C. olitorius*. Furthermore, less water application can reduce amount of fertiliser leached thereby reducing fertiliser application and cost. Low water application means less maintenance of irrigation systems. This was however, the opposite for *B. vulgaris* where yields were higher in higher moisture regimes of 100% ETc. In a similar study, Slabbert et al. (2012) in screening reported that a relatively higher leaf area and relative water content was maintained by the six-major indigenous leafy vegetable than in *B. vulgaris*. Farmers could, however benefit from growing *V. unguiculata* through its nitrogen fixing properties, high leaf yield and reduction in soil erosion due to its extensive soil cover. The development of a wider choice of crops, including crops adapted to dry areas is critical if the growing human population of South Africa will continue to obtain its food from local production. If global warming persists, areas currently under irrigation could in future be without water supply, which means that cultivation will require the use of drought tolerant crops. Moisture deficit affects plant growth, development, yield and quality of crops under field conditions (Luvaha et al., 2008). When soil moisture is limited, photosynthetic rate, the respiration process, ion uptake and subsequently sugars (carbohydrates) and nutrient metabolism decrease and thus plant growth is also affected (Jaleel et al., 2009).

Conclusion

The present study showed that water stress leads to reduced yield in some ALVs. In *A. cruentus* plants were bigger (plant height) while yields were improved by application of 60% ETc. Considering that application above 60% ETc reduced yield there is a possibility that the optimum level may still be below 60% ETc which will be very economic for producing these crops. In both *A. cruentus* and *B. vulgaris*, yield significantly increased with increase in water application from 30% ETc to 60% ETc. For *Vigna unguiculata* and *C. olitorius*, CF, CCI, leaf number and yield as well as plant height were not affected by moisture stress and this indicated that these crops can be produced under limited water conditions. For *Vigna unguiculata* stem fresh mass improved by application of 100% ETc. However, this can be recommended when fodder is the end product because only the stems were improved while leaf yield was not affected. Using 60% ETc may be ideal for *A. cruentus* and *B. vulgaris* production, while 30% ETc can be recommended for *V. unguiculata* and *C. olitorius*. Further work needs to be done to explore performance of various plant species under different water regimes or stress severity. In addition, trials covering multi-site as well as different varieties in South Africa are necessary because variation in water requirement with location has been reported.

Acknowledgments

The authors acknowledge funding from the Department of Rural Development, National Research Foundation, Pretoria, South Africa and Agricultural Research Council – Vegetable and Ornamental Plants, South Africa.

References

Abdel Rahman, A.A., Shalaby, A.F., El Monayeri, M.O., 1971. Effect of moisture stress on metabolic products and ion accumulation. Plant and Soil 34: 65 90.

Abidoye, T.O., 2004. Effects of soil moisture content on growth and yield of cowpea (*Vigna unguiculata L.Walp*). B. Agric. Dissertation, University of Ilorin, Nigeria.

Aderolu, A.M., 2000. The effect of water stress at different growth stages on yield and seed quality of cowpea varieties. B. Agric Project University of Ilorin Nigeria.

Alam, S.M., 1999. Nutrient uptake by plants under stress conditions. In: Pessarakli, M. (ed): Handbook of plant and crop stress, pp. 285-313. Marcel Dekker, New York.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and Drainage Paper no. 56. Rome: Food and Agriculture Organization of the United Nations.

Ashraf, M.Y, Azmi, A.R., Khan, A.H., Ala, S.A., 1994. Effect of water stress on total phenols, peroxidase activity and chlorophyll content in wheat (*Triticum aestivum* L.) genotypes under soil water deficits. Acta Physiologiae Plantarum. 16, 185-191.

Ayodele, V.I., Fawusi, M.O.A., 1989. Studies on drought susceptibility of *Corchorus olitorius*: I. Effects of stressing plant at mid vegetative stage on dry matter yield and yield components of two cultivars of *C. olitorius*. Biotronics 18: 23-27.

Ayodele, V.I., Fawusi, M.O.A., 1990. Studies on drought susceptibility of *Corchorus olitorius*: II. Effect of moisture stress at different physiological stages on vegetative growth and seed yield of *C. olitorius* cv. Oniyaya. Biotronics 19:33-37

Beletse, Y.G., du Plooy, C.P. van Rensburg, J.W.S., 2012. Water requirement of eight indigenous vegetables. In: Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Oelofse A, Van Averbeke W, Eds.; Water Research Commission: Pretoria, South Africa.

Bennie, A.T.P., Hensley, M., 2001. Maximizing precipitation utilization in dry land agriculture in South Africa – a review. Journal of Hydrology 241, 124-139.

Chaudhuri, K., Choudhuri, M.A., 1997. Effect of short-term NaCl stress on water relations and gas exchange of two jute species. Biologia Plantarum. 40: 373-380.

Dunham, R.J., Nye, P.H., 1976. The influence of water content on the uptake of ions by roots. III. Phosphate, potassium, calcium and magnesium uptake and concentration gradients in soil. Journal of Applied Ecology, 13: 957–981.

Faber, M., Phungula, M.A.S., Venter, S.L., Dhanat, M.A., Benade, A.J.S., 2002. Home gardens focusing on the production of yellow and dark green leafy vegetables increase the serum retinol concentrations of 2–5-year-old children in South Africa. The American Journal of Clinical Nutrition. 76: 1048-1054.

Fasinmirin, J.T., 2001. Nitrogen and water use of irrigated jute mallow. Unpublished M. Eng Thesis. Department of Agricultural Engineering, Federal University of Technology, Akure, Nigeria

Fasinmirin, J.T., Olufayo, A.A., 2009. Yield and water use efficiency of jute mallow *Corchorus olitorius* under varying soil water management strategies. Journal of Medicinal Plants Research Vol. 3(4): pp. 186-191,

Fawusi, M.O.A., Ormrod, D.P., Eastham, A.M., 1984. Response to water stress of *Celosia argentea* and *Corchorus olitorius* in controlled environments. Scientia Horticulturae 22: 163-171

Frederick, J.R., Camp, C.R., Bauer, P.J., 2001. Drought-stress effects on branch and main

Griffiths, K.M., Behboudian, M.H., & Dingle, M., 1992. Irrigation management and fruit quality in Asian pea. HortScience 27:672

Grubben, G.J.H., 2004. *Amaranthus cruentus* L. In *PROTA 2, Vegetables/Légumes*; Grubben, G.J.H., Denton, O.A., Eds.; PROTA: Wageningen, The Netherlands; pp. 71–72.

IturbeOrmaetxe, I., Escuredo, P.R., Arrese-Igor, C., Becana, M. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiology.,116: 173–181

Jafar, M.S., Nourmohammadi, G., Maleki, A., 2004. Effect of water deficit on seedling, plantlets and compatible solutes of forage sorghum cv. Speed feed 4th International Crop Science Congress, Brisbane, Australia, 26 Sep-1Oct .

Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Somasundaram, R., Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agricultural Biology 11: 100-105.

Kiani, S.P., Maury, P., Sarrafi, A., Grieu, P., 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annus*) under well-watered and water-stressed conditions. Plant Science 175 565-573

Luvaha, E., Netondo, G.W., Ouma, G., 2008. Effect of water deficit on the physiological and morphological characteristics of mango (*Mangifera indica*) rootstock seedlings. American Journal of Plant Physiology. 3(1): 115.

Mabhaudhi, T., 2012. Drought tolerance and water–use of selected South African landraces of Taro (*Colocasia esculenta*L. Schott) and Bambara groundnut (*Vigna Subterranea*L. Verdc). Ph. D Thesis, University of KwaZulu Natal, Durban, South Africa.

Mabhaudhi, T., Modi, A.T., Beletse, Y.G., 2013. Growth, phonological and yield responses of a Bambara groundnut *(Vigna subter-ranea* L. Verdc) landrace to imposed water stress: ll. Rain shelter conditions. Water SA 39*:* 191-198.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y., 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science 4: 580-585.

Maksymiec, W., Baszynski, T., 1996. Chlorophyll fluorescence in primary leaves of excess Cu treated runner bean plants depends on their growth stages and the duration of Cu-action. Plant Physiology 149, 196-200.

Masarirambi, M.T., Dlamini, Z., Manyatsi, A.M., Wahome, P.K., Oseni, T.O, Shongwe, V.D., 2012. Soil water requirements of amaranth (*Amaranthus hybridus*) grown in a greenhouse in a semi-arid, sub-tropical environment. American-Eurasian Journal Agriculture & Environment Science 12: 932-936

Maseko, I., Beletse, Y.G., Nogemane, N., du Plooy, C.P., Mabhaudhi T., 2015. Growth, physiology and yield responses of *Amaranthus cruentus*, *Corchorus olitorius* and *Vigna unguiculata* to plant density under drip-irrigated commercial production. South African Journal of Plant and Soil. 32 (2), 87-94

Maseko, I., Beletse, Y.G., Nogemane, N., du Plooy, C.P., Musimwa, T.R., Mabhaudhi, T., 2017. Productivity of non-heading Chinese cabbage (*Brassica rapa* subsp. *chinensis*) under different agronomic management factors. South African Journal of Plant and Soil, 34 (4): 275-282.

Maseko, I., Ncube, B., Mabhaudhi, T., Tesfay, S., Chimonyo, V.G.P., Araya, H.T., Fessehazion, M., Du Plooy. C.P., 2019. Nutritional quality of selected African leafy vegetables cultivated under varying water regimes and different harvests. South African Journal of Botany doi.org/10.1016/j.sajb.2019.06.016

Mavengahama, S., McLachlan, M., de Clercq, W., 2013. The role of wild vegetable species in household food security in maize based subsistence cropping systems. Food Security, 5, 103–122

Mbatha, T.P., Modi, A.T., 2010. Response of local mustard germplasm to water stress. South African Journal of Plant and Soil27: 328-330.

Mensha, J.K., Obadoni, B.O., Eroutor, P.G.,\ Onome, I.F. 2006. Simulated flooding and drought effects on germination, growth and yield parameters of sesame (*Sesamum indicum*). African Journal of Biotechnology. 5: 1249-1253.

Mitchell, J.H., Siamhan, D., Wamala, M.H., Risimeri, J.B., Chinyamakobvu, E., Henderson, S.A., Fukai, S., 1998. The use of seedling leaf death score for evaluation of drought resistance of rice. Field Crops Research 55: 129-139.

Muthomi, J., Musyimi., D.M., 2009. Growth responses of African nightshades (*Solanum Scabrum Mill*) seedlings to water deficit. Journal of Agricultural and Biological Science4: 24–31.

Neluheni, K., Du Plooy, C.P., Mayaba, N., 2009. Yield response of leafy amaranths to different irrigation regimes. African crop science conference proceedings. 8: 1619

Nielson, D.W., Nelson, N.O., 1998. Black bean sensitivity to water stress at various growth stages. Crop Science. 38: 422-427.

Nkaa, F.A., Nwokeocha, O.W., Ihuoma, O., 2014. Effect of phosphorus fertilizer on growth and yield of cowpea (*Vigna unguiculata*). Journal of Pharmacy and Biological Sciences 9: 74-82.

Nyathi, M.K., Annandale, J.G., Beletse, Y.G., Beukes. D.J., Du Plooy, C.P., Pretorius B, van Halsema, G.E., 2016. Nutritional water productivity of traditional vegetables crops. WRC report No.2171/1/16.

Nyathi, M.K., van Halsema, G.E., Beletse, Y.G., Annandale, J.G., Struike P.C., 2018a. Calibration and validation of the AquaCrop model for repeatedly harvested leafy vegetables grown under different irrigation regimes. Agricultural Water Management208: 107-119

Nyathi, M.K., van Halsema, G.E., Beletse, Y.G., Annandale, J.G., Struike P.C., 2018b. Nutritional water productivity of selected leafy vegetables. Agricultural Water Management. 209: 111-122

Oelofse, A, van Averbeke, W., 2012. Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Water Research Commission: Pretoria, South Africa.

Oktem, A., 2008. Effect of water shortage on yield, and protein and mineral compositions of drip irrigated sweet corn in sustainable agricultural systems. Agricultural Water Management, 95(9): 1003-1010.

Ommen, O.E., Donnelly, A., Vanhoutvin, S., van Oijen, M., Manderscheid, R., 1999**.** Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentrations and other environmental stresses within the ESPACE-wheat project. European Journal of Agronomy10: 197-203.

Palada, M.C., Chang, L.C., 2003. Suggested Cultural Practices for Vegetable Amaranth. In: *International Cooperates Guide*; AVRDC Pub #03-552; AVRDC-The World Vegetable Center: Shanhua, Taiwan, p. 4.

Pascale, S.D., Paradiso, R., Barbieri, G., 2001. Recovery of physiological parameters in gladiolus under water stress. Colture Protette, 30(7):65-69

Saleh, S., Liu, G., Liu, M., Ji, Y., He, H., Gruda, N., 2018. Effect of Irrigation on Growth, Yield, and Chemical Composition of Two Green Bean Cultivars. Horticulturae 4, 1–10

Schippers, R.R., 2000. African Indigenous Vegetables. In: An Overview of the Cultivated Species; Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation: Chatham, UK.

Shiwachi, H., Komoda, M., Koshio, K., Takahashi, H., 2008. Effect of soil moisture stress on the growth of *Corchorus olitorius* . African Journal of Agricultural Research 4 (4): pp. 289-293,

Singh, B.P. Whitehead, W.F., 1992. Response of vegetable amaranth to differing soil pH and moisture regimes. Acta Horticulturae 318: 225-229.

Slabbert, M.M., Sosibo, M.S., van Averbeke, W., 2012. The response of six African leafy vegetables to drought and heat stress. In Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa,

Slabbert, R., Van Den Heever, E., 2007. Selection of traditional crops for improved drought tolerance in leafy amaranth: Moving toward sustainable food supply. Acta Horticulture 752, 281-286

Torrecillas, A., Alarcon, J.J., 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. Plant Physiology162: 281–289

Turner, N.C., 1986. Crop water deficits: a decade of progress. Advances in Agronomy. 39: 1-51.

Van Averbeke, W., Netshithuthuni, C., 2010. Effect of irrigation scheduling on leaf yield of non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*). South African Journal of Plant and Soil 27: 322–327

Van Rensburg, W.S.J., Van Averbeke, W., Slabbert, R., Faber, M., Van Jaarsveld, P., Van Heerden, I., Wenhold, F., Oelofse, A., 2007. African leafy vegetables in South Africa. Water SA, 33: 317–326.

Vurayai, R., Emongor, V., Moseki, B., 2011. Effect of water stress imposed at different growth and development stages on morphological traits and yield of Bambara Groundnut (*Vigna subterranea*). American Journal of Plant Physiology. 6 (1) 17–27.

Watanabe, N.C., Hakoyama, T.T., Singh, B.B., 1997. Evaluation methods for drought tolerance of cowpea. In: Advances in cowpea research. Singh, B.B., D.R. Mohan Raj, K.E. Dashiell and L.E.N. Jackai (eds). Copublication of the International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria

Yarnia, M., 2010. Sowing dates and density evaluation of amaranth (cv. Koniz) as a new crop. Advances in Environmental Biology 4: 41–46

Zulu, N.S., Modi, A.T., 2010. A preliminary study to determine water stress tolerance in wild melon (*Citrullus lanatus*). South African Journal of Plant and Soil 27: 334-336

CHAPTER 4

Productivity of selected African leafy vegetables to varying water regimes. Rain-shelter conditions.

I. Maseko[1*], T. Mabhaudhi[2], S. Tesfay[1], B. Ncube[3], V.G.P. Chimonyo[2], H. T. Araya[3], M. Fessehazion[3], C.P. Du Plooy[3]
[1]Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa; [2]Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa;[3]Agricultural Research Council, Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria 0001, South Africa;
*Correspondence: innocentmsk94@gmail.com; Tel: +27-(0)-33-260-6108

Abstract.

African leafy vegetables (ALVs) are rich in nutrients and can offer a wider choice of crops adapted to dry areas of South Africa. However, research on the productivity of various ALVs under limited water availability remains limited and sporadic. The effect of irrigation levels on growth, physiology and yield of *V. unguiculata*, *C. olitorius*, *A. cruentus* and a reference crop *B. vulgaris* were evaluated under a rain shelter at Roodeplaat, Pretoria over two summer seasons, 2015/2016 and 2016/2017. A randomised complete block design was used with: irrigation level and four crops, replicated three times. Vegetables species used as planting material were: *A. cruentus*, *C. olitorius*, *V. unguiculata* and *B. vulgaris*. The irrigation levels were: 30%, 60% and 100% of crop water requirement (ETc). Leaf number, plant height, chlorophyll content index (CCI), chlorophyll fluorescence (CF), and yield were measured in situ. In *A. cruentus* and *C. olitorius*, limited water availability of 30% ETc was shown to lower yield although leaf number, plant height and chlorophyll content index was shown to be unaffected. Comparable, in *B. vulgaris var. cicla* leaf number and yield were reduced by water stress. For *Vigna unguiculata,* CF, CCI, plant height, leaf number, and yield was not affected by water stress and this indicated that it can be produced under limited water compared to *B. vulgaris*. Using 60% ETc was suitable for production of *A. cruentus, C. olitorius* and *B. vulgaris var. cicla*, whereas 30% ETc is recommended for *V. unguiculata*. The yield results of *V. unguiculata* indicates it performs better, while yield of *A. cruentus* and *C. olitorius* comparable to that of *B. vulgaris* under similar conditions indicating the potential for marginal production.

Keywords: irrigation, production, yield

4.1 Introduction

South Africa is a water stressed country (Mabhaudhi et al., 2013) that faces challenges of population growth including food and nutrition insecurity (Oelofse and van Averbeke, 2012). Most smallholder communities live in marginal areas where crops struggle to survive and face challenges of water scarcity and malnutrition (Oelofse and van Averbeke, 2012). Furthermore, commercial or irrigated agriculture takes place under water scarcity and water availability is likely to drop below benchmark of 1000 m^3 $person^{-1}$ $year^{-1}$ (Annandale et al., 2011). African leafy vegetables (ALVs) offer alternatives both to small holder and commercial farmers because they have dense nutrients and more tolerant to abiotic stresses such as drought, heat stress, pests and diseases (Van Averbeke et al., 2012; DAFF, 2004). ALVs contribute to both micronutrients and bioactive compounds to diets (Smith and Eyzaguirre, 2007). They contain nutrients such as calcium, iron, vitamin A, vitamin C, fibre and proteins (Mavengahama, 2013). Furthermore, they are good sources of antioxidants such as flavonoids, tannins and other polyphenolic constituents (Afolayan and Jimoh, 2009).

South Africa has more than 100 different species of ALVs that have been identified; however, few groups of leafy vegetable species are still utilised (Van Rensburg et al., 2007). *Cochorus olitorius* (jute mallow), *Amaranthus cruentus* (pigweed) and *Vigna unguiculata* (cowpea) are among the major groups that are utilized. *Amaranthus* are reported to be tolerant to adverse environmental effects (Dieleman et al., 1996; Ghorbani et al., 1999). They have been growing wild in arid and semi-arid ecological regions, which means that they could be more tolerant to low water and high temperature conditions (Modi, 2006). Although cowpea is relatively drought tolerant, it has been shown that water stress reduces essential physiological and biochemical processes that affect growth and productivity (Pimentel, 2004; Costa et al., 2008; Lobato et al., 2008). Water stress in cowpea also occurs within genotypes (de Ronde and Spreeth, 2007). *Corchorus olitorius* is susceptible to moisture stress owing to its shallow rooting depth which can be prevented by using irrigation (Fasinmirin, 2001). African leaf vegetables have been reported to have advantages over exotic vegetable species, because of their adaptability to marginal agricultural production areas and their ability to provide dietary diversity in poor rural communities (Maseko et al., 2018). Inclusion of ALVs in cropping systems can contribute to climate change adaptation, the environment, and employment creation in poor rural communities (Mabhaudhi et al., 2016). However, their adoption is currently low because of limited research on their yield response to water.

ALVs have been documented to address some of the challenges South Africa faces in terms of water scarcity and malnutrition; however, there is lack of information on their yield response to water (Maseko et al., 2018; Nyathi et al*.,* 2018a). Studies conducted in South Africa to determine the water requirements of selected ALVs showed that although adequate amount of water is needed to produce marketable yield (Beletse et al., 2012; Nyathi et al., 2016) there is possibility of producing ALVs (Slabert et al., 2012) under limited water conditions. Recent studies conducted in South Africa on nutritional water productivity of *Amaranthus*, *Cleome* and *B. vulgaris* reported yield reduction in water stress conditions (Nyathi et al. 2018b). ALVs are also reported to produce yield comparable to that of *Beta vulgaris* var. *cicla* under similar conditions (Nyathi et al*.,* 2018b). Since a lot of different species of ALVs exist (Maseko et al., 2018), with a wide genetic diversity in growth habit, leaf shape, leaf colour, leaf size, plant size (Van Rensburg et al., 2007), there is need for further research on selectedALVs. These include *A. cruentus*, *C. olitorius* and *V. unguiculata* in comparison to *B. vulgaris* under the same locality. The greater number of species for people to select from, as well as a wider diversity of desirable traits can lead to successful commercilisation because farmers have a wide range to choose species that are better adapted for their region within South Africa. The objective of the study is to evaluate the productivity and yield of *A. cruentus*, *C. olitorius*, *V. unguiculata* and a reference vegetable crop,*B. vulgaris* under varying water regimes.

4.2 Material and Methods

4.2.1 Plant material

Seeds of *A. cruentus* and *C. olitorius* were obtained from the seed bank of the Agricultural Research Council (ARC) - Roodeplaat, Vegetable and Ornamental Plant Institute (VOPI). *V. unguiculata* (Bechuana white, a runner type) and Swiss chard (*B. vulgaris*) cultivar 'Ford Hook Giant' were obtained from Hygrotech Seed Pty. Ltd., South Africa. No treatment was done to the seeds.

4.2.2 Site description

Trials were planted at Roodeplaat, Pretoria (25º60´S; 28º35´E) during the summer seasons of 2015/2016 and 2016/2017. Soils in the rain shelter was classified as loamy sand (USDA taxonomic system). Soil physical characteristics were used to generate parameters for amount of water available at field capacity (FC), permanent wilting point (PWP), and saturation (SAT), as well as the saturated hydraulic conductivity using the Soil Water Characteristics Hydraulic Properties Calculator ® (Version 6.02.74, USDA Agricultural Research Services). Daily maximum and minimum temperature averages were 28.5˚C and 15˚C in summer (November – April) (Agricultural Research Council – Institute of Soil Climate and Weather).

Rainfall was excluded since the rain shelter is designed to close when rainfall starts. The field capacity of the soil was 146 mm^{-1} and the permanent wilting point was 75 mm m^{-1}.

4.2.3 Experimental design

The experimental design was a factorial experiment arranged in a randomised completely block design; individual plot size in the rain shelter was 6 m^2, with plant spacing of 0.3 m x 0.3 m. There were two factors: irrigation level and four crops, replicated three times. Vegetables species used as planting material were: *Amaranthus cruentus*, *Corchorus olitorius*, *Vigna unguiculata* (cowpea) and *Beta vulgaris* (Swiss chard). The irrigation levels were: 30% (Deficit irrigation), 60% (Moderate stress) and 100% (Well-watered) of crop water requirement (ETc). Swiss chard was chosen because it is a commercialised leafy vegetable that is highly nutritious which contains high levels of Fe, Zn and β-carotene (Mavengahama et al., 2013).

4.2.4 Irrigation

Drip irrigation was used to apply water in the rain shelter. The system consisted of a pump, filters, solenoid valves, water meter, control box, online drippers, 200 litre watertank, main line, sub-main lines and laterals. The system was designed to allow for a maximum operating pressure of 200 kPa with average discharge of 2 l/hour per emitter. Drip lines were spaced according to the plant spacing (0.3 m x 0.3 m). A black 200 µm thick polyethylene sheet was trenched at a depth of 1 m to separate the plots to prevent water seepage and lateral movement of water between plots.

Irrigation scheduling was based on reference evapotranspiration (ET) and a crop factor (Allen et al. 1998). Reference evapotranspiration (ET_o) values were obtained from an automatic weather station (AWS); the AWS calculates ET_o daily according to the Penman–Monteith's method (Allen et al., 1998, Mabhaudhi et al., 2014). Crop coefficient (K_c) values used were for spinach as described by Allen et al. (1998) whereby $Kc_{initial} = 0.7$, $Kc_{med} = 1$ and $K_{clate} = 0.95$. Using these values of K_c and ET_o from the AWS, crop water requirement (ETc) was then calculated as follows as described by Allen et al. (1998):

$$ET_c = ET_o*K_c$$

where, ET_c = crop water requirement

ET_o= reference evapotranspiration, and

K_c = crop factor.

During the first two weeks all treatments received the same amount of water to establish the plants and thereafter the irrigation treatments were imposed. Irrigation was applied three times every week and during the mornings to ensure water availability during peak periods of demand in the day. The total amount of irrigation water applied, taking into consideration the initial watering whileranged from 622 mm (100% ETc-well watered), 373 mm (60% ETc-medium watered) and 186 mm for (30% ETc-stress) for 2015/16. During 2016/17 season, watering ranged from 556 mm (100% ETc), 333 mm (60% ETc) and 166 mm for (30% ETc). The soil water status during the growing period was monitored using Theta probes.

4.2.5 Agronomic practices

Soil samples were taken from the field prior to land preparation at a depth between 0.3 m to 0.6 m and submitted for soil fertility analysis at the Agricultural Research Council- Institute of Soil, Climate and Water (ARC-ISCW). Land preparation included digging and harrowing to achieve a fine seedbed. Nitrogen (limestone ammonium nitrate (LAN) 28% N) was applied according to results of soil fertility analysis for 2015/2016 and 2016/2017 both seasons (Table 1).

Table 4.1: Soil physico-chemical analysis results of the soil used in the study

K	**Ca**	**Mg**	**Na**	**P**	**pH**	**N-NO3**	**N-NH4**
				mg/kg			
105	1412	221	67	67.7	7,4	5.44	3.42

Application rates were: 125 kg ha^{-1} N for *A. cruentus* and *C. olitorius,* 150 kg ha^{-1} N for *B. vulgaris* and 135 kg ha^{-1} N for *V. unguiculata* for both seasons. Nitrogen was applied by banding in three split applications. The first application was at transplanting/sowing (50%), second at 4 weeks after transplanting/sowing (25%) and the last at (25%) 8 weeks after transplanting/sowing. Double super phosphate was applied at 20 kg (10.5 % P) at planting for season 1 for all the crops. During second season at 63 P kg ha^{-1} for *B. vulgaris*, 55 kg ha^{-1} P for *A. cruentus* and *C. olitorius and* 75 kg ha^{-1}P for *V. unguiculata* at planting. Potassium was deemed sufficient based on results of soil fertility analyses for both seasons. Seedlings of *A. cruentus*, *B. vulgaris* and *C. olitorius* were grown in 250 cavity polystyrene trays filled with a commercial growing medium, Hygromix® (Hygrotech Seed Pty. Ltd., South Africa) and covered with vermiculate to minimize water losses from the above surface. Seedlings were transplanted at four weeks after sowing. *V. unguiculata* was sown directly using seed at a rate of one (1) seed per station because the germination percentage was high based on results of previous standard germination tests carried out at the experimental site. Routine weeding and scouting for pests and diseases were done to ensure best management practices for the trials. Seedlings were planted at an inter-row and intra row spacing of 0.3 m x 0.3 m (111,111 plants ha^{-1})

4.2.6 Data collection

Data collection was done on the inner rows for both seasons to prevent border effects. A total of twelve (12) plants per replication were tagged for data collection for growth and physiology parameters. All measurements were done on leaves that had at least 50% green leaf area. Plant height, leaf number, chlorophyll content index (CCI) and chlorophyll fluorescence (CF) were measured starting from four weeks after transplanting (WAT). Plant height was measured using a measuring tape from the ground level to the tip or apex of the tallest stem. Chlorophyll content index was determined on the adaxial surface using the CCM-200 *Plus* chlorophyll content meter (Opti-Sciences, Inc., USA). All measurements were done before irrigation and during mid-day.

Harvesting commenced at six (6) weeks after transplanting (WAT) or sowing and every two weeks thereafter. The sample size for yield was 1 m^2 for each replicate for both seasons. During each harvest, *C. olitorius* and *A. cruentus* yield were determined by cutting the mass of above ground portion of the plant leaving 0.2 m of plant height above ground level. For *V. unguiculata*, harvesting was done by picking three to four fresh marketable leaves including their tender stems towards the growing tip of each runner, leaving the first and second growing leaves from the tip. Marketable leaves in *V. unguiculata* were defined as fresh or green tender leaves. The harvested portion was then partitioned into leaves and stems. For *B. vulgaris* during each harvest, yields were determined by picking fresh marketable leaves. Marketable leaves were defined as fresh green and tender leaves that were large enough to be marketable starting from the fifth true leaf. At first harvest the small lower leaves were removed to promote growth. In order to obtain accurate results, plants were weighed within an hour to avoid loss of water. Dry matter content was obtained by oven drying at 70°C for 48 hours. Yield per hectare was obtained by conversion from measurements taken at 1 m^2 per replicate.

Soil water content (SWC) was monitored using ML-2X Theta Probes connected to a DL-6 data logger (Delta-T Devices, UK) in the rain shelters at varying depths. The frequency of data collection for SWC using the Theta probes was every day. Crop water productivity was determined as follows:

Water productivity = Biomass / ETc

Where: Crop water productivity was in kg m^{-3},

Biomass = FM (fresh matter) and DM (dry matter) yields above ground in (t ha^{-1}, and

ETc = crop evapotranspiration/ water-use/ crop water requirement in m^3.

4.2.7 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS software for Windows (IBM SPSS, version 25.0, Chicago, IL, USA). Where there were significant differences ($P \leq 0.05$), the means were further separated using Duncan's multiple range test (DMRT).

4.3 Results and discussion

4.3.1 Meteorological conditions and soil water content

Figure 1 shows the soil water content measurements from the three water regimes. The measurements confirmed that there were indeed differences between the three water regimes.

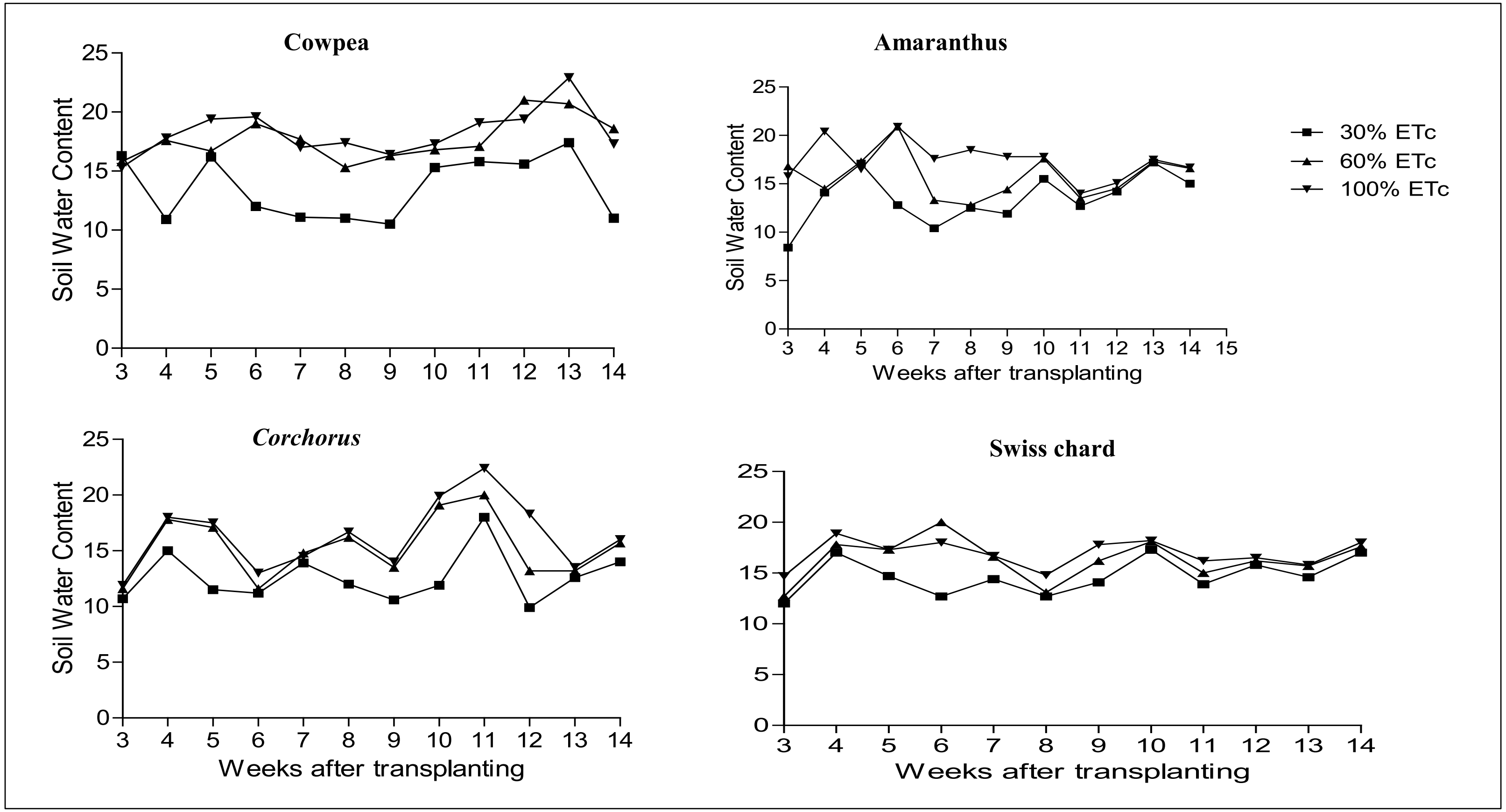


Figure 4.1. Volumetric soil water content observed from 3 WAT showing differences between the 30%, 60% and 100% ETc water regimes.

In the 2015-16 experiment, the amount of irrigation water applied was slightly higher than in the 2016-17 experiment although the difference was negligible (Table 4.2). The ET_o was slightly higher for 2015-16 compared to 2016-17. The 2015-16 season had higher temperature with average of 32.47 °C while during the 2015-16 the average temperature was 29.95°C. Minimum temperature, radiation and wind speed were similar for both seasons. The weather data was consistent for both seasons.

Table 4.2. Summary of monthly averages for climaticvariables during the growing season of ALVs

Season 2016-17 Month	$^{a}T_x$ (°C)	$^{b}T_n$ (°C)	Total radiation ($MJ\ m^{-2}\ day^{-1}$)	Wind speed ($m\ s^{-1}$)	$^{c}ET_o$
October	30,90	13,32	25,18	1,15	163,12
November	29,40	15,49	24,70	0,83	148,48
December	30,14	17,39	24,31	0,87	155,51
January	29,36	17,24	23,02	0,89	146,04
Season 2015-16					
October	32,58	14,16	25,17	0,69	161,52
November	31,77	13,95	27,88	1,15	176,03
December	33,88	18,09	26,54	0,94	176,96
January	31,67	17,63	25,68	0,87	165,89

[a]Maximum temperature; [b]Minimum temperature; cFAO reference evapotranspiration; *. Monthly averages and totals were calculated from hourly data. Note: meteorological variables do not include rainfall, because it was excluded in the rainshelter

4.3.2 Growth parameters

4.3.2.1 Plant height and leaf number

Plant height of *A. cruentus* was not significantly (P>0.05) affected by different water regimes for both seasons (Table 4.3). Despite lack of statistical significance, the trend was an increase in plant height with increase in water application for the first season while during the second season the trend was an increase from 30% ETc to 60% ETc and then a decline at 100% ETc (Table 4.3). The present study did not show any significant difference although other researchers have reported decreasing plant height with low soil moisture under controlled environments in *A. hybridus* (Masarirambi et al., 2012) and *A. tricolor* (Singh and Whitehead, 1992). Differences observed may be due to variation in plant species used since stress tolerance varies with species or stage of plant growth or level of stress (Slabbert et al., 2012).

Table 4.3. Effect of irrigation on growth of selected African leafy vegetables for two seasons

Plants	Parameters	Irrigation levels					
		2015/16 summer (Season 1)			2016/2017 summer (Season 2)		
		30% ET_c	60% ET_c	100% ET_c	30% ET_c	60% ET_c	100% ET_c
A. cruentus	Plant height (cm)	52.1^{a}	63.8^{a}	68.3^{a}	29.5^{a}	37.5^{a}	34.6^{a}
	Leaf number	80^{a}	103^{a}	111^{a}	69^{a}	64^{a}	74^{a}
C. olitorius	Plant height (cm)	41.1^{a}	34.3^{a}	41.6^{a}	41.8^{a}	39.6^{a}	51.4^{a}
	Leaf number	128^{a}	139^{a}	149^{a}	46^{a}	44^{a}	67^{a}
V. unguiculata	Plant height (cm)	65.4^{a}	74.3^{a}	77.0^{a}	31.3^{a}	30.5^{a}	23.7^{a}
	Leaf number	89^{a}	95^{a}	86^{a}	49^{a}	48^{a}	36^{a}
B. vulgaris	Plant height (cm)	22.9^{a}	21.1^{a}	20.7^{a}	27.5^{a}	24.3^{a}	21.5^{a}
	Leaf number	10^{a}	11^{a}	11a	7^{a}	13^{b}	9^{a}

*Means followed by the same letters within a row are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Although not significant for both seasons, at 100% ETc, plants had a higher leaf number than at lower water application during the second season in *A. cruentus*. For the first season leaf number increased with increase in water application as observed in plant height. Although not statistically significant, for both seasons, the trend suggested that limiting water application could lead to reduced leaf number and plant height. From the study, *A. cruentus* growth was favoured at 30% ETc to 60% ETc although better growth could be expected when the crop was irrigated at 100% ETc.

In *C. olitorius*, plant height and leaf number were higher in 100% ETc compared to limited water application of 30% ETc to 60% ETc for both season, however, the difference observed were not significant (P>0.05) for all seasons (Table 4.3). The present study showed that *C. olitorius* was able to grow under soil moisture stress condition which concurs with Shiwachi et al. (2008). Similarly, other researchers have reported that *C. olitorius* was shown to be tolerant to soil moisture and NaCl stress (Chaudhuri and Choudhuri, 1997; Fawusi et al., 1984; Ayodele and Fawusi, 1989; 1990). Distribution of *C. olitorius* in arid-regions is thought to be attributed to its tolerance to soil moisture stress.

There was no significant (P>0.05) difference in leaf number and plant height of *V. unguiculata* for both season (Table 4.3). Although not significant (P>0.05), plant height and leaf number increased from 30% ETc to 60% ETc and then declined at 100% ETc during the first season. The trend observed concurs with Aderolu (2000) who reported that water stress affected number of leaves for cowpea. During the second season the results were not consistent, where leaf number and plant height increased with decrease in water application. Cowpea has been found to be one of the most drought tolerant crops (Singh et al., 1997).

Irrigation regimes did not significantly (P>0.05) affect plant height of *B. vulgaris* in both seasons (Table 4.3). Significant (P<0.05) differences were observed for leaf number during the second season, although no significant differences were recorded during the first season (Table 4.3). Leaf number increased significantly from 30% ETc to 60% ETc then declined significantly (P<0.05) at 100% ETc. Water stress was shown to reduce plant height, leaf number and area in ALVs such as wild mustard (Mbatha and Modi, 2010) and wild melon (Zulu and Modi, 2010). Water stress impairs mitosis, elongation and expansion, resulting in reduced leaf number and reduced crop growth (Kaya et al., 2006).

4.3.3 Crop physiology

4.3.3.1 Chlorophyll Content Index (CCI)

Chlorophyll content index was not significantly (P>0.05) affected by varying water regimes in *A. cruentus*, *C. olitorius*, *B. vulgaris* and *V. unguiculata* for both seasons (Figure 4.2). In *C. olitorius* and *V. unguiculata*, CCI increased with increase in water application for both season although not statistically significant (P>0.05). A similar trend was observed for *A. cruentus* and *B. vulgaris* although in some instances the trend was an increase in CCI from 30 up to a 60% ETc then a decline. Researchers have reported various responses of CCI in plants. Chlorophyll content was shown to decrease in sunflower plants subjected to water stress (Kiani et al., 2008). Vurayai et al. (2011) working on pot trials, reported that water stress did not have a significant effect on chlorophyll content index (CCI) of Bambara groundnut landraces; they concluded that CCI was not reduced by water stress at all stages of growth. Lack of significant differences in *V. unguiculata* among treatments may be due to the ability of plants to maximise resources even at a limited water application of 30% ETc. Therefore, varying irrigation application in *V. unguiculata* did not compromise leaf colour or greenness of the leaf. According to Ashley (1993), drought tolerance is the ability of a plant to live, grow and yield satisfactorily with a limited soil water supply or under periodic water deficiencies.

There were no significant (P>0.05) differences in Chlorophyll fluorescence (CF) in response to varying water regimes in *A. cruentus*, *C. olitorius*, *B. vulgaris* and *V. unguiculata* (Data not shown). Despite lack of statistical significance, there was a tendency of CF in all crops to increase from 30% ETc to 60% ETc up to 100% ETc. The lack of differences for CF may be because experiments were conducted under optimum fertilisation. No significant differences (P > 0.05) between water regimes, suggests that CF was not as sensitive to water stress.

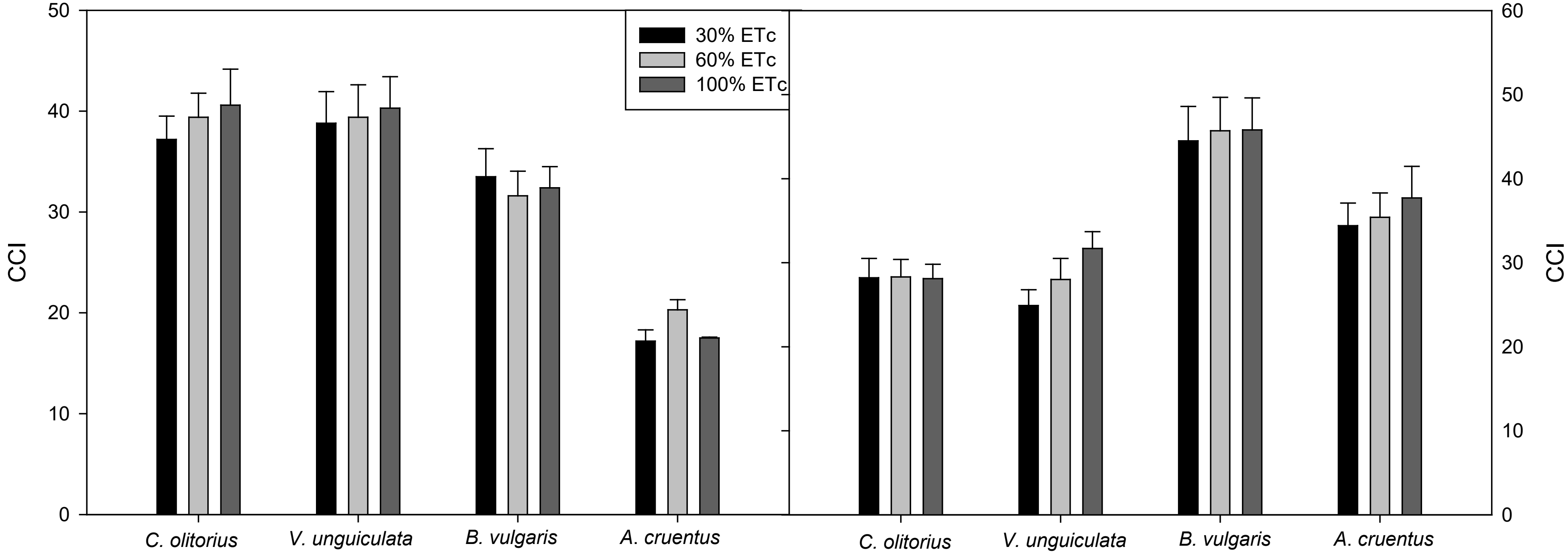


Figure 4.2. Effect of irrigation on chloropyl content Index of selected African leafy vegetables for two seasons

4.3.4 Yield parameters

4.3.4.1. Total fresh and dry yield

Yield in *A. cruentus* was significantly (P<0.05) affected by water regimes during both seasons (Table 4.4). Fresh mass of stems, leaves and leaf dry matter increased significantly (P<0.05) with increase in water application from 30% ETc to 60% ETc, then remained the same at 100% ETc for both seasons. Results concur with wit previous reports that irrigation improved biomass yield in amaranth (Nyathi et al*.*, 2016). Saleh et al. (2018) also reported that green bean growth parameters and pod yield increased with increasing water application from 60 to 80% of ETc while further increase up to 100% of ETc did not improve yield. Water deficit often causes plant water stress, which has a negative effect on growth and quality of plants and would cause substantial reductions in yield (Wang et al., 2003). Similar observation was made by Beletse et al. (2012) where medium watered plants had better yield that well watered plants. Higher yield was obtained in the 60% ETc treatment than in the 100% ETc irrigation treatment. The reduced yield obtained in the 100% ETc treatment could be attributed to the high frequency of irrigation applied to replenish the soil water deficit, which may have caused nutrient leaching from the root zone (Beletse et al., 2012). Lower yields in limited water application for *A. cruentus* concurs with previous researchers who reported that drought tolerance in amaranth depends on the species (Nehuleni, 2007; Palada and Chang, 2006; Schippers, 2000). Yarnia et al. (2010) also reported that applying low levels of irrigation leads to reduction in yield. According to Beletse et al. (2012) yields obtained under water-stressed conditions may lack the quality needed to market the produce. Although results of growth parameters (leaf number and plant height) were not significant, the trend was consistent with yield results.

In *C. olitorius* leaf dry matter content (first season) and fresh leaf mass (second season) were significantly (P<0.05) affected by water regimes (Table 2.4). Leaf dry matter and fresh leaf mass increased significantly (P<0.05) with increase in water application from 30% ETc to 60% ETc, further application of water to 100% ETc did not improve yield. The same trend was observed in other measured yield components for both seasons although not significant (P>0.05). Fasinmirin and Olufayo (2009) reported that above ground biomass increased with amount of water application when grown under irrigated conditions.

Table 4.4. Effect of irrigation on the yield of selected African leafy vegetables obtained from two growing seasons

Crops	Plant parts (t. ha^{-1})	Irrigation levels					
		2015/16 summer (Season 1)			2016/2017 summer (Season 2)		
		30% ETc	60% ETc	100% ETc	30% ETc	60% ETc	100% ETc
A. cruentus	FM stem + leaves	4.11^{a}	10.84^{b}	7.85^{ab}	2.87^{a}	4.35^{ab}	5.00^{b}
	FM leaves	3.17^{a}	4.14^{a}	3.71^{a}	1.66^{a}	1.97^{ab}	2.45^{b}
	FM stem	2.92^{a}	4.67^{a}	3.60^{a}	1.32^{a}	1.86^{ab}	2.48^{b}
	DM leaves	0.54^{a}	0.87^{b}	0.71^{ab}	0.38^{a}	0.50^{a}	0.54^{a}
	DM stem	0.45^{a}	0.70^{a}	0.52^{a}	0.38^{a}	0.39^{a}	0.48^{a}
C. olitorius	FM stem + leaves	4.43^{a}	7.21^{a}	6.79^{a}	1.95^{a}	3.68a	4.04^{a}
	FM leaves	2.05^{a}	2.74^{a}	2.62^{a}	0.83^{a}	1.46^{ab}	1.70^{b}
	FM stem	2.40^{a}	3.71^{a}	3.40^{a}	1.21^{a}	1.32^{a}	1.25^{a}
	DM leaves	0.50^{a}	0.63^{ab}	0.66^{b}	0.33^{a}	0.40^{a}	0.43a
	DM stem	0.45^{a}	0.43^{a}	0.48^{a}	0.33^{a}	0.37^{a}	0.36^{a}
V. unguiculata	FM stem + leaves	5.04^{a}	5.72^{a}	6.90^{a}	4.93a	7.34^{a}	5.76^{a}
	FM leaves	3.03^{a}	3.34^{a}	3.81^{a}	2.28^{a}	3.60^{a}	2.91^{a}
	FM stem	2.05^{a}	2.39^{a}	3.05^{a}	2.56^{a}	3.42^{a}	2.93
	DM leaves	0.62^{a}	0.68^{a}	0.68^{a}	0.59	0.54^{a}	0.51
	DM stem	0.36^{a}	0.39^{a}	0.43^{a}	0.57^{a}	0.44^{a}	0.44^{a}
B. vulgaris	FM leaves	4.53^{a}	6.91^{ab}	10.26^{b}	4.08^{a}	6.44^{b}	8.67^{b}
	DM leaves	0.83^{a}	0.74^{a}	1.04^{a}	0.61^{a}	0.72^{ab}	0.86^{b}
	Leaf number	40^{a}	51^{a}	57^{a}	28^{a}	38^{a}	37^{a}

*Means followed by the same letters within a row are not significantly different according to Duncan's multiple range tests at P≤0.05. FM=Fresh mass, DM =Dry mass

Results concur with reports that *C. olitorius* is susceptible to moisture stress owing to its shallow rooting depth which can be prevented by using irrigation (Fasinmirin, 2001). Taylor and Wepper (1990) reported that the yield of *C. olitorius* was enhanced when irrigation was used in conjunction with rainfall to reduce soil moisture stress. When evaporation rates are high, frequent irrigations are required to maintain plant available water at levels necessary to maximize growth and yield (Connor et al., 1985; Whitfield et al., 1986).

Yield components in *V. unguiculata* increased with increase in water regimes from 30% ETc to 60% ETc andup to 100% ETc during first season; however, statistical analysis showed that there were no significant differences (Table 4.4). During the second season, yield components increased from 30% ETc to 60% ETc, and then remained unchanged at 100% ETc; however; no significant differences were observed. The results concur with Slabbert et al. (2012) who reported that *V. unguiculata* is among the most tolerant ALVs. Studies conducted elsewhere have shown that cowpea (Singh et al., 2003; Singh et al., 1997) is tolerant to adverse climatic conditions. Present results indicate potential of *V. unguiculata* production under deficit irrigation. *Vigna unguiculata* grew well under deficit irrigation of 30% ETc without losing quality of the leaves. Drought stress did not have an influence on biomass. The results contradict with Hayatu and Mukhtar (2010) who reported that drought stress significantly reduced plant above ground biomass in cowpea genotypes. Variation in results may be attributed to variation in species used or climatic condition among other factors. Nkaa et al. (2014) reported that different cowpea varieties perform different under various stress conditions.

In *B. vulgaris* fresh mass and dry mass yield was significantly ($P<0.05$) affected by water regimes during both seasons (Table 4.4). Fresh mass of stems, leaves and leaf dry matter increased significantly ($P<0.05$) with increase in water application from 30% ETc to 60% ETc and up to 100% ETc for both season. For both season applications of 100% ETc produced double the amount of biomass compared to 30% ETc. Highest fresh leaf weight was obtained from the 100% ETc treatment, which indicated that *B. vulgaris* favoured high levels of soil water availability for optimum growth and development. Similarly, Van Averbeke and Netshithuthuni (2010) reported that *Brassica* species such as Chinese cabbage are sensitive to water stress. Sammis and Wu (1989) reported that cabbage marketable yield increased linearly with increased water application. Sanchez et al. (1994) found that cabbage production was optimized when crops were irrigated for evapotranspiration (ET) replacement while both deficit and excess irrigation reduced yield. Statistical analysis showed that there was no difference at 60% ETc to 100% ETc and therefore it will be economic for farmers to adapt 60% ETc for *B. vulgaris*.

Overall, the results of this study indicated that the ALVs had a higher degree of drought tolerance than the reference crop *B. vulgaris*. Ranking for drought tolerance starting with the most tolerant could be: *V.unguiculata*, *C. olitorius*, *A. cruentus* and *B. vulgaris.* Growth and yield of *V. unguiculata* were not affected by varying water application compared to *B. vulgaris.* Yield in *A. cruentus*, *C. olitorius* and *B. vulgaris* showed similar trends, an increase from 30% ETc to 60% ETc, and then remaining the same at 100% ETc. Considering that application of 60% ETc and 100% ETc yielded same results according to statistical analysis, therefore application of 60% ETc is more economic for both crops. The yield results found in *A. cruentus* are often comparable, and in some cases were better than *B. vulgaris.* Yield in *A. cruentus* doubled when 60% ETc was applied while in *B. vulgaris*, a double in yield was obtained when 100% ETc was applied compared to 30% ETc. Our findings on yield response to limited water availability were consistent with results of crop growth. Reduction in yield in well irrigated plants (*C. olitorius*, *A. cruentus* and *B. vulgaris*) can be due to increased susceptibility of soil to water logging which reduces aeration within the soil (Jenson et al. 1990). Sharma et al. (1990) also stated that crop growth and yield were improved when application of water can be controlled to what the plant actually needs.

4.3.5 Water productivity

A. cruentus grown in the 30% ETc irrigation treatment produced the least average biomass yield on fresh and dry weight basis (Table 4.5). Fresh biomass yield for both seasons averaged 6.42 t ha^{-1} (100% ETc) and 7.59 t ha^{-1} (60% ETc) (Table 5). Average crop water productivity for *A. cruentus* increased from 30% ETc (1.98 kg m^{-3}) to 60% ETc (2.15 kgm^{-3}) then dropped at 100 % ETc 1.09 kgm^{-3}). Although water productivity for dry mass decreased as applied irrigation water increased the maximum marketable fresh mass yield was obtained in the 60% ETc treatment (Table 4.5). Therefore results indicate that 60% ETc irrigation treatment was more water productive than all other treatments in terms of fresh biomass yield. Amaranth plants show lower water loss rates and greater water use efficiency than many other C4 plants, and more so in dry conditions (Moran and Showler, 2006; Liu and Stutzel, 2002).

Table 4.5. Average total above ground fresh mass and dry yield, irrigation water use and crop water productivity of selected African leafy vegetables for two seasons (2015/2016 and 2016/2017).

African Leaf Vegetables	Well-Watered (100 ETc)			Medium-Watered (60 ETc)			Deficit Irrigation (30 ETc)		
	Average total above ground fresh yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity [1,] ($kg\ m^{-3}$)	Average total above ground fresh yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($k\ gm^{-3}$)	Average total above ground Fresh yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($k\ gm^{-3}$)
A. cruentus	6.42	589	1.09[a]	7.59	353	2.15[a]	3.49	176	1.98[a]
C. olitorius	5.42	589	0.91[a]	5.44	353	1.50[a]	3.18	176	1.80[a]
V.unguiculata	6.33	589	1.07[a]	6.53	353	1.84[b]	4.98	176	2.83[b]
B. vulgaris	9.46	589	1.60[b]	6.67	353	1.89[b]	4.30	176	2.40[a]

African Leaf Vegetables	Well-Watered (100 ETc)			Medium-Watered (60 ETc)			Deficit Irrigation (30 ETc)		
	Average total above ground dry matter yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($kg\ m^{-3}$)	Average total above ground dry matter yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($k\ gm^{-3}$)	Average total above ground dry matter yield ($t\ ha^{-1}$)	Average irrigation water use (mm)	Crop water productivity ($k\ gm^{-3}$)
A. cruentus	0.56	589	0.10[a]	0.61	353	0.17[a]	0.43	176	0.24[a]
C. olitorius	0.48	589	0.08[a]	0.45	353	0.12[a]	0.40	176	0.22[a]
V.unguiculata	0.51	589	0.09[a]	0.51	353	0.14[a]	0.53	176	0.30[a]
B. vulgaris	0.95	589	0.16[a]	0.73	353	0.20[a]	0.70	176	0.40[a]

Amaranth has been often described as a drought tolerant crop (Zavitkovski and Ferrell, 1968; Liu and Stutzel, 2002) capable of maintaining normal physiological processes under stress. Findings on yield response to limited water availability in *A. cruentus* were consistent with results of water productivity on fresh mass basis. Results were also consistent with the findings of Beletse et al. (2012) who observed higher water productivity in water limited treatments in comparison to well water treatments. Improving water productivity can make a contribution to global food production and poverty alleviation.

Corchorus olitorius yield obtained from the irrigation treatments were in the range of 3.18, 5.44 and 5.42 t ha^{-1} on a fresh weight basis for 30, 60 and 100% ETc (Table 5). The highest yield was obtained in the well irrigated treatment 100% ETc showing a positive effect on increased water application. A tendency for yield to decrease was observed as irrigation was reduced from 100% ETc to 30% ETc. The highest water productivity was obtained in the driest irrigation treatment (30% ETc). Results concur with Nyathi et al. (2016) who reported that ALVS are productive under limited water conditions. On the contrary, Fasinmirin and Olufayo (2009) reported higher biomass yield and WUE of *C. olitorius* can be achieved when the crop is grown at full irrigation. The difference in water productivity between the 100% ETc and 30% ETc reported previous may be due to variation in climatic conditions, specie and degree of severity. Deficit irrigation compromised leaf quality of *C. olitorius* because it favours good application of water for its growth and development (Beletse et al., 2012). Water deficit reduces crop productivity; causing economic losses (Oelofse and van Averbeke, 2012).

Highest yields of *V. unguiculata* leaves were obtained in the 60 and 100% ETc irrigation treatments, on fresh weight basis (Table 5). Drought stress has been reported to decrease water use efficiency (WUE), leaf production and root proliferation; and consequently crop productivity (Farooq et al., 2009). Maximum yield was attained in the 60% ETc, but maximum water productivity was obtained where deficit irrigation (30% ETc) was applied. *V. unguiculata* seems to grow at deficit irrigation (30% ETc) without losing marketable quality of the leaves. According to Beletse et al. (2012) if the crop is grown for seed or bean production it has to be well irrigated to get optimum yield. Water use efficiency is an important trait for improving drought tolerance in cowpea as it saves considerable amount of irrigation water. An improvement in water use efficiency would significantly enhance total biomass production as well as yield at a given level of soil water availability

The average total yield of *B. vulgaris* obtained for both seasons experiments ranged between 4.30 t ha^{-1}, 6. 67 and 9.46 t ha^{-1} on fresh weight (FW) basis for 30, 60 and 100% ETc respectively (Table 4.5). The fresh mass yield obtained from the 30% ETc treatment was not of marketable quality. Therefore, irrigating *B. vulgaris* at this level of water stress is not recommended, because both yield and quality were compromised. Maximum crop water productivity was obtained in the 30% ETc, where deficit irrigation was applied. Water use efficiency has been reported to increase with decreasing water supply (Mabhaudhi et al., 2014; Songsri et al., 2013). Water productivity decreased as applied irrigation water increased but maximum marketable fresh mass yield was obtained in the 100% ETc treatment which was statistical similar to 60% ETc. Results of the study showed that irrigating at 60 and 100% ETc gave higher above-ground yield and better quality leaves compared to other treatments to the30% ETcMaximum crop water productivity was obtained in the driest treatment (30% ETc) and decreased when applied irrigation water increased but yield was compromised.

The ALVs differed in their response to drought stress because plant response to drought depends on plant species and stress severity. Results from the 2 year data showed that ALVs performed comparable or better than *B. vulgaris* as far as water productivity was concerned. At deficit irrigation (30% ETc) *V. unguiculata* produced the highest amount of biomass per cubic metre of water followed by *B. vulgaris*, *A. cruentus* and *C. olitorius*. At 60% ETc, *A. cruentus* produced the highest amount of biomass per cubic metre of water followed by *B vulgaris*, *V. unguiculata* and *C. olitorius*. In the *B. vulgaris* irrigation experiment, highest leaf fresh weight was obtained from the 100% ETc treatment and this indicates that *B. vulgaris* favours regular application of water for optimum growth and development, confirming the findings reported by Van Averbeke and Netshithuthuni (2010). Nyathi et al. (2016) reported that results of water productivity of ALVs were comparable to those of *B.vulgaris*.

If farmers are to select a preferred crop among the three ALVs crops studied, they should consider yield, cost of inputs and irrigation set up among other factors. At limited water level of 30% ETc, *V. unguiculata* performed similar in terms of growth and yield compared to other water treatments**.** This suggests that production of this crop is still possible under limited water supply. This confirms to the study by Beletse et al. (2012) in which *V. unguiculata* was ranked as one of the drought tolerant crops compared to *B. vulgaris*. *V. unguiculata* production was optimised in terms of reduced amount of water use under limited water supply. Furthermore, limited water supply can be efficient in terms of use of less fertiliser which cannot be leached and low maintenance of irrigation systems. At higher water application the systems will have to be running for a long time compared to limited water application. If farmers decide to grow *V. unguiculata*, the benefits include reduced soil erosion, and improved soil status due to nitrogen fixation.

For *A. cruentus* and *C. olitorius* application of 30% ETc resulted in reduced yield therefore production is feasible at 60% ETc. In *B. vulgaris* yield was higher in water regimes of 100% ETc which was statistically similar to 60% ETc. This concurs with the report that water deficit affects growth, development, yield and quality of plants in the greenhouse and field conditions (Luvaha et al., 2008). The development of a wider choice of crops adapted to dry areas is critical because of global warming threats, decrease in water supply and demand to feed an increasing population. Research results on water productivity can help in decision-making options of vegetable growers in terms of calculating gross returns. This gives important insights into economic water productivity per cubic metre of water applied. Where irrigation water is in limited supply or where irrigation is expensive, irrigation management methods are needed which result in less water use while maintaining adequate yields of the economic product.

4.4 Conclusions

Water stress reduced yield for *A. cruentus*, *C. olitorius* and *B. vulgaris* compared to the well-watered treatment although other growth and physiological parameters were not affected. Considering that the objective of every farmer is to achieve high yield under all conditions, more so under drought stress, therefore yield is a very important aspect. In *V. unguiculata*, all measured parameters were not compromised implying that it performed better than other ALVs including *B. vulgaris* under limited water application**.** Results concur with the current notion that wild vegetables can perform better or comparable to vegetable species such as *B. vulgaris* var. *cicla* under similar conditions. Yield followed a similar trend in *A. cruentus*, *C. olitorius* and *B. vulgaris,* an increase with increase in water application from 30% ETc to 60% ETc, and then remained the same at 100% ETc. A further increase in water application led to diminishing returns because optimum production had been reached. Int the present study the optimum level was reached at 60% ETc which is recommended for the three crops. However, there is a possibility that the level of water application can still be lower than in the current study making them even better adapted to marginal areas. Use of low water application reduces irrigation maintenance cost as the irrigation systems can operate at low pressure and leaching of nutrients is reduced. It will also translate to low cost saving since some of the water can be diverted to other crops. Highest water productivity was obtained by deficit irrigation (30% ETc) but deficit irrigation compromised leaf quality in *B. vulgaris*, *C. olitorius* and *A. cruentus*. The highest biomass per metre cube of water was obtained in *V. unguiculata* and *A. cruentus* compared to *B. vulgaris* in terms of fresh mass weight. There is need to conduct studies under open field conditions because sometimes results obtained in closed systems such as rain shelter do not translate well under field conditions. Future studies should explore performance of various plant species under different water regimes or stress severity. Since drought seldom occurs in isolation, and mostly interacts with a variety of other abiotic and biotic stresses such as temperatures.and incidence, disease it is important that these factors are studied simultaneously. Since species have been reported to show a drought tolerance in various stages of development, or with time, it is important to use a variety of screening techniques (cellular level and whole plant level) to make sound conclusions concerning the general drought tolerance of a given plant. Further work needs to be done on using fertiliser application methods such as fertigation along irrigation which could possibly reduce fertiliser application rates, thereby reducing fertiliser costs and increasing sustainability of the enterprise. In addition, multi-location trials and studies of different varieties in South Africa are required.

Acknowledgments

The authors acknowledge funding from the South African Department of Rural Development and Agricultural Research Council – Vegetable and Ornamental Plant Institute

Conflicts of Interest: The authors declare no conflict of interest.

References

Aderolu, A.M., 2000. The effect of water stress at different growth stages on yield and seed quality of cowpea varieties. B. Agric Project University of Ilorin. Nigeria p. 68.

Afolayan, A.J., Jimoh, F.O., 2009. Nutritional quality of some wild leafy vegetables in South Africa. Int. J. Food Sci. Nutr. 60, 424-431.

Agbemafle, R., Owusu-Sekyere, J.D., Bart-Plange, A., 2015. Effect of deficit irrigation and storage on the nutritional composition of tomato (*Lycopersicon esculentum* Mill. cv. *Pectomech*) Croatian Journal of Food Technology, Biotechnology and Nutrition 10 (1-2), 59-65

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and Drainage Paper no. 56. Rome: Food and Agriculture Organization of the United Nations.

Annandale, J.G., Stirzaker, R.J., Singels, A., Van Der Laan, M., Laker, M.C., 2011. Irrigation schedule research, South African experiences and future prospects. Water SA 37: 751-763.

Ashley, J., 1993. Drought and crop adaptation. In: Rowland J.R.J (ed.), Dryland Farming in Africa. Macmillan Press Ltd, UK, pp. 46-67.

Ayodele, V.I., Fawusi, M.O.A., 1989. Studies on drought susceptibility of *Corchorus olitorius* L.: I. Effects of stressing plant at mid vegetative stage on dry matter yield and yield components of two cultivars of *C. olitorius*. Biotronics 18: 23-27.

Ayodele, V.I., Fawusi, M.O.A., 1990. Studies on drought susceptibility of *Corchorus olitorius* L.: II. Effect of moisture stress at different physiological stages on vegetative growth and seed yield of *C. olitorius* cv. Oniyaya. Biotronics 19: 33-37

Beletse, Y.G., du Plooy, C.P., van Rensburg, J.W.S., 2012. Water requirement of eight indigenous vegetables. In Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa.

Chaudhuri, K., Choudhuri., M.A., 1997. Effect of short-term NaCl stress on water relations and gas exchange of two jute species. Biol Plantarum. 40: 373-380.

Connor, D.J., Jones, T.R., Palta, J.A., 1985. Response of sunflower to strategies of Irrigation. In Growth, yield and the efficiency of water use. Field Crops Res. 10: 15-36.

Costa, R.C.L., Lobato, A.K.S, Oliveira Neto, C.F., Maia, P.S.P., Alves, G.S.R., Laughinhouse, H.D., 2008. Biochemical and physiological responses in two *Vigna unguiculata (L) Walp*. cultivars under water stress. J. Agron. 7(1): 98-101.

De Ronde, J.A., Spreeth, M.H., 2007. Development and evaluation of drought resistant mutant germ-plasm of *Vigna unguiculata*. Water SA 33(3): 381-386

Department of Agriculture, Fisheries and Forestry, 2004. Strategic Plan for the Department of Agriculture Consolidating the Partnership for Poverty Eradication, Accelerated Growth and Wealth Creation; Directorate Agricultural Information Services: Pretoria, South Africa.

Dieleman, A., Hamill, A.S., Fox, G.C., Swanton, C.J., 1996. Decision rules for post emergency control of pigweed (*Amaranthus spp*) in Soybean (*Glycine max*). Weed Sci 44: 126-132.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29, 185-212.

Fasinmirin, J.T., 2001. Nitrogen and Water Use of Irrigated Jute Mallow. Unpublished M. Eng Thesis. Department of Agricultural Engineering, Federal University of Technology, Akure, Nigeria pp. 4 – 63.

Fasinmirin, J.T., Olufayo, A.A., 2009. Yield and water use efficiency of jute mallow *Corchorus olitorius* under varying soil water management strategies. Journal of Medicinal Plants Research 3(4):186-191, April, 2009 Available online at http://www.academicjournals.org/JMPR ISSN 1996-0875© 2009 Academic Journals

Fawusi, M.O.A., Ormrod, D.P., Eastham, A.M., 1984. Response to water stress of *Celosia argentea* and *Corchorus olitorius* in controlled environments. Sci. Hortic 22: 163-171

Ghorbani, R., Seel, W., Leifert, C., 1999. Effects of environmental factors on germination and emergency of Amaranthus retroflexus. Weed Sci 48, 505510.

Hayatu, M., Mukhtar, F.B., 2010. Physiological responses of some drought resistant cowpea genotypes (*Vigna unguiculata (l.) walp*) to water stress. Bayero J Pure Appl Sci3: 69-75.

Jenson, M.E., Burman, R.D., Allen, R.G., 1990. Evaporation and Irrigation water Requirements ASCE Manual and Rep. On Egrg. Pract. No. 70. ASCE New York.

Kaya, M.D., Okcub, G., Ataka, M., Cikilic, Y., Kolsaricia, O., 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annus L*). Eur J Agron 24: 291-295

Kiani, S.p., Maury, P., Sarrafi, A., Grieu, P., 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annus*) under well-watered and water-stressed conditions. Plant Sci. 175: 565–573.

Liu, F., Stutzel, H., 2002. Leaf water relations of vegetable amaranth (*Amaranthus spp.*) in response to soil drying. Eur J Agron 16: 137150.

Lobato, A.K.S., Oliveira Neto, C.F., Costa, R.C.L., Santos Filho, B.G., Cruz, F.J.R., Laughing house, H.D., 2008. Biochemical and physiological behaviour of *Vigna unguiculata* (L) Walp, under water stress during the vegetative phase. Asian J. Plant Sci. 7(1): 44-49

Luvaha, E., Netondo, G.W., Ouma, G., 2008. Effect of water deficit on the physiological and morphological characteristics of mango (*Mangifera indica*) rootstock seedlings. American Journal of Plant Physiol3(1): 115.

Mabhaudhi, T., Modi, A.T., Beletse, Y.G., 2014. Parameterisation and evaluation of the FAO-AquaCrop model for a South African taro (*Colocasia esculenta* L) landrace. Agric. For. Meteorol 192–193, 132–139.

Mabhaudhi, T., O'Reilly, P., Walker, S., 2016. The role of underutilised crops in Southern African farming systems, a scoping study. Sust 8: 302

Masarirambi, M.T., Dlamini, Z., Manyatsi, A.M., Wahome, P.K., Oseni, T.O., Shongwe, V.D., 2012. Soil water requirements of amaranth (*Amaranthus hybridus*) grown in a greenhouse in a semi-arid, sub-tropical environment. Am Eurasian J Agric Environ Sci 12: 932-936

Maseko, I., Mabhaudhi, T., Tesfay, S., Araya, H.T., Fezzehazion, M., Du Plooy, C.P.D., 2018. African Leafy vegetables: a review of status, production and utilization in South Africa. Sust. 10, 16.

Mavengahama, S., 2013. The Contribution of Indigenous Vegetables to Food Security and Nutrition within Selected Sites in South Africa. Ph.D. Thesis, Stellenbosch University, Cape Town, South Africa.

Mbatha, T.P., Modi, A.T., 2010. Response of local mustard germplasm to water stress. S. Afr. J. Plant Soil. 27: 328-330.

Modi, A.T., 2006. Growth temperature and plant age influence on nutritional quality of *Amaranthus* leaves and seed germination capacity. Water South Africa 33, 03784738.

Moran, P.J., Showler, A.T., 2006. *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* symptoms on *Amaranthus* spp. under South Texas conditions. Plant Dis. 91, 12-16.

Neluheni, K., Du Plooy, C.P., Mayaba, N., 2007. Yield response of leafy amaranths to different irrigation regimes. Afr. Crop Sci. Conf. Proc., *8*, 1619

Nkaa, F.A., Nwokeocha, O.W., Ihuoma, O., 2014. Effect of Phosphorus fertilizer on growth and yield of cowpea (*Vigna unguiculata*). J Pharm Biol Sci. 9: 74-82.

Nyathi, M.K., Annandale, J.G., Beletse., Y.G., Beukes, D.J., Du Plooy, C.P., Pretorius, B., van Halsema, G.E., 2016. Nutritional water productivity of traditional vegetables crops. WRC report No.2171/1/16.

Nyathi, M.K., van Halsema, G.E., Beletse, Y.G., Annandale, J.G., , Struike P.C., 2018a. Calibration and validation of the AquaCrop model for repeatedly harvested leafy vegetables grown under different irrigation regimes. Agric Water Manag 208: 107-119 https://doi.org/10.1016/j.agwat.2018.06.012

Nyathi, M.K., van Halsema, G.E.,Beletse, Y.G., Annandale, J.G., , Struike P.C., 2018b. Nutritional water productivity of selected leafy vegetables. Agric Water Manag 209,:111-122

Oelofse, A., van Averbeke, W., 2012. *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Water Research Commission: Pretoria, South Africa.

Palada, M.C., Chang, L.C., 2003. Suggested Cultural Practices for Vegetable Amaranth. In: *International Cooperates Guide*; AVRDC Pub #03-552; AVRDC-The World Vegetable Center: Shanhua, Taiwan, p. 4.

Pimentel, C., 2004. The relation of the plant with water. EDUR. Seropedica.

Saleh, S., Liu, G., Liu, M., Ji, Y., He, H., Gruda, N., 2018. Effect of Irrigation on Growth, Yield, and Chemical Composition of Two Green Bean Cultivars. Horticulturae 4, 1–10

Sammis, T., Wu, I.P., 1989. Deficit irrigation effects on head cabbage production. Agric Water Manag, 16: 229-239.

Sanchez, C.A., Roth, R.L., Gardner, B.R., 1994. Irrigation and nitrogen management for sprinkler irrigated cabbage on sand. J Am Soc Hortic Sci., 119: 427-433

Schippers, R.R., 2000. African Indigenous Vegetables. In:*An Overview of the Cultivated Species*; Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation: Chatham, UK.

Sharma, B.D., Cheema, S.S., Kar, S., 1990. Water and nitrogen uptake of wheat as related to nitrogen application rate and irrigation water regime fert. Newsl. 35(9): 31–35.

Shiwachi, H., Komoda, M., Koshio, K., Takahashi, H., 2008. Effect of soil moisture stress on the growth of *Corchorus olitorius L*. Afr J Agr Res Vol. 4 (4), pp. 289-293, Available online at http://www.academicjournals.org/AJAR ISSN 1991-637X © 2009 Academic Journals

Singh, B.B., Ajeigbe, H.A., Tarawali, S., Fernandez-Rivera, S., Abubakar, M., 2003. Improving the production and utilization of cowpea as food and fodder. Field Crops Res., *84*, 169–177

Singh, B.B., Chambliss, O.L., Sharina, B., 1997. Recent advances in cowpea breeding. in: Advances in cowpea research. B.B. Singh, B.R Mohan Raji, K.E Dasiell and L.E.N. Jackiat (eds) co-pub. Of IITA and JIRCAS IITA Ibadan, Nigeria.

Singh, B.P., Whitehead, W.F., 1992. Response of vegetable amaranth to differing soil pH and moisture regimes. Acta Horticulturae 318: 225-229.

Slabbert, M.M., Sosibo, M.S., van Averbeke, W., 2012. The response of six African leafy vegetables to drought and heat stress. In Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa,

Smith, I.F., Eyzaguirre, P., 2007. African leafy vegetables: Their role in the World Health Organization's global fruit and vegetables initiative. Plant Food, 7, 3–5.

Songsri, P., Jogloy, S., Junjittakarn, J., Kesmala, T., Vorasoot, N., Holbrook, C.c., Pananothai, A., 2013. Association of stomatal conductance and root distribution with water use efficiency of peanuts under different soil water regimes. Aust. J. Crop Sci. *7* 948-955.

Taylor, H.M., Wepper, B., 1990. Limitation to efficient water use in Crop Production. American Society of Agron. Madison, W.I

Van Averbeke, W., Chabalala, M.P., Okorogbona, A.O.M., Rumania, T.D., Azeez, J.O., Slabbert, M.M., 2012. Plant nutrient requirements of African leafy vegetables. In Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Oelofse, A., Van Averbeke, W., Eds.; Water Research Commission: Pretoria, South Africa,

Van Averbeke, W., Netshithuthuni, C., 2010. Effect of irrigation scheduling on leaf yield of non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*). *S Afr J Plant Soil* 27: 322-327.

Van Rensburg, W.S.J, Van Averbeke, W., Slabbert, R., Faber, M., Van Jaarsveld, P., Van Heerden, I., Wenhold, F., Oelofse, A., 2007. African leafy vegetables in South Africa. Water SA, 33, 317–326.

Vurayai, R., Emongor, V., Moseki, B., 2011. Effect of water stress imposed at different growth and development stages on morphological traits and yield of Bambara Groundnut (*Vigna subterranea L. Verdc*). Am. J. Plant Physiol. 6 (1) 17–27.

Wang, W., B. Vinocur and A. Altman. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. 218:1-14.

Whitfield, D.M., Wright, G.C., Gyles, O.A., Taylor, A.J., 1986. Growth of Lucerne (*Medicago sativa L*) in response to frequency of Irrigation and gypsum application on a heavy clay soil: Irrig. Sci. 7: 37– 52.

Yarnia, M., 2010. Sowing dates and density evaluation of amaranth (Cv. Koniz) as a New Crop: Advances in Environmental Biology, 4(1): 41-46.

Zavitkovski, J., Ferrell, W.K., 1968. Effect of drought upon rates of photosynthesis, respiration and transpiration of seedlings of two ecotypes of Douglas fir. Two- and three-month-old seedlings. Botanical Gazette 129, 346-350

Zulu, N.S., Modi, A.T., 2010. A preliminary study to determine water stress tolerance in wild melon (*Citrullus lanatus L*). *S*. Afr. J. Plant Soil, 27: 334-336.

Chapter 5

Nutritional quality of selected African leafy vegetables cultivated under varying water regimes and different harvests.

Innocent Maseko[1], Bhekumthetho Ncube[3*], Tafadzwanashe Mabhaudhi[1], Samson Tesfay[2], Vimbayi G.P. Chimonyo[1], Hintsa T. Araya[3], Melake Fessehazion[3], Christian P. Du Plooy[3]

[1] *Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa.*

[2] *Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209, South Africa.*

[3] *Agricultural Research Council – Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria, 0001, South Africa*

***Correspondence**

Dr B Ncube, Agricultural Research Council – Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria, 0001, South Africa. E-mail address: bbkncube@yahoo.com. Tel: +27 12 808 8000

Abstract

African leafy vegetables (ALVs) are considered rich in micronutrients and adapted to marginal production areas than their exotic counterparts. However, information on ALV nutritional content when grown under limited moisture is scant in the literature. In this study, we evaluated the nutritional composition of three ALVs (*Amaranthus cruentus* L., *Corchorus olitorius* L, and *Vigna unguiculata* (L.) Walp) – to varying water regimes using *Beta vulgaris* L. as a reference crop. The experimental trial was carried out at the Agricultural Research Council (ARC) in Roodeplaat, Pretoria over two summer seasons, 2015-2016 and 2016-2017. The irrigation levels were: 30%, 60% and 100% of crop water requirement (ETc) and nutrients were analysed at each harvest. From the nutritional analysis, under severe drought conditions (30% ETc) Ca and Mg were high in *A. cruentus* and *C. olitorius* while only Ca was high in *B. vulgaris*. The following were also observed: Na, K and Zn in *A. cruentus*, Zn in *C. olitorius*, P and K in *V. unguiculata*, Na and Zn in *Beta vulgaris* increased with increase in water application from 30 to 60 % ETc. Further increase in water application did not improve the nutrient content. Leaf Fe, Zn, Mn, Mg, Ca increased as time of harvesting increased from 6 weeks to 8 weeks, with no further increase at 10 weeks in *A. cruentus*, *V. unguiculata* and *B. vulgaris*. In *C. olitorius*, Fe, Zn, Mn, Mg and Na were high when harvested early at 6 weeks than during late harvesting at 8 weeks and 10 weeks. Early and medium harvesting has potential to retain nutrient in leafy vegetables. Application of 60% ETc led to improved nutritional yield in all crops while concentration of nutrient under water stress indicates the potential of production in marginal areas.

List of abbreviations

ALV	African leafy vegetables
ARC	Agricultural Research council
ARC-ISCW	Agricultural Research Council–Institute for Soil, Climate and Water
DMRT	Duncan's multiple range test
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometric
LAN	Limestone ammonium nitrate
WATP	Weeks after transplanting

Keywords*:* Leafy vegetables, Nutritional composition, Water stress

5.1 Introduction

South Africa faces challenges of food insecurity at household level collectively known as "hidden hunger" (Faber and Wenhold, 2007; Maunder and Meaker 2007;). Nutrition insecurity ("hidden hunger") in South Africa includes iron, iodine and zinc deficiency (Oelofse and van Averbeke 2012). White and Braodleyi (2009) also reported that Mg and Cu deficiencies also constitute "hidden hunger" and can be reduced by production of edible vegetative organs with increased concentrations of these nutrients. South Africa is a dry country with some areas experiencing shortages of drinking water and crop production mostly practiced under water deficit (Annandale et al. 2011; Mabhaudhi 2013; Nyathi et al. 2018). Studies have shown that African leafy vegetables (ALVs) can contribute to addressing gaps in nutrition and inadequate availability of water because they are nutrient dense and adapted to marginal areas of production (Oelofse and van Averbeke, 2012). However, information on nutritional value and yield of ALVs grown under limited water availability is scant.

Studies conducted in other regions reports that a decrease in the amount of water in the soil reduces the amount of minerals absorbed by the roots and hence reduces the mineral content (Pascale et al. 2001). Saleh et al. (2018) reported N, P, K Fe, Zn, and Cu to be increasing with increase in soil water regimes from 60% ETc to 80% ETc, then remained constant at 100% in green bean. Other researchers observed a reduction in K due to water stress in *Gongrolema latifolium* (Osuagwu and Edeoga 2012), *Dalbergonia sisso* (Singh and Singh 2004) and *Lycopersicon esculentum* (Nahar and Gretzmachar 2002). Luoh et al. (2014) found no significant difference in leaf calcium content of *A. cruentus* and *A. hypochondriacus* grown under water-deficient (water when plants showed signs of wilting) conditions in the greenhouse. Agbemafle et al. (2015) observed that Fe contents decreased with deficit irrigation in *Lycopersicon esculentum*. The concentration of minerals K, Na, Fe and Zn in *Lycopersicon esculentum* was observed to increase with increasing level of irrigation water from 70% ETc, 80% ETc, 90% ETc up to 100% ETc (Agbemafle et al., 2015). The information on the amount of nutrients reported in various research studies vary considerably and sometimes even within the same crop species. This is possibly due to variation in production systems, soil fertility, age of plant or time of harvest and seasonal variations in leafy vegetables (Giri et al. 1984; Khader and Rama 2003; Mavengahama 2013). To further advance knowledge and information on the nutritional response of these vegetables to varied (mostly marginal) environments, the need to conduct controlled trials under similar environmental settings would make for conclusive and sound recommendations.

African leafy vegetables are reported to be among the major contributors of micronutrients in diets as they contain significant amounts of calcium, zinc and iron (Odhav et al. 2007, Vorster et al. 2008). *Corchorus olitorius*, *Vigna unguiculata* and *Amaranthus cruentus* are rich in minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese (Oelofse and van Averbeke 2012). The nutrient levels found in leafy vegetables are often comparable, and in some cases higher than those of exotic vegetables such as cabbage and Swiss chard (Nesamvuni et al. 2001; Van Der Walt 2005; Ndlovu et al. 2008; Afolayan and Jimoh 2009; Oelofse and van Averbeke 2012). The information provided by some of these studies is limited by the fact that they have tried to make comparisons of leafy vegetables and Swiss chard when grown in different environmental conditions (Nyathi et al. 2018).

However, adopting recommendations based on the few preliminary studies is challenging as South Africa has a high diversity of ALVs that are available for consumption and preference varies with province. Hence there is need to conduct extensive trials on a wider range of leafy vegetables under similar experimental settings. The larger the number of species for people to select from, as well as a wider diversity of desirable traits can lead to successful commercialization of these vegetables. Swiss chard is often chosen as a reference crop because it is a widely accepted leafy vegetable that is commercialized (Mavengahama et al. 2013).

The aim of the current study was to evaluate the nutritional quality and water productivity of *Amaranthus cruentus*, *Corchorus olitorius*, *Vigna unguiculata* to varying moisture regimes.

5.2. Materials and methods

5.2.1. Plant material and growth conditions

AVLs were grown as a field trial at the Agricultural Research Council (ARC) - Vegetable and Ornamental Plants (VOP) farm, Roodeplaat, Pretoria (25°35' S; 28°21' E; 1164 masl) under the various water regimes (100% ETc, 60% ETc and 30% ETc) 2015/2016 and 2016/2017 summer seasons. Fertiliser was applied according to soil analysis results done at the Agricultural Research Council–Institute for Soil, Climate and Water (ARC–ISCW), Acardia, Pretoria (Table .5.1).

Table 5.1**.** Physical and chemical characteristics of the soil in the experimental field

Soil attribute	2015-16 summer season	2016-17 summer season
P (mg kg^{-1})	40.0	5.9
K (mg kg^{-1})	227.0	250.0
Ca (mg kg^{-1})	825.0	696.0
Mg (mg kg^{-1})	240.0	273.0
Na (mg kg^{-1})	34.0	17.8
Exchangeable cation Ca (%)	60.2	53.8
Exchangeable cation Mg (%)	29.2	35.1
Exchangeable cation K (%)	8.5	9.9
Exchangeable cation Na (%)	2.2	1.2
pH	7.0	6.9
Clay (%)	18.0	20.0
Silt (%)	6.0	4.0
Sand (%)	76.0	76.0
$N\text{-}NO_3$	6.2	6.4
$N\text{-}NH_4$	5.5	3.1

Leaf growth was monitored throughout the growing period and harvested during the early morning of the trial. The leaves were harvested at six (6), eight (8) and 10 weeks after transplanting (WATP) and packed in an upright position in clean plastic crates and immediately transported to the laboratory (100m from the harvesting site) for processing and harvesting. Each treatment had 3 replicates, each containing approximately 300g of fresh leaves.

5.2.2 Agronomic practices

Nitrogen (limestone ammonium nitrate (LAN), 28% N) was applied according to results of soil nutrient analysis for 2015-2016 and 2016-2017 seasons. Application rates were: 125 kg ha^{-1} N for *A. cruentus* and *C. olitorius,* 150 kg ha^{-1} N for *B. vulgaris* and 135 kg N ha^{-1} for *V. unguiculata* for both seasons. Nitrogen was applied by banding in three split applications. The first application was at transplanting/sowing (50%), second at 4 weeks after transplanting/sowing (25%) and the last (25%) at 8 weeks after transplanting/sowing. Double Super Phosphate was applied at 20 kg (10.5 % P) at planting for season 1 for all the crops. During second season at 63 kg ha^{-1} P for *B. vulgaris*, 55 kg ha^{-1} P for *A. cruentus* and *C. olitorius* and 75 kg ha^{-1} P for *V. unguiculata* at planting. Potassium was deemed sufficient based on results of soil fertility analyses for both seasons.

5.2.3 Analysis of nutritional composition

Leaf samples were dried in an oven at 50 °C for 48 h. Dried samples for each treatment were prepared for the analysis of macro and micro nutrients (sodium (Na), phosphorus (P), potasium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) by grinding them into a fine powder. The macronutrients P, K, Ca, Mg and Ca and micronutrients Fe, Cu, Zn and Mn were analysed at the Agricultural Research Council-Institute of Soil, Climate and Water (ARC-ISCW) laboratory in Pretoria for both seasons. Samples (1 g) were digested by first muffling in a muffle furnace at 500°C for 2 hrs before adding 3 ml of 50% aqueous (dionised) nitric acid (v/v) (after cooling) and heating the mixture again at 100°C in a hot plate until dry. Ten millilitres of 50% HCL(v/v) were then added into the sample which then further diluted with deionised water and analysed for the various nutritional components in triplicate and all nutrient concentrations expressed as mg/kg.

An aliquot of the digest solution was used for the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometric) determination of chemical parameters. The ICP-OES is a multi-element instrument. The instrument used was an Agilent 725 (700 Series) simultaneous instrument, where all the elements (and all wavelengths) are determined simultaneously. Several elements were determined at more than one wavelength, allowing confirmation of the values, with no increase in analysis time or consumption of digest solution. Each element was measured at one or two appropriate emission wavelengths, chosen for high sensitivity and lack of spectral interferences. The instrument was set up and operated according to the recommended procedures in the instrument manual and optimised conditions. The instrument was calibrated against a series of standard solutions, containing all the elements of interest.

5.2.4 Data analysis

Data were subjected to one way analysis of variance (ANOVA) using SPSS software for Windows (IBM SPSS, version 25, Chicago, IL, USA). Where there were significant differences ($P \leq 0.05$), the means were further separated using Duncan's multiple range test (DMRT).

5.3 Results and discussion

5.3.1 Effect of irrigation on Mg, Ca, Na, P and K

Calcium plays a role in plant growth and development because of its role in cell physiology such as cell division (Shao et al. 2008) and its concentration is affected by water stress. With regard to Ca, although only *A. cruentus* (2015-2016 season), *C. olitorius* (2016-2017 season) and *B. vulgaris* (2016-2017season), showed significant changes in Ca levels between treatments, the observed general characteristic trend was that high levels of calcium were either alternating between the most severe water stress (30% ETc) and the well-watered (100 ETc) treatments across all crops in this study (Table 5.2).

Akinci and Lösel (2012) reported higher levels of leaf calcium content at low water levels due to increased water stress as plants develop a selective uptake for specific elements. De carvolho and Savaria (2005) reported that water stress caused a decrease in calcium content of *Lupins lopinus albus* and *Lopinus metabilis*. Decrease of Ca uptake under drought conditions may be attributed to depressed absorption (Ciríaco da Silva et al. 2011) and reduction in transpiration (Sardans et al. 2008). Similarly, Saleh et al. (2018) working on green bean reported lower Ca content at lower water level of 60% ETc relative to 80% ETc and 100% ETc in their trials.

Table 5.2. Effect of irrigation regimes on the levels of macro elements of selected African leafy vegetables from two growing seasons

Crop	Irrigation	2015/16 summer (Season 1)					2016/2017 summer (Season 2)				
		Concentration (mg/kg)									
		Mg	Ca	Na	P	K	Mg	Ca	Na	P	K
A. cruentus	30% ETc	12500.1^{a}	34200.1^{a}	350.95^{b}	4500.4^{a}	29100.1^{b}	14062.85^{a}	32090.90^{a}	1454.04^{a}	4515.47^{b}	37245.69^{a}
	60% ETc	10400.3^{b}	26200.1^{b}	1118.63^{a}	5100.6^{a}	39800.4^{a}	13214.27^{a}	30933.46^{a}	328.12^{a}	5095.97^{a}	38293.86^{a}
	100% ETc	11000.5^{a}	28400.6^{a}	346.51^{b}	4800.7^{a}	37600.1^{a}	15536.73^{a}	31775.73^{a}	642.65^{a}	4948.02^{a}	37245.69^{a}
C. olitorius	30% ETc	3600.4^{a}	16600.0^{a}	254.35^{a}	5600.5^{c}	30200.9^{a}	3720.75^{a}	17554.97^{a}	3997.10^{a}	5438.39^{a}	37772.79^{a}
	60% ETc	2900.1^{b}	17000.1^{a}	264.55^{a}	6100.3^{b}	28600.5^{a}	3076.96^{b}	15118.35^{b}	1639.37^{a}	6072.29^{a}	32868.26^{b}
	100% ETc	3400.8^{a}	17500.8^{a}	222.79^{a}	6900.8^{a}	26700.5^{a}	2708.79^{c}	12598.87^{c}	3997.09^{a}	5389.61^{a}	30475.67^{c}
V.unguiculata	30% ETc	5100.7^{a}	21500.3^{a}	222.15^{a}	3700.7^{b}	18000.4^{b}	4537.58^{a}	16912.80^{a}	319.79^{b}	4856.15^{a}	
	60% ETc	5100.6^{a}	20900.7^{a}	236.07^{a}	3800.7^{ab}	19400^{ab}	5029.28^{a}	16771.77^{a}	718.33^{a}	5354.64^{a}	-
	100% ETc	4600.5^{a}	21500.9^{a}	235.76^{a}	4200.3^{a}	22100.0^{a}	4775.81^{a}	14409.81^{a}	429.17^{a}	6331.02^{a}	-
B. vulgaris	30% ETc	7100.2^{a}	9300.6^{a}	40388.79^{a}	4300.5^{a}	33400.7^{a}	6894.66^{a}	9978.26^{a}	28727.07^{b}	4580.68^{a}	33459.85^{a}
	60% ETc	9200.1^{a}	11200.5^{a}	30667.43^{a}	8600.3^{a}	33600.8^{a}	7017.77^{a}	8835.74^{b}	35177.29^{a}	4358.71^{a}	40275.87^{a}
	100% ETc	8100.5^{a}	9500.5^{a}	28950.73^{a}	6700.4^{a}	32800.6^{a}	8554.32^{a}	10782.32^{a}	35825.61^{a}	3830.42^{a}	42830.60^{a}

*Means followed by the same letters within a column for each treatment are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

- There was no data for K for second season.

Luoh et al. (2014) on the other hand found no significant difference in leaf calcium content of A. cruentus and A. hypochondriacus under water-deficient levels in greenhouse conditions. The concentration in the soil is reported to be directly linked to its concentration in the plant (Ciríaco da Silva et al. 2011). Magnesium plays a role in the central atom of chlorophyll molecules, energy conservation and conversion and protein synthesis (Amtmann and Blatt 2009) and its uptake is affected by drought or irregular water availability (Ciríaco da Silva et al. 2011). With the exception of *B. vulgaris*, Mg levels were consistently higher (*C. olitorius* and *A. cruentus*), though not significantly different, under water stress than in well-watered treatments in all crops in the 2015-2016 season in this study. Nahar and Gretzmachar (2002) reported that the uptake of magnesium by tomato plants was significantly reduced under water stress. When plants are stressed to low internal water potential, uptake of nutrients decreases due to diminishing absorbing power of roots (Dunham and Nye 1976), an explanation of which could be advanced to explain the observed trend in this study. Generally, for both seasons and in all crops (except for Ca in *C. olitorius* 2016-2017 season), Ca and Mg levels were comparable with no significant differences between 30% ETc and 100% ETc irrigation treatment levels in this study. In light of these results and from an economic viewpoint, its suffice to deduce or draw recommendations that application of 30% ETc irrigation levels for the vegetable crops under study would be an economic water-saving strategy without compromising on nutritional yield and quality. Calcium and Mg are important nutritional elements in human diets with beneficial roles such as growth and maintenance of bones, teeth and muscles (Turan et al. 2003).

In two out of the four crops in this study, leaf K (first season) and Na (second season) increased significantly ($P < 0.05$) with water application from 30% ETc to 60% ETc and further increase in water application did not increase nutritional yield. Although not significant in some crops, this trend was generally characteristic of all crops under study. Previous researchers reported similar findings to the current study, a reduction in K due to water stress in *Lycopersicon esculentum* (Nahar and Gretzmachar 2002; Agbemafle et al., 2015), *Gongrolema latifolium* (Osuagwu and Edeoga 2012) and *Dalbergonia sisso* (Singh and Singh 2004). On a season to season comparison, Na seemed to show some significantly huge fluctuations, for example 350.95 mg/kg in the first season increasing more than four-fold to 14062.85 mg/kg at 30% ETc irrigation in *A. cruentus* while on the other hand increasing from 118.63 to 328.12 mg/kg at 60% ETc irrigation from season one to season two for the same crop. In *C. olitorius*, Na levels increased by more than 11-fold from season one to season two in 30- and 100% ETc irrigation levels and by more than 6-fold in 60% ETc irrigation level. This multiple-fold decrease and increase in sodium levels between treatments and season may point to the sensitivity of the element to the interactive combination of environmental factors affecting plant survival and growth. Sodium serves to concentrate carbon dioxide and to promote metabolism and hence its uptake by plants may be affected by water availability. The highest potassium concentration was found in *B. vulgaris*, while the element occurred in more or less the same quantities in *A. cruentus* and *C. olitorius* but at much lower levels in *V. unguiculata*. High potassium coupled with a low sodium content, as observed in *B. vulgaris* has been reported to serve a protective role against numerous diseases (Arlington et al. 1992). African leafy vegetables can meet the daily requirements of potassium for an adult and be useful in the management of hypertension and other cardiovascular diseases.

Leaf P content also followed a similar trend as observed in K and Na, with significant ($P < 0.05$) differences observed during the second season (Table 5.2). This observation concurs with that of Saleh et al. (2018) who observed that P increased with increase in water regimes from 60% ETc to 80% ETc, then remained constant at 100% in green bean. The results for P content in *C. olitorius* were also similar to those of *A. cruentus*, showing a significant ($P < 0.05$) increase from 30% ETc to 60% ETc with no further significant increase when water was increased to 100% ETc. Faye et al. (2006) reported that phosphate ions move through soils through diffusion and a decrease in soil content decreases P mobility hence drought causes a reduction in P absorption and transport in plants. From the soil, P is a highly immobile nutrient element and is thus either required in high amounts and as closer to the plant roots as possible for ease of uptake. Within the plant, the element P holds key physiological and metabolic roles such as conserving and transferring energy in the cell metabolism that are fundamental to plant growth and survival (Jin et al. 2006). Phosphorus also forms a key component of the universal energy carrier molecule ATP in all living systems including plants and is also an integral chemical component of some amino acids and nucleic acid (Ciríaco da Silva et al. 2011). As opposed to Mg and Ca, the elements K, Na and P in higher levels can be obtained in moderate to well-watered (60-100% ETc) conditions although the quantities obtained under high water stress conditions (30% ETc) were still significantly comparable in almost all the crops in the current study. Based on the general observation in this study, it remains logical to draw similar recommendations for K, Na and P to that of Ca and Mg, with a general observation that it is economical to grow these vegetable crops under limited moisture conditions (30% ETc to 60% ETc) and still be able to obtain good quality nutritional content.

5.3.2 Effect of irrigation on Cu and Mn

Manganese is involved in electron transport and therefore it is involved in photosynthesis, respiration and the activation of several enzymes and its uptake is affected by low moisture in the soil (Ciríaco da Silva et al. 2011). Copper participates in electron transport in photosynthesis, mitochondrial respiration and in response to oxidative stress. Effects of drought on Cu uptake and distribution in higher plants is limited (Ciríaco da Silva et al. 2011). In *A. cruentus* and *C. olitorius* leaf samples of Cu and Mn were not significantly (P > 0.05) affected by irrigation water regimes although there was a general tendency of increase from 30% ETc to 60% ETc followed by a decline at 100% ETc for both seasons (Table 5.3).

Table 5.3. Effect irrigation regimes on the levels of Cu and Mn on selected African leafy vegetables from two growing seasons

Crops	Irrigation	2015/16 summer (Season 1)		2016/2017 summer (Season 2)	
		Concentration (mg/kg)			
		Mn	Cu	Mn	Cu
A. cruentus	30% ETc	137.81^{a}	9.26^{a}	133.61^{a}	10.56^{a}
	60% ETc	203.93^{a}	9.58^{a}	190.69^{a}	10.24^{a}
	100% ETc	206.84^{a}	8.27^{a}	175.32^{a}	10.37^{a}
C. olitorius	30% ETc	75.32^{a}	9.45^{a}	78.52^{a}	9.99^{a}
	60% ETc	78.60^{a}	8.31^{a}	120.86^{a}	9.98^{a}
	100% ETc	70.60^{a}	8.96^{a}	54.90^{a}	8.87^{a}
V. unguiculata	30% ETc	153.83^{a}	8.42^{a}	121.67^{a}	7.85^{a}
	60% ETc	135.89^{a}	7.73^{a}	124.82^{a}	8.96^{a}
	100% ETc	126.87^{a}	7.89^{a}	109.26^{a}	9.40^{a}
B. vulgaris	30% ETc	192.85^{a}	12.03^{a}	168.62^{a}	12.11^{a}
	60% ETc	249.88^{a}	13.25^{a}	170.90^{a}	12.35^{a}
	100% ETc	241.21^{a}	13.49^{a}	146.86^{a}	9.79^{a}

*Means followed by the same letters within a column for each treatment are not significantly different according to Duncan's multiple range test at P≤0.05.

The ability of *A. cruentus* and *C. olitorius* to concentrate Cu and Mn under low soil moisture conditions agrees with the notion that the two crops can be produced under limited moisture availability. Although not significant, the trend suggests that high levels of stress can reduce yield quality. Singh and Singh (2004) reported that increasing water stress decreased the level of copper in *Dalbergonia sisso* leaves. Brown et al. (2006) reported reductions of Mg in both the roots and shoots of *Spartina alterniflora* (coastal smooth cordgrass) under drought conditions. Lack of significant response at higher soil moisture content may possibly be due to leaching of nutrients at higher water application.

In *V. unguiculata* and *B. vulgaris* leaf Cu and Mn were not significantly (P > 0.05) affected by water regimes although a general increase from 30% ETc to 60% ETc followed by a decline at 100% ETc for both seasons (Table 5.3) was noted.

Results for Cu and Mn were higher in *B. vulgaris* compared to all ALVs which disagree with the notion that wild vegetables always have a greater inherent ability to accumulate micronutrients from the soil than the widely cultivated exotic vegetables.

5.3.3 Effect of irrigation on Zn and Fe

Although required in minute quantities in plants, Zn and Fe are important micro elements in human nutrition. Iron plays a role in the prevention of anemia while zinc plays a role in vitamin A and vitamin E metabolism (FAO, 2004). Zinc was significantly (P < 0.05) affected by water regimes in *A. cruentus*, *C. olitorius* and *B. vulgaris* (Figure 1). Zn content increased significantly (P < 0.05) with increase in water regimes from 30% ETc to 60% ETc, then remained constant at 100% ETc in *A. cruentus* (both seasons), *C. olitorius* (second season) and *B. vulgaris* (both season). Nyathi et al. (2016) and Saleh et al. (2018) reported similar results in amaranths species and green beans respectively where Zn content decreased with increase in water stress. Similarly, the concentration of Zn in *Lycopersicon esculentum* was reported to decrease with decreasing level of irrigation from 100% ETc to 70% ETc (Agbemafle et al. 2015). In *V. unguiculata*, leaf Zn did not show any significant (P>0.05) response to water application. Similarly, Pirzad et al. (2012) showed that different water applications had no significant effect on zinc uptake of German chamomile (*Matricaria chamomilla* L).

On the other hand, leaf Fe concentration did not show any significant (P>0.05) response to water application for both seasons in all crops. Research indicates that Fe content varies with moisture availability; under wet soil Fe availability is higher for plants compared to drought conditions (Sardans et al. 2008). Nyathi et al. (2016) reported a significant decrease in Fe content in amaranths with increase in water stress. Birnin-Yauri et al. (2011) reported a higher Fe value for rainy season in amaranth arguing that this weather condition favours Fe accumulation without further elaboration on the quality of the season. Although this may be partly true in light of the results obtained in this current and other previous studies, it can be deduced that low water levels does favour Fe accumulation in plants. Variation in the reported Fe content of the current study with those of other studies may be due to variation in climatic conditions, levels of water stress applied, plant species and/or the inherent ability of the plants' mechanisms to concentrate nutrients at low water level.

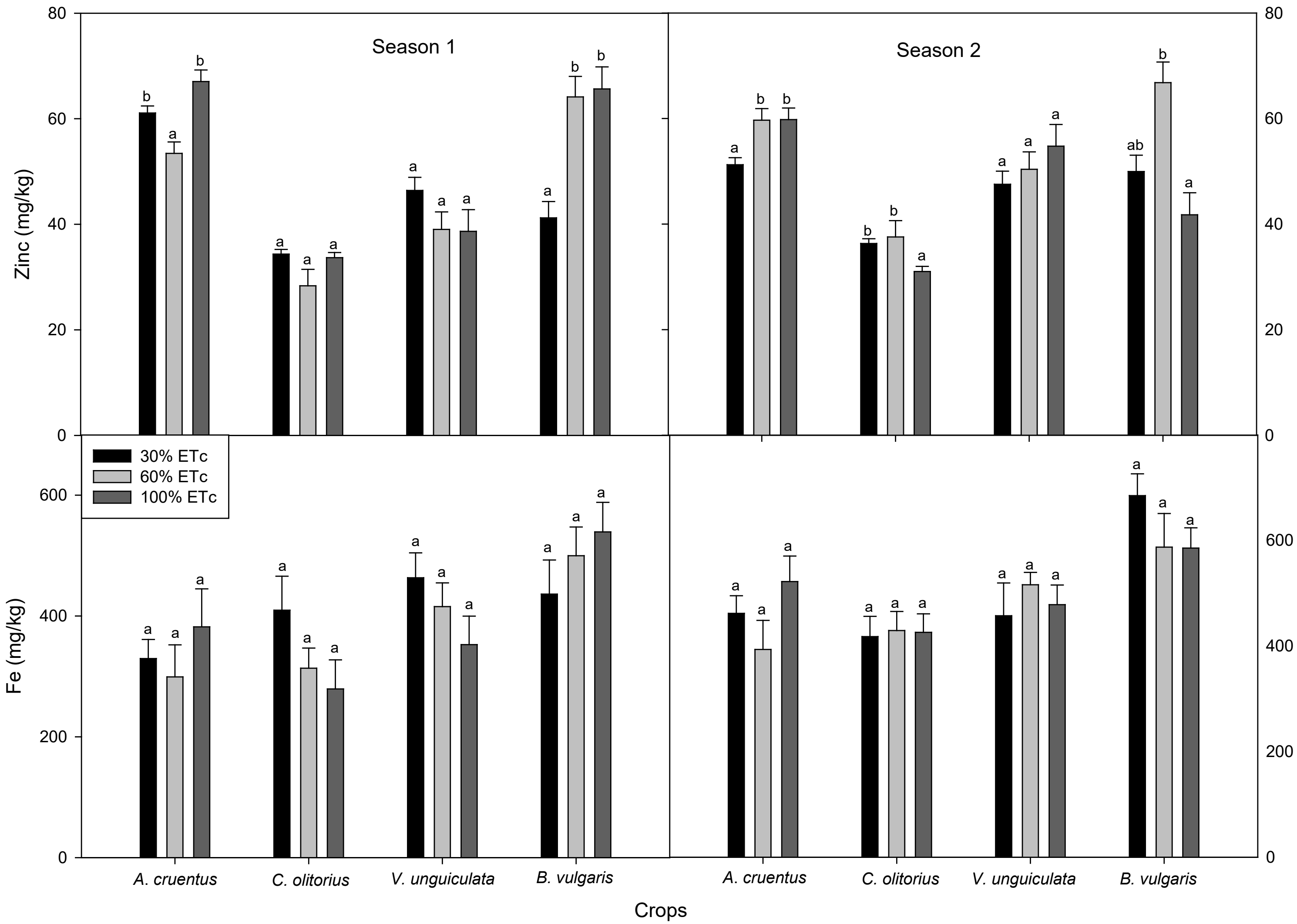


Figure 5.1. Effect of irrigation regimes on the levels of Fe and Zn from two growing seasons

These attributes play a central role in determining whether a crop is able to sustain economic production under high water stress. Be that as it may and notwithstanding the observed variations in this study, all four crops in this current study showed comparable accumulation of Zn and Fe levels under low soil water conditions of 30% ETc. *B. vulgaris* had higher Fe concentrations than all ALVs while Zn concentrations in *B. vulgaris* and *A. cruentus* were comparable and also higher than *V. unguiculata* and *C. olitorius*. Anaemia, due to hookworms and iron deficiency, is a widely-occurring problem and iron is required for haemoglobin formation (Kaya and Incekara 2000). Consumption of ALVs like *V. unguiculata* and *A. cruentus*, which are comparatively high in iron content, in adequate amounts may help to alleviate some of the nutritional problems associated with iron deficiency. The amounts of Fe in the present study correlates and are comparative with those obtained from previous work where *V. amyglidina*, *C. tora* and *C. olitorius* leaves were reported to contain 277 mg/kg and 204 mg/kg and 840 mg/kg of iron respectively (Barminas et al. 1998; Anyoola et al. 2010; Asaolu and Asaolu 2010). The levels of Zn in the current study also corroborate and are comparative with those obtained from previous work. Barminas et al. (1998) reported *C. tora* to contain an average of 209 mg/kg Zn while *C. tridens*, *Amaranthus spinosus* and *Adansonia digitata* had 123, 68 and 224 mg/kg zinc, respectively.

5.3.4 Effect of harvesting time on Fe, Zn, Mn, Mg, Ca P, and Na

African leaf vegetables are harvested from crop fields at different stages of plant growth. For most ALVs there is a preferred stage of plant development when flavour and palatability are favourable for human consumption. Research has indicated that levels of nutrients and toxic substances in vegetables are influenced by stages of plant development (Khader and Rama 2003; Modi et al. 2006). A number of ALVs are consumed at different stages of maturity, but limited information is available on their mineral content at different stages of maturity (Khader and Rama 2003). In *A. cruentus* trace elements were significantly ($P<0.05$) affected by harvesting (Table 5.4). The trend observed for Fe and Zn was an increase as time of harvesting increased from 6 weeks to 8 weeks for both seasons and then declined at 10 weeks during the second season. The results of the present study concur with Khader and Rama (1998) who observed a decrease in Zinc content with increasing maturity stages in *Amaranthus* and *Spineces* species. Other researchers reported similar results (Flyman and Afolayan 2008; Amanabo et al. 2011).

Table 5.4. Effect of harvest intervals on mineral composition of selected African leafy vegetables from two growing seasons

Crop	Harvest	2016/2017 growing season - Concentration (mg/kg)						
		Fe	Zn	Ca	Mn	Na	Mg	P
A. cruentus	6 Weeks	510.73^{a}	56.65^{a}	23372.52^{c}	109.62^{b}	699.52^{a}	11426.62^{b}	5027.30^{a}
	8 Weeks	538.59^{a}	62.43^{a}	31582.36^{b}	147.76^{b}	629.75^{a}	16108.04^{a}	4995.53^{b}
	10 Weeks	287.79^{b}	51.66^{b}	39845.25^{a}	242.23^{a}	495.53^{a}	15279.19^{a}	4536.61^{b}
C. olitorius	6 Weeks	579.44^{a}	39.17^{a}	14901.85^{a}	59.29^{a}	580.50^{a}	5963.20^{a}	247.81^{b}
	8 Weeks	350.37^{b}	34.99^{a}	15632.08^{a}	51.47^{a}	542.77^{a}	5118.45^{a}	918.86^{a}
	10 Weeks	342.27^{b}	30.74^{b}	14738.25^{a}	41.41^{b}	297.27^{a}	5460.15^{a}	238.19^{b}
V. unguiculata	6 Weeks	118.80^{b}	8.50^{b}	4452.00^{b}	118.80^{b}	247.81^{b}	4452.00^{b}	5963.20^{a}
	8 Weeks	142.34^{a}	10.50^{a}	5602.64^{a}	142.34^{a}	918.86^{a}	5602.64^{a}	5118.45^{a}
	10 Weeks	94.59^{c}	7.18^{c}	4288.01^{b}	94.59^{c}	238.19^{b}	4288.01^{b}	5460.15^{a}
B. vulgaris	6 Weeks	630.90^{a}	57.80^{a}	9200.02^{b}	149.36^{b}	32181.88^{a}	7872.57^{a}	4395.28^{b}
	8 Weeks	658.85^{a}	64.34^{a}	11632.71^{a}	237.01^{a}	37118.12^{a}	8872.11^{a}	5416.24^{a}
	10 Weeks	516.34^{a}	36.35^{b}	8763.57^{b}	100.01^{c}	30429.95^{b}	5722.06^{b}	2958.28^{c}
		2015/2016 growing season - Concentration (mg/kg)						
A. cruentus	6 Weeks	261.25^{b}	58.15^{a}	26224.86^{a}	87.01^{b}	319.04^{a}	11455.63^{a}	5840.32^{a}
	8 Weeks	422.24^{a}	62.89^{a}	32999.37^{a}	278.71^{a}	799.80^{a}	11105.07^{a}	3774.96^{b}
C. olitorius	6 Weeks	471.27^{a}	30.45^{a}	18377.02^{a}	86.82^{a}	284.36^{a}	3093.20^{a}	6192.92^{a}
	8 Weeks	197.02^{b}	33.80^{a}	15697.72^{b}	62.85^{b}	207.05^{b}	3495.47^{a}	6185.66^{a}
V. unguiculata	6 Weeks	492.80^{a}	44.77^{a}	21287.28^{a}	116.11^{b}	196.85^{b}	4924.39^{a}	3676.49^{a}
	8 Weeks	328.24^{a}	36.93^{a}	18646.73^{a}	161.62^{a}	276.31^{a}	4541.91^{a}	3772.61^{a}
B. vulgaris	6 Weeks	241.77^{b}	55.19^{a}	8895.73^{b}	186.71^{a}	26780.76^{b}	6075.48^{a}	4468.9^{b}
	8 Weeks	754.25^{a}	60.35^{a}	11136.88^{a}	269.23^{a}	39890.53^{a}	10186.32^{a}	8689.01^{a}

*Means followed by the same letters within a column for each treatment are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

Lanyasunya et al. (2007) observed that the rapid uptake of minerals by plants during early growth and the gradual dilution that occurs as plants mature would have been responsible for the decrease in some of the mineral content. Modi et al. (2006) found that iron concentration in A. cruetus increased significantly with age. Variation on results may be due to many factors such as soil composition and pH, water availability to the plant, weather conditions prevailing during the growth of the plant and the variety of the plant. During the second season leaf Ca and Mn significantly ($P<0.05$) increased as harvesting time increased although during the first season the differences were not significant (Table 5.4). These findings are in agreement with previous researchers who reported that calcium accumulated in more mature parts of the plant than in the younger parts of the plant (Loneragan 1968; Loneragan and Snowball 1969). Modi (2007) also reported that the calcium content increased in leaves of *A. hybridus* and *A. tricolor* with plant age.

Leaf P levels decreased significantly as harvesting time increased. Magnesium content increased significantly ($P<0.05$) from harvesting at 6 weeks to 8 weeks for both seasons and decreased significantly ($P<0.05$) at 10 weeks during the second season. This result was in agreement with results of Singh and Saxena (1972). They also found an increase in Mg content from 15 days to 30 days and a decrease in Mg content from 30 days to 45 days in most of the leafy vegetables studied. Leaf P content decreased significantly as time of harvesting increased. Similar results were obtained by Khader and Rama (2003) where P content was higher where growth rate was higher. Khader and Rama (2003) attributed the results to synthesis of new protoplasm in young leaves than in older leaves.

Leaf Fe, Zn, Mn, Mg and Na content in *C. olitorius* were significantly ($P<0.05$) affected by harvesting time (Table 5.4). Leaf Fe, Zn, Mn, Mg and Na were significantly ($P<0.05$) higher in early harvest (6 weeks) compared to late harvesting at 8 weeks and 10 weeks during the second season. A similar trend for Fe, Mn, and Na was observed during the first season (Table 4). P content increased significantly ($P<0.05$) from harvesting at 6 weeks to 8 weeks and decreased significantly ($P<0.05$) at 10 weeks during the second season although there were no significant differences during the first season (Table 5.4). Researchers have reported variations in nutrients such as Fe, Mn, Zn and Cu contents due to age of plant or time of harvest in leafy vegetables (Khader and Rama 2003). Phosphate content increased significantly ($P<0.05$) from harvesting at 6 weeks to 8 weeks and decreased significantly ($P<0.05$) at 10 weeks. This result concurs with the results of Giri et al. (1984) who reported that P increased with the age of the Chekurmeni plant.

Leaf Fe, Zn, Mn, Mg and Na content in *V. unguiculata* were significantly (P<0.05) affected by harvesting time (Table 5.4). Leaf Fe, Zn, Ca, Mn, Mg and Na significantly (P<0.05) increased as time of harvesting increased from 6 weeks to 8 weeks and then declined at 10 weeks during the second season. Similar results were obtained for Na and Mn with regard to increase from 6 weeks to 8 weeks during the first season. The trend was similar to that observed for *A. cruentus*. These findings were similar to previous reports, with an increase in iron content at each stage in *V. unguiculata* as the plant matured from 21 to 57 days after sowing the seeds (Flyman and Afolayan 2008). The increasing trend of iron suggests that the mineral may be indissociable ion and accumulates as age increase. Giri et al.(1984) also found a continuous increase in calcium content from 3 months to one year of age in chekurmenis leaves. It may be due to the immobile nature of the calcium and failure to retranslocate from older parts of the plant to the growing parts of the plant**.** Bello et al. (2011) reported that *Amaranthus* species, when harvested several times, are more productive than plants harvested once. Results of Na concur with other authors who reported an increase in Na concentration in *V. unguiculata* followed by a decrease with age (Flyman and Afolayan 2008; Mahala et al. 2012). Materechera and Medupe (2006) recommended leaves to be harvested every two weeks in *A. hybridus*.

Leaf Fe, Zn, Mn, Mg and Na levels in *B. vulgaris* were significantly affected by harvesting time (Table 4). Leaf Fe, Zn, Mn, Mg, Ca P, and Na content significantly (P<0.05) increased as time of harvesting increased from 6 weeks to 8 weeks for both seasons and then declined at 10 weeks during the second season. Giri et al.(1984) reported that phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), copper (Cu) and manganese (Mn) increased with the age of the Chekurmeni plant. Similar results were reported by Singh and Saxena (1972). They found an increase in Ca and Mg content with time up to a point followed by a decline. The increase may have been due to the Mg ion being in an unfixed or dissociable form that accumulates with age. The trend was similar to that observed for *A. cruentus* and *V. unguiculata.* African leafy vegetables can provide a continuous source of nutritious leaves to enrich the staple food over time because they can be harvested more than once. For *A. cruentus* and *C. olitorius* minerals were concentrated on early harvest while in *V. unguiculata* and *B. vulgaris* they increased with time up to a point then declined. During the second season, the crops were harvested more than the previous season based on the interest of results from he first season. It will be recommended in future to study multiple harvesting (above 3 harvest from the study) consistently since ALVs can be harvested as many times. This will give indication as what stage of a plant a crop performs better in terms of nutrients. Knowing the optimum point of nutrient accumulation at harvest will help optimise resources such as fertiliser and water. For example, if harvesting a crop 4 or 5 times is not productive in terms of nutrient composition, it will be worthy to harvest once to save resources.

If farmers are to select a preferred crop among the three ALVs crops studied, they should consider nutritional yield and benefits gained from the saving of water among other factors. At limited water level of 30% ETc, *V. unguiculata* performed better than all crops under study. This confirms that *V. unguiculata* is one of the drought tolerant crops compared to *B. vulgaris var. cicla*. In *V. unguiculata* production was optimised in terms of reduced amount of water use under limited water supply. *A. cruentus* and *C. olitorius* performed similar to *B. vulgaris* with optimum nutritional yield at 60% ETc. Application of 60% ETc is still a water-saving strategy because it is below the water requirements. This suggests that production of these crops is still possible under limited water supply especially for home consumption. The results obtained for nutrients were consistent to biomass yield (not shown) because quality is produced in the field. Therefore, the promotion of production of ALVs in South Africa can include addressing the notion of “more crop per drop”, thus the production of more food per millimeter of water used without compromising yield quality.

5.4 Conclusion

In the current study, water stress reduced nutrients for selected African leafy vegetables relative to the medium and well-watered treatment. Fe, Zn, Na and Cu were not affected by varying water regimes in *V. unguiculata.* Ca and Mg were higher in water limited conditions of 30% ETc compared to well-watered conditions in *A. cruentus* and *C. olitorius*. The ability of these crops to concentrate trace elements even under low water availability indicates the possibility of production even under limited water conditions. The different vegetable species investigated demonstrated different abilities to concentrate Mn, Cu, Fe, Na and K in the order *B. vulgaris* > *A. cruentus* > *C. olitorius* > *V.unguiculata*. The trend *A. cruentus* > *C. olitorius* > *V.unguiculata* > *.B. vulgaris* was observed for the Ca and P while for Zn and Mg the trend was *A. cruentus* > *B. vulgaris* > *V.unguiculata* > *C. olitorius*. *A. cruentus*, *C. olitorius* and *B.vulgaris* are recommended to be irrigated at 60 % ETc, because 30% ETc reduced yield while 100% ETc did not have any additional benefits. *V. unguiculata* is recommended to be irrigated at 30% ETc considering that most of the nutrient were not affected. Leaf Fe, Zn, Mn, Mg, Ca increased as time of harvesting increased from 6 weeks to

8 weeks, with no further increase at 10 weeks in *A. cruentus*, *V. unguiculata* and *B. vulgaris*. In *C. olitorius*, Fe, Zn, Mn, Mg and Na were high when harvested early at 6 weeks than during late harvesting at 8 weeks and 10 weeks. Early and medium harvesting has potential to retain nutrient in leafy vegetables. The present study shows that ALVs perform comparably, and in some cases better than their exotic vegetables such as *B. vulgaris*. Further studies are needed to assess nutritional composition of many ALVs under various water management strategies in different locations, climates and soils.

Acknowledgements

The authors acknowledge funding from the Department of Rural Development, National Research Foundation, Pretoria, South Africa and Agricultural Research Council – Vegetable and Ornamental Plants, South Africa.

References

Afolayan, A.J., Jimoh, F., 2009. Nutritional quality of some wild leafy vegetables in South Africa. Int. J. Food Sci. Nutr. 60, 424–431.

Agbemafle, R., Owusu-Sekyere, J.D., Bart-Plange, A., 2015. Effect of deficit irrigation and storage on the nutritional composition of tomato (*Lycopersicon esculentum* Mill. cv. pectomech) Croat. J. Food Tech. Biotech. Nutr. 10, 59–65.

Akinci, S., Lösel, D.M., 2012. Plant water-stress response mechanisms. In: Md, I., Rahman, M., Hasegawa, H. (Eds.), Water Stress. In Tech, Rijeka, Croatia, pp. 15–42.

Amanabo, M., Johnson, A.O., Matthew, I.S., Ezenwa, H.O.A., Emmanuel, O.O., 2011. Effect of heading on some micronutrients, anti-nutrients and toxic substances in Amaranthus cruentus grown in Minna, Niger State, Nigeria. Am. J. Food Nutr. 1, 147–154.

Amtmann, A., Blatt, M.R., 2009. Regulation of macronutrient transport. New Phytol. 181, 35–52.

Annandale, J.G., Stirzaker, R.J., Singels, A., Van Der Laan, M., Laker, M.C., 2011. Irrigation schedule research, South African experiences and future prospects. Water SA 37, 751–763.

Anyoola, P.B., Adeyeye, A., Onawumi, O.O., 2010. Trace element and major evaluation of *Spondias mombin*, *Vernonia anygdalina* and *Momordica charantia* leaves. Pak. J. Nutr. 9, 755–758.

Asaolu, S., Asaolu, M., 2010. Trace metal distribution in Nigerian leafy vegetables. Pak. J. Nutr. 9, 91–92.

Barminas, J., Charles, M., Emmanuel, D., 1998. Mineral composition of non-conventional leafy vegetables. Plant Food. Hum. Nutr. 53, 29–36.

Bello, Z., Walker, S., Tfwala, C., 2011. Influence of water supply and harvesting frequency on production of leafy amaranth in a semi-arid region of South Africa. Afr. Crop Sci. Conf. Proc. 381–385.

Birnin-Yauri, U., Yahaya, Y., Bagudo, B., Noma, S., 2011. Seasonal variation in nutrient content of some selected vegetables from Wamakko, Sokoto State, Nigeria. J. Soil Sci. Environ. Manage. 2, 117–125.

Brown, C., Pezeshki, S., DeLaune, R., 2006. The effects of salinity and soil drying on nutrient uptake and growth of *Spartina alterniflora* in a simulated tidal system. Environ. Exp. Bot. 58, 140–148.

Da Silva, E.C., Nogueira, R., da Silva, M.A., de Albuquerque, M.B., 2011. Drought stress and plant nutrition. Plant Stress 5, 32–41.

De Carvalho, I.M.M.S., 2005. Effects of water stress on the proximate composition and mineral contents of seeds of two lupins (*Lupinus albus* and *Lupinus mutabilis*). J. Food Qual. 28, 325–332.

Dunham, R., Nye, P., 1976. The influence of soil water content on the uptake of ions by roots. III. Phosphate, potassium, calcium and magnesium uptake and concentration gradients in soil. J. Appl. Ecol. 967–984.

Faber, M., Wenhold, F., 2007. Nutrition in contemporary South Africa. Water SA 33.

Faye, I., Diouf, O., Guisse, A., Sene, M., Diallo, N., 2006. Characterizing root responses to low phosphorus in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Agron. J. 98, 1187–1194.

Flyman, M.V., Afolayan, A.J., 2008. Effect of plant maturity on the mineral content of the leaves of *Momordica balsamina* L. and *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. J. Food Qual. 31, 661–671.

Food and Agriculture Organization, 2004. The State of Food Insecurity in the World. Economic and Social Department, Rome, Italy.

Giri, J., Bhuvaneswari, V., Rajeswari, D., 1984. Changes in the nutritive value of chekkurmenis at different stages of growth [*Sauropus androgynus*, a green leafy vegetables]. Ind. J. Nutr. Diet. 21, 419–423.

Jin, J., Wang, G., Liu, X., Pan, X., Herbert, S., Tang, C., 2006. Interaction between phosphorus nutrition and drought on grain yield, and assimilation of phosphorus and nitrogen in two soybean cultivars differing in protein concentration in grains. J. Plant Nutr. 29, 1433–1449.

Kaya, I., Incekara, N., 2000. Contents of some wild plants species consumed as food in Aegean region. J. Turk. Weed Sci. 3, 56–64.

Khader, V., Rama, S., 1998. Selected mineral content of common leafy vegetables consumed in India at different stages of maturity. Plant Foods Hum. Nutr. 53, 71–81.

Khader, V., Rama, S., 2003. Effect of maturity on macromineral content of selected leafy vegetables. Asia Pac. J. Clin. Nutr. 12, 45–49.

Loneragan, J., 1968. Nutrient requirements of plants. Nature 220, 1307.

Loneragan, J., Snowball, K., 1969. Calcium requirements of plants. Aust. J. Agr. Res. 20, 465–478.

Luoh, J.W., Begg, C.B., Symonds, R.C., Ledesma, D., Yang, R.-Y., 2014. Nutritional yield of African indigenous vegetables in water-deficient and water-sufficient conditions. Food Nutr. Sci. 5, 812.

Mabhaudhi, T., Modi, A., Beletse, Y., 2013. Response of taro (*Colocasia esculenta* L. Schott) landraces to varying water regimes under a rainshelter. Agric Water Manag 121, 102–112.

Mahala, A., Amasiab, S., Yousif, M.A., Elsadig, A., 2012. Effect of plant age on DM yield and nutritive value of some leguminous plants (*Cyamopsis tetragonoloba*, *Lablab purpureus*) and Clitoria (Clitoria ternatea). Int. Res. J. Agric. Sci. Soil Sci. 2, 502–508.

Materechera, S., Medupe, M., 2006. Effects of cutting frequency and nitrogen from fertilizer and cattle manure on growth and yield of leaf amaranth (*Amaranthus hybridus*) in a South African semi-arid environment. Biol. Agric. Hortic. 23, 251–262.

Maunder, E., Meaker, J., 2007. The current and potential contribution of home-grown vegetables to diets in South Africa. Water SA 33.

Mavengahama, S., 2013. The Contribution of Indigenous Vegetables to Food Security and Nutrition within Selected Sites in South Africa. Ph.D Thesis. Stellenbosch University, Cape Town, South Africa.

Modi, M., Modi, A., Hendriks, S., 2006. Potential role for wild vegetables in household food security: a preliminary case study in Kwazulu-Natal, South Africa. Afr. J. Food Agric. Nutr. Dev. 6, 1–13.

Nahar, K., Gretzmacher, R., 2002. Effect of water stress on nutrient uptake, yield and quality of tomato (*Lycopersicon esculentum* Mill.) under subtropical conditions. Bodenkultur 53, 45–51.

Nesamvuni, C., Steyn, N., Potgieter, M., 2001. Nutritional value of wild, leafy plants consumed by the Vhavenda. S. Afr. J. Sci. 97, 51–54.

Nyathi, M.K., Annandale, J.G., Beletse, Y.G., Beukes, D.J., Du Plooy, C.P., Pretorius, B., van Halsema, G.E., 2016. Nutritional water productivity of traditional vegetables crops. WRC report No.2171/1/16.

Nyathi, M., Van Halsema, G., Beletse, Y., Annandale, J., Struik, P., 2018. Nutritional water productivity of selected leafy vegetables. Agric Water Manag. 209, 111–122.

Odhav, B., Beekrum, S., Akula, U., Baijnath, H., 2007. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J. Food Compos. Anal. 20, 430–435.

Oelofse, A., Van Averbeke, W., 2012. Nutritional value and water use of African leafy vegetables for improved livelihoods - WRC Project No. K5/1579. Pretoria.

Osuagwu, G.G.E., Edeoga, H.O., 2012. The influence of water stress (drought) on the mineral and vitamin content of the leaves of *Gongronema latifolium* (Benth). Int. J. Med. Aromat. Plants 2 (2), 301–309.

Pascale, S., Paradiso, R., Barbieri, G., 2001. Recovery of physiological parameters in *Gladiolus* under water stress. Colture Protette 30, 65–69.

Pirzad, A., Darvishzadeh, R., Bernousi, I., Hassani, A., Sivritepe, N., 2012. Influence of water deficit on iron and zinc uptake by *Matricaria chamomilla* L. Chil. J. Agric. Res. 72, 232. Rahimi-Madiseh, M., Lorigoini, Z., Zamani-Gharaghoshi, H., Rafieian-Kopaei, M., 2017. *Berberis vulgaris*: specifications and traditional uses. Iran. J. Basic Med. Sci 20 (5), 569.

Lanyasunya, Wang, T., Rong, H., Mukisira, E., Abdulrazak, S., Kibitok, N., 2007. Effect of maturity and fertilizer application on in vitro gas production characteristics of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* Roth. Asian J. Anim. Vet. Adv. 6, 943–949.

Saleh, S., Liu, G., Liu, M., Ji, Y., He, H., Gruda, N., 2018. Effect of irrigation on growth, yield, and chemical composition of two green bean cultivars. Horticulturae 4, 3.

Sardans, J., Penuelas, J., Ogaya, R., 2008. Drought's impact on Ca, Fe, Mg, Mo and S concentration and accumulation patterns in the plants and soil of a Mediterranean evergreen *Quercus ilex* forest. Biogeochemistry 87, 49–69.

Shao, H.-B., Song, W.-Y., Chu, L.-Y., 2008. Advances of calcium signals involved in plant anti-drought. C. R. Biol. 331, 587–596.

Singh, P., Saxena, S., 1972. Effect of maturity on oxalate and cation contents of 6 leafy vegetables. Ind. J. Nutr. Diet. 9, 269.

Singh, B., Singh, G., 2004. Influence of soil water regime on nutrient mobility and uptake by *Dalbergia sissoo* seedlings. Trop. Ecol. 45, 337–340.

Turan, M., Kordali, S., Zengin, H., Dursun, A., Sezen, Y., 2003. Macro and micro mineral content of some wild edible leaves consumed in Eastern Anatolia. Acta Agric. Scand. B-S P 53, 129–137.

Van Der Walt, A.M., Mossanda, A.M., Jivan, K.S.A., Swart, S.D., Bezuidenhout, C.C., 2005. Indigenous African food plants: Vehicles of diseases or source of protection. IAJIKS Indilinga 4 p. 279.

Vorster, H.J., Stevens, J.B., Steyn, G.J., 2008. Production systems of traditional leafy vegetables: challenges for research and extension. S. Afr. J. Agric. Ext. 37, 85–96.

White, P.J., Broadley, M.R., 2009. Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182, 49–84.

CHAPTER 6

Postharvest drying maintains phenolic, flavonoid and gallotannin content of some cultivated African Leafy Vegetables.

I. Maseko[b,*], T. Mabhaudhi[b], B. Ncube[c], S. Tesfay[a], H.T. Araya[c], M.K. Fessehazion[c], V.G.P. Chimonyo[b], A.R. Ndhlala[c], C.P. Du Plooy[c]

[a]Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

[b]Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth & Environmental Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, Pietermaritzburg, 3209, South Africa

[c]Agricultural Research Council, Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria 0001, South Africa

*Correspondence: 215082595@stu.ukzn.ac.za; innocentmsk94@gmail.com; Tel: +27-(0)-33-260-6108

Abstract

The study investigated the effect of three irrigation regimes (30%, 60% and 100% of crop water requirement (ETc) and three drying (sun, oven, shade) methods on phenolic, flavonoids and gallatannin content of leafy vegetables. *Corchorus olitorius* grown under full irrigation and subjected to sun drying (100% ET_c x sun) had significantly higher total phenolic content followed by medium stress subjected to shade drying (60% ET_c x shade). Water stressed plants then shade and sun dried retained better gallotannins content than other treatments. *Amaranthus cruentus* grown under drought and shade dried (30% ET_c x shade) retained better total phenolic, flavonoid and gallotannin content. Drought stress and sun drying also performed better for *A. cruentus* (30% ET_c x sun) in terms of all phenolic components measured. In *Vigna unguiculata*, total phenolic content was high in water-stressed plants subjected to sun drying (30% ET_c x sun), results were similar to well-watered plants subjected to shade drying (100% ET_c x shade). Furthermore, sun drying retained better flavonoid and gallotannin content than shade and oven drying. In *Beta vulgaris*, well irrigated plants and shade or oven dried (100% ET_c x shade or oven) performed similar to stressed plants subjected to sun drying (30% ET_c x sun) in phenolics.

Shade dried leaves had better flavonoid while drought stress had better gallotannins content compared to other treatments in *B. vulgaris*. The three leaf vegetables can grow under drought stress then sun and shade dried without compromising their phenolic content.

Keywords: water stress, wild vegetables, processing, production, yield

*To whom correspondence should be addressed (Email: innocentmsk94@gmail.com)

6.1 Introduction

African leafy vegetables (ALVs) are important food and nutritional security crops which can contribute to addressing gaps in nutrition because they are nutrient-dense and requires less water for production (Oelofse and van Averbeke 2012). *Amaranthus cruentus* L., *Corchorus olitorius* L. and *Vigna unguiculata* L.Walp are among the major ALVs of great importance in South Africa. They are good dietary sources of calcium, iron, antioxidants such as flavonoids, tannins and other polyphenolic constituents (Moyo *et al*. 2012). These bioactive compounds provide strong protective effects against diseases such as cancer, arthritis, emphysema, retinopathy, neuro-degenerative cardiovascular diseases, atherosclerosis and cataracts (Sarker and Opa 2018; Kaur and Kapoor 2002). Despite their importance, cultivation of ALVs has not been widely adopted in South Africa. The main constraint to increased production, marketing and consumption of these crops is the high perishability in the fresh form and seasonality (Smith and Eyzaguirre 2007; Voster *et al*. 2005). Most ALVs are available in summer during the rainy season (Modi *et al*. 2006).

To overcome perishability and seasonal shortages, households apply preservation techniques that reduce their biological properties (Maseko *et al*. 2018). Drying is a way of processing leaf vegetables to ensure their availability during periods of short supply (Smith and Eyzaguirre 2007, Vorster *et al*. 2007) especially if packaging is unaffordable. Drying reduces microbiological activity through reduced moisture content in the food. There are several drying methods that can be utilised for leafy vegetables which include sun drying, solar drying, vacuum drying, oven drying, and freeze-drying (Fellows 2009). The most common natural drying method includes sun and shade drying. Sun drying method is the simplest, affordable and easily accessible means for resource-constrained households to preserve seasonal foods (Masarirambi *et al*. 2010; Bhila *et al*. 2010). Despite being cheaper, sun drying is reported to cause high nutrient losses, requires a longer drying period and is prone to contamination (Faber *et al*. 2010; Bankole *et al*. 2005). Shade drying is also a natural drying method that maintain better nutritional quality although it takes many days to dry to constant weight (Rajeswari 2010). Shade dried samples have been reported to have the highest nutrient retention followed by sun drying and oven dried samples (Joshi and Mehta 2010). Oven drying is reported to retain more carotene than sun drying; reduces drying time, allows for even heat distribution and improves sensory attributes such as colour and texture (Mdziniso *et al*. 2006). However, oven drying is expensive for resource-constrained rural households who do not have ready access to electricity.

In South Africa, sun drying of fresh leaves and sun drying of blanched leaves is the major method of processing leafy vegetables to make them available during periods of scarcity (Smith and Eyzaguirre 2007). The rural households usually store vegetables in a dried state in order to use them during times when they are not readily available (Misra *et al*. 2008; Smith and Eyzaguirre 2007). Van Averbeke *et al*. (2007) reported that electrification of the rural areas has introduced the new preservation technology, of freezing of leaves. However, most households are constrained with no access to a fridge or freezers (Van der Hoeven *et al*. 2013) and ovens hence freezing and oven drying methods are no options for them.

Presently, there is very limited literature on the various drying methods for ALVs in South Africa. Most of the nutrients reported from drying have been based on collecting various samples from farmers or samples purchased from the market to conduct bioassays. Some of the drying methods reported was based on survey studies with no structured and controlled experimentations conducted. This, however, makes it difficult to make nutritional recommendations as the field conditions in which the plants were produced has not been considered. South Africa is a dry country and crop production is mostly practiced under water deficit (Nyathi *et al*. 2018; Mabhaudhi *et al*. 2013; Annandale *et al*. 2011). For successful promotion and utilisation of ALVs, there is a need to conduct trials on production factors such as drought effect and postharvest factors and thus develop and promote locally processing techniques. Drought stress has been reported to enhance phenolic acids, flavonoids and antioxidants in *A. tricolor* (Sarkar and Oba 2018), phenolic content and antioxidant activity in leafy lettuce varieties (Malejane *et al*. 2018). Currently there is scanty information regarding drought stress and drying effects on bioactive compounds such as phenolic, flavonoids and gallotannins of ALVs in South Africa. The present study was undertaken to determine the effect of water stress and drying methods on the total phenolic, flavonoid and gallotannin content of *Amaranthus cruentus*, *Corchorus olitorius*, *Vigna unguiculata* and a reference vegetable crop– *Beta vulgaris* L.var. *cicla*.

6.2 Material and Method

6.2.1 Plant material and growth conditions

ALVs were grown in an open field trial at the Agricultural Research Council (ARC) - Vegetable and Ornamental Plants (VOP) farm, Roodeplaat, Pretoria (25°35' S; 28°21' E; 1164 masl) under varied irrigation regimes during 2015/2016 summer season. The vegetables species used as planting material were: *Amaranthus cruentus* (Amaranth), *Corchorus olitorius* (Jute mallow), *Vigna unguiculata* (cowpea variety Bechuana white, a runner type) and *Beta vulgaris* var. *cicla* (Swiss chard cultivar ‘Ford Hook Giant’). The irrigation levels were: 30% (drought stress), 60% (medium stress) and 100% (well-watered) of crop water requirement (ETc). The vegetable leaves were harvested at six (6) weeks after transplanting (WATP) from each irrigation treatment and packed in an upright position in clean plastic crates and transported to the storage facility where they were packed according to the drying methods.

6.2.2 Collection and drying of plant samples

Samples of *Amaranthus cruentus*, *Corchorus olitorius*, *Vigna unguiculata* (Bechuana white, a runner type) and *Beta vulgaris var. cicla* from each irrigation treatment (30%, 60% and 100% ETc) were subjected to three drying methods. The drying methods were: shade (28°C), sun (35°) and oven (45°C). Sun drying: fresh leafy vegetables were evenly spread on a tray and left to dry in the sun. Oven drying: the vegetables were oven dried at 45°C for 48 hours until completely dried. Shade drying: leaves were dried in a closed shade which protected the drying vegetables from the direct sunlight. The room selected for shade drying was well ventilated. The temperature range was (ambient temperature 25-35°C). Natural current of air was used for shadow drying the leaves. In all drying methods, leaves were turned occasionally until constant weight was attained. The dried leaves were ground and the powder was then sieved manually by using sieve with size 250 mm. Around 3 g of powder sample was used to test the particle size using particle size analyser. The dried samples were then used for the required analysis.

6.2.3 Sample Preparation

Dried plant materials were ground into powders and extracted (1:20 w/v) with 50% aqueous methanol in an ultrasonic bath for 1 h. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The resulting fresh extracts were then used in the phytochemical analysis.

6.2.4 Bioassays

6.2.4.1 Determination of total phenolic and flavonoid content

Folin Ciocalteu (Folin C) assay as described by Makkar (1999) with slight modifications was used to determine amounts of total phenolic compounds in plant samples. Fifty microlitres of each extract were transferred into test tubes then 950 µl of distilled water were added followed by 1 N Folin C reagent (500 µl) and 2% sodium carbonate (2.5 ml). Under room temperature for 40 min the test mixtures were incubated. The absorbance was read at 725 nm using a UV-vis spectrophotometer (Varian Cary 50, Australia) against a blank consisting of aqueous methanol instead of extract. Total phenolic concentrations were expressed in mg gallic acid equivalents (GAE) per g dry weight (DW). Total flavonoid content was determined following the vanillin assay Makkar (1999) and expressed as µg catechin equivalents (CTE) per g DW. Extracts (50 µl), were made up to 1 ml with methanol in test tubes before adding 2.5 ml methanolic-HCl (95:5, v/v) and 2.5 ml vanillin reagent (1 g 100 ml^{-1} acetic acid). Similar preparations of a blank that contained methanol instead of plant extracts were made. Absorbance was read at 500 nm using a UV-vis spectrophotometer after 20 min at room temperature.

6.2.4.2 Determination of gallotannin content

Hydrolysable tannin as gallotannins was determined using method of Makkar (1999) with slight modifications according to Ndhlala *et al*. (2007). Sample extracts (50 µl) were made up to 1 ml with distilled water (in triplicate). Sulphuric acid (100 µl, 0.4 N) and 600 µl of rhodanine were added to the diluted extracts. Incubation at room temperature was done for 5 min, and then 200 µl of potassium hydroxide (0.5 N) was added followed by 4 ml distilled water after a further 2.5 min. The mixtures were incubated at room temperature for 15 min, and then absorbance was done at 520 nm using a UV-visible spectrophotometer against a blank that contained 50% aqueous methanol instead of plant extract. For standard curve, freshly prepared stock of gallic acid solution (0.1 mg/ml in 0.2 N sulphuric acids) was. Gallotannin concentrations were expressed as gallic acid equivalents (GAE), derived from a standard curve.

6.2.5 Statistical analyses

All data were subjected to two-way analysis of variance (ANOVA) using SPSS software for Windows (IBM SPSS, version 25.0, Chicago, IL, USA). Where there were significant differences (P≤0.05), the means were further separated using Duncan's multiple range test (DMRT).

6.3 Results and discussion

6.3.1 Total Phenolics

Phenolic compounds play a major role in the prevention of cancer and cardiovascular diseases (Moyo *et al*. 2012). They are secondary metabolites that are synthesized by plants during normal development and in response to stress conditions (Naczk and Shahidi 2004). In the present study, total phenolic content increased significantly (P<0.05) in response to the interaction effect between irrigation and drying in *C. olitorius* (Figure 6.1). Total phenolic content increased with increase in the amount of irrigation water applied in sun drying; while an increase with increase in water application from 30 to 60% ETc followed by a decline was observed in shade drying. Leaves that were grown in well irrigated plots and sun dried (100% ETc x sun drying) produced higher phenolic of 17.5 mg/gDW which was also not significantly different to 16.5 mg/gDW obtained in medium irrigated *C. olitorius* and shade dried (100/60% ETc x shade drying). Leaves from all water rgimes subjected to oven drying had lowest phenolic content (7.9 mg/gDW) relative to sun and shade dried leaves (Figure 6.1). Results are in agreement with those of Mphahlele *et al*. (2016) who reported decrease in phenolic compounds due to degradation at elevated temperature such as during oven drying process. Postharvest processing using sun or shade is economic for resource-poor farmers compared to oven drying were resources in terms of oven and electricity are needed. Variation in the results of oven dried samples compared to sun and shade drying may be due to variation in drying temperature used.

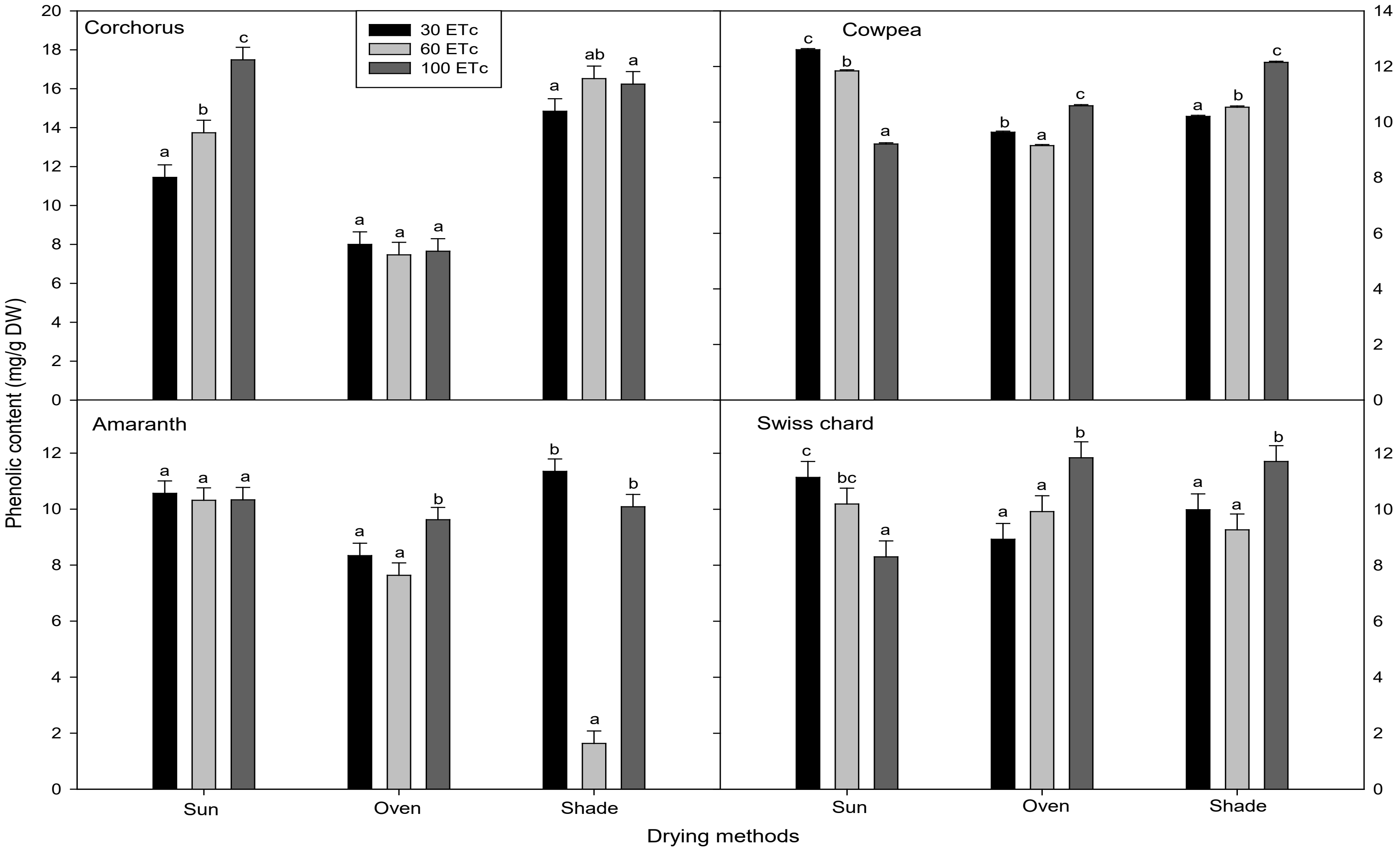


Figure 6.1. Interaction effect of irrigation and drying on phenolic content of Amaranthus cruentus, Corchorus olitorius, Vigna unguiculata and Beta vulgaris var. Figure cicla.

Rababah *et al*. (2015) observed a decrease in phenolic content in herbs due to oven drying. Quality is produced in the field hence plants grown under favourable environment translated to better yield quality. In terms of cost production to farmers, leaves grown under medium stress and shade dried (60% ETc x shade drying) performed better than all treatments on total phenolic content. In terms of phenolic content *C. olitorius* cultivation under drought-stressed areas such as semi-arid and drought-prone area is less feasible.

Phenolic acids of amaranths species such as *A. tricolor* have been reported to be good sources of natural antioxidant as they detoxify reactive oxygen species (ROS) in the human body (Sarker and Oba 2018; Venskutonis *et al*. 2013). Similar results were obtained in the present study, total phenolic content significantly ($P<0.05$) increased in response to interaction of irrigation and drying in *A. cruentus* (Figure 6.1).

Treatment combination of drought stress and shade drying (30% ETc x shade drying) had higher total phenolic content of 11.34 mg/gDW compared to other treatments combinations which had as lower as 7.6 mg/gDW. Water stress condition could have triggered production of secondary metabolites (Naczk and Shahidi 2004) in vegetables in the current study. Plant phenolics are the most widely distributed secondary metabolites that are involved in the response to stress (Cheynier *et al*. 2013; Ncube *et al*. 2013). Drought stress has been reported to enhance phenolic acids and antioxidant capacity of *Amaranthus* (Sarker and Oba 2018). Phenolic compounds accumulation is also affected by types and severity of stress (Ncube *et al*. 2013) that suggest the vairiation in results for the species under study. All treatment combination with oven drying had lower total phenolic relative to sun and shade drying. Results are similar to findings of Joshi and Mehta (2010) who reported that shade dried samples have the highest nutrient retention compared to sun and oven dried samples. Sun and shade drying are cost effective especially for poor farmers where there is no electricity. Based on the results of high total phenolic in limited irrigated plants, *A. cruentus* can be produced in marginal areas hence a promising crop for farmers of semi-arid and dry areas (Sarker and Oba 2018). Furthermore, postharvest processing of *A. cruentus* using sun/shade is economic for resources poor farmers compared to oven drying were resources in terms of oven and electricity are needed.

Interaction effect between the drying and irrigation on the total phenolics content of *V. unguiculata* was noted in this study (Figure 6.1). Irrigated plants that were shade and sun dried better maintained phenolic content than oven dried treatment. Zhang *et al*. (2009) reported severe loss of total flavonoids and total phenolics in oven dried bitter melon leaves compared to freeze-dried product. Thermal treatment has been reported to have an effect on the depletion of polyphenols in food products (Kaur and Kapoor, 2001). In sun dried treatments, total phenolic levels decreased as amount of water applied increased while the reverse effect was true for shade dried treatments, a phenomenon that suggest shade drying to be preserving phenolic content in the dried vegetative products. Total phenolic content was high in treatments obtained from limited water and sun dried (12.6 mg/gDW in 30% ETc x sun drying) which was statistically not different to well-irrigated and shade dried samples (12.1 mg/gDW- 100% ETc x shade drying). Phenolic compounds are synthesized by plants during normal development and in response to stress conditions (Naczk and Shahidi 2004). Similar results of total phenolic content in limited and well-watered conditions under different drying conditions asserts to shade drying as being superior and ideal in preserving the phenolic content in stored vegetables. Possible explanation could be drought drying method (shade) preserves the state of phenolic content as opposed to other drying methods.

The results of total phenolics content in response to interaction of irrigation and drying in *Beta vulgaris* are presented in Figure 6.1. The study showed that well irrigated treatments subjected to shade or oven dried (11.7 mg/gDW in 100% ETc x shade and 11.8 mg/gDW in 100% ETc x oven drying) had higher total phenolic levels than other treatments. Higher phenolic compounds are synthesized by plants during normal development a (Naczk and

Shahidi 2004). Drought stressed plants subjected to sun drying (11.1 mg/gDW 30% ETc x sun drying) was the third promising treatment. Similar observation of increase in total phenolic concentration in water stressed plants was reported in lettuce (Min *et al*. 2010). Total phenolic content decreased with increase water application for sun dried samples while the opposite was true for oven drying. Oven drying yielded results comparable to those of shade drying possibly due to controlled uniform heating resulting in increased availability of nutrients**.** Mdziniso *et al*. (2006) also found that oven drying reduces drying time and improves some sensory attributes like colour and texture. Higher phenolic content in well-watered conditions in *Beta vulgaris* compared to drought stressed plants in *A. cruentus* and *V. unguiculata* indicate that production of secondary metabolites (such as phenolics) vary from species involved, as species have different mechanisms of interactions with stress environments (Ncube *et al*. 2013).

6.1.3.2 Flavonoid content

Flavonoids have a variety of biological activities because of their antioxidant effect; they protect against coronary heart disease, stroke and cancer and also produce colour and flavour in food (Mampholo *et al*. 2015). Total flavonoid content in *C. olitorius* was not affected by water regimes and drying method. There was no interaction effect recorded and the results on independent factors are presented in Table 6.1. Although no significant differences were observed, plants grown under stress (30% ETc x shade drying) and shade dried produced the highest flavonoid content of 4.02 mg/gDW relative to other treatments. Higher flavonoids content under limited water combined with shade drying indicates the possibility of cultivating the crop under drought-stressed conditions and utilising cheaper method of preservation to retain nutrients.

Table 6.1. Independent influence of irrigation and drying on polyphenolic content of selected African leafy vegetables.

Crops	**Compounds**	**Irrigation levels (mg/gDW)**			**Drying (mg/gDW)**		
		30% ET_c	**60% ET_c**	**100% ET_c**	**Shade**	**Sun**	**Oven**
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
A. cruentus	Phenols	10.08±0.36^{a}	6.52±0.36^{b}	10.01±0.36^{a}	7.68±0.36^{c}	10.40±0.36^{a}	8.53±0.52^{b}
	Flavonoids	3.99±0.03^{a}	3.88±0.03^{b}	3.99±0.03^{b}	3.92±0.03^{b}	3.92±0.03^{b}	4.03±0.03^{a}
	Gallotannins	10.04±0.19^{a}	9.62±0.19^{b}	9.58±0.19^{b}	9.08±0.19^{b}	10.55±0.19^{a}	9.61±0.19^{b}
C. olitorius	Phenols	11.42±0.52^{a}	12.57±0.52^{b}	13.79±0.52^{c}	15.86±0.52^{a}	14.22±0.52^{b}	7.70±0.52^{c}
	Flavonoids	3.94±0.04^{a}	3.86±0.04^{b}	3.85±0.04^{c}	3.94±0.04^{a}	3.86±0.04^{a}	3.85±0.04^{a}
	Gallotannins	9.40±0.18^{a}	9.29±0.18^{a}	8.86±0.18^{b}	9.39±0.18^{a}	9.52±0.18^{a}	8.64±0.52^{b}
V. unguiculata	Phenols	10.81±0.32^{a}	10.51±0.32^{a}	10.64±0.32^{a}	15.86±0.32^{a}	11.21±0.32^{a}	9.79±0.32^{a}
	Flavonoids	3.87±0.40^{a}	3.82±0.40^{a}	3.87±0.40^{a}	3.88±0.40^{a}	3.76±0.40^{a}	3.85±0.40^{a}
	Gallotannins	10.37±0.33^{a}	9.56±0.33^{b}	13.79±0.33^{a}	10.77±0.33^{a}	14.22±0. 33^{a}	9.59±0.33^{a}
B. vulgaris L	Phenols	10.01±0.56^{a}	9.78±0.56^{a}	10.61±0.56^{a}	10.31±0.56^{a}	9.87±0.56^{a}	10.22±0.56^{a}
	Flavonoids	3.98±0.40^{a}	3.96±0.33^{a}	3.96±0.33^{a}	4.08±0.33^{a}	3.84±0. 33^{b}	3.97±0.33^{c}
	Gallotannins	10.20±0.13^{a}	9.57±0.13^{c}	9.86±0.13^{b}	9.85±0.13^{a}	9.92±0.13^{a}	9.87±0.13^{a}

* Mean values (±SE) in rows with different letters are significantly different ($p < 0.05$; $n = 3$) according to Duncan's multiple range tests.

Amaranthus species such as *tricolor* are sources of natural antioxidants such as flavonoids that serve some protective against a number of conditions, such as cancer and cardiovascular diseases (Venskutonis *et al*. 2012). Various factors such as drought (Sarker and Oba 2018) and drying method (Joshi and Mehta, 2010) are reported to influence accumulation of nutritional and bioactive compounds in plants. Results on total flavonoid content in response to the interaction effect between irrigation and drying in *A. cruentus* are presented in Figure 6.2.

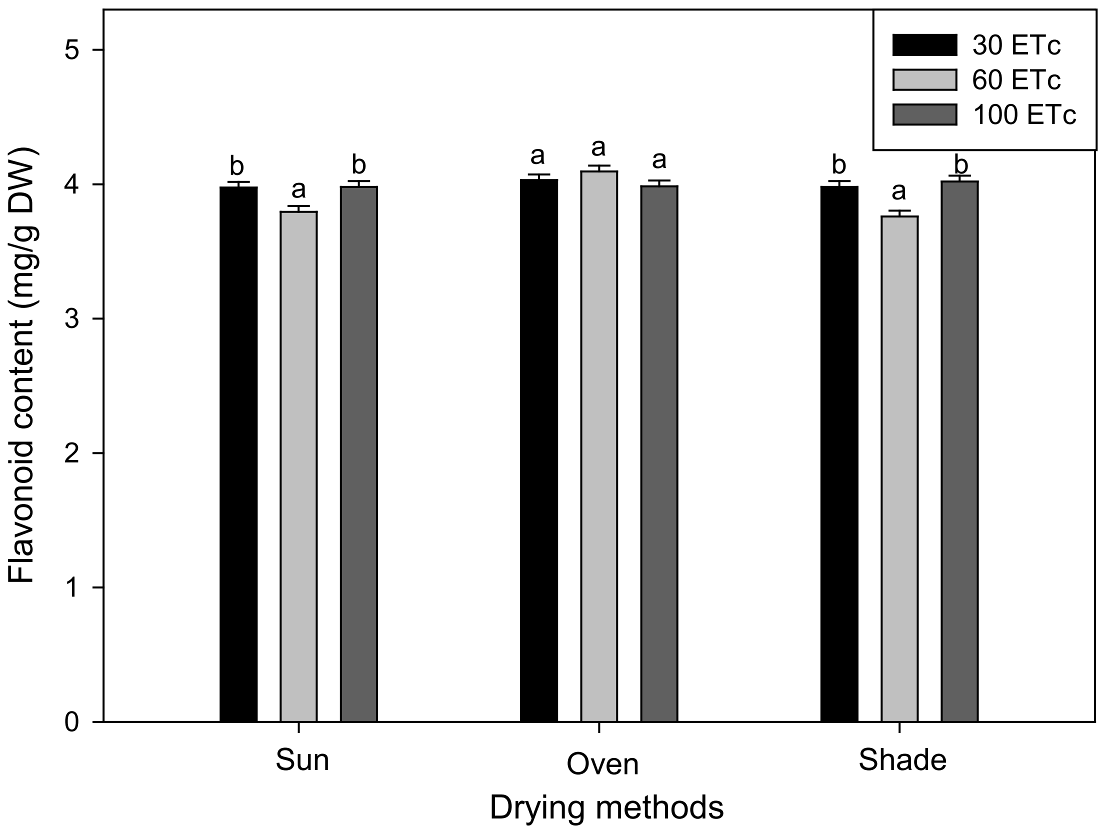


Figure 6.2. Interaction effect of irrigation and drying method on flavonoid content in *Amaranthus cruentus.*

Total flavonoid content was higher or statistically similar in stressed plants subjected to all three drying (30% ETc x sun, oven, and shade drying) methods compared to other treatment combination. Flavonoids accumulation may represent a defense against the increased oxidative stress produced by drought, because flavonoids respond to various kinds of adverse environmental conditions and play several protective roles (García-Calderón *et al*. 2015). Although oven drying is one of the recommended methods in preserving agricultural produce due to even drying temperature that retains aesthetic physical quality attributes, nutritional degradation has been reported due to high drying temperature (Joshi and Mehta 2010). Furthermore, it is costly for resource-constrained households as it requires the usually inaccessible resources such as ovens and electricity. Higher levels of flavonoids in stressed plants could be attributed deficit irrigation that influences the abiotic stress condition that stimulates the biosynthesis of phytochemicals in plants and improves their levels (Malejane e*t al*. 2018). Drought stress has been reported to enhance flavonoids in amaranths species such as *A. tricolor* (Sarker and Oba 2018). The results reported in this current study are consistent with those on phenolic and gallotannins, in which the interactive effect of drought stress and shade drying overall performed better in all bioactive compounds. Shade (room temperature) drying has been reported to retain higher amounts of total phenolics, antioxidant activity and flavonoids than oven drying (Rababah *et al*. 2015). Furthermore, postharvest processing of *A. cruentus* using shade is economic for resources poor farmers compared to oven drying that require resources such as oven and electricity.

Independently, drying as a factor significantly ($P<0.05$) affected total flavonoid content in *V. unguiculata* and no interaction effect was observed (Table 6.1). Total flavonoid content was higher in sun dried samples compared to shade and oven drying. Although sun drying exposes samples to direct sun exposure and contamination, it still remains one of the major options for rural households that have limited resources (Voster *et al*. 2007). This is because sun drying is the simplest, affordable and easily accessible means for poor households to preserve seasonal foods (Masarirambi *et al*. 2010). Sun drying is less-resource demanding as the sun is freely accessible and is less time consuming for marginalised populations. Total phenolics, antioxidant activity and flavonoids content in herbs decreased apparently by oven dried compared to shade drying (Rababah *et al*. 2015).

In *B. vulgaris*, drying significantly ($P<0.05$) affected total flavonoid content although no significant interaction effect was observed (Table 6.1). Shade drying had the highest flavonoid content, followed by sun drying and oven drying respectively and the differences between these methods were significant. Joshi and Mehta (2010) reported similar findings that shade dried samples have the highest nutrient retention compared to sun and oven dried samples. Rababah *et al*. (2015) reported the loss of flavonoids to be less in shade drying than oven drying possibly due to drying time and temperature (Schieber *et al*. 2001). Heating may breakdown some phytochemicals which affect cell wall integrity and cause a migration of some flavonoids component (Rababah *et al*. 2015). Lack of response of flavonoids due to water stress maybe due to intermolecular conversion of flavonoids to phenolic that occur under higher stress levels (Ncube *et al*. 2013).

6.3.3 Total Gallotannins

Gallotannins are the simplest hydrolysable tannins, containing a polyphenolic and a polyol residue (Gan *et al*. 2018). They possess useful bioactivities, including antioxidant, cardiovascular protective and anti-diabetic properties (Gan *et al*. 2018). Various factors influence production or modification of plant secondary metabolites such as gallotannins. In the present study, there was a significant ($P<0.05$) increase in gallotannin content due to interaction of irrigation and drying in *C. olitorius* (Figure 6.3).

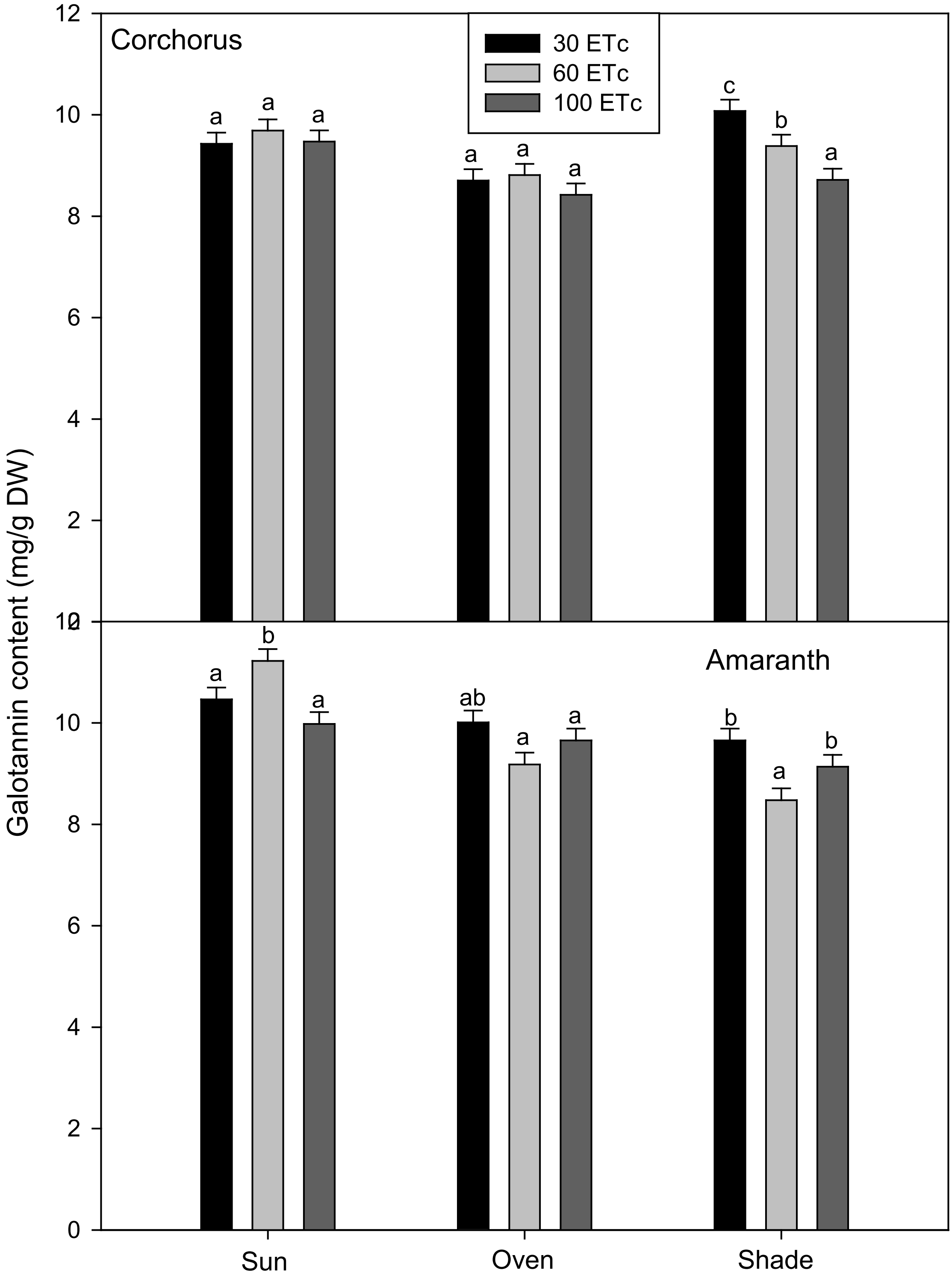


Figure 6.3. Interaction effect of irrigation and drying method on gallotannin content in *Amaranthus cruentus* and *C. olitorius* leaves

Total gallotannin content was lower in oven dried samples compared to sun and shade dried ones in all irrigation regimes. The treatment that performed better was the drought stressed plants subjected to shade drying (30% ETc x shade drying) with 10.2 mg/gDW as the highest relative to 8.42 mg/gDW which was the lowest in well irrigated plants subjected to oven drying (100% ETc x ovendrying). Overall observation was that, all stressed plants had better gallatannin retention than medium/well watered plants. The possible reason to high gallotannin content in water-stress plant material could be that deficit irrigation influences the abiotic stress condition that stimulates the biosynthesis of these phytochemicals in plants (Malejana *et al*. 2018). Ncube *et al*. (2013) reported that under severe stress conditions that dehydrate tissues, flavonoids are converted into tannins (gallatannins) to deal with most devastating stress. This could account for the results observed in *C. olitorius* because Flavonoids were not significantly affected possible due to conversion to gallatannins. Other treatment combination that produced higher gallotannin were plants grown under medium water conditions and subjected either to sun or shade drying (60% ET_c x shade/sun drying). Shade and sun drying treatment combinations had the highest total gallotannins content which were significantly different to those from oven drying. Joshi and Mehta (2010) reported similar findings on nutrient retention for various drying methods.

The concentrations of secondary metabolites in vegetables is influenced by many factors, including soil, irrigation, and other climatic conditions (Chandra *et al*. 2014). Total gallotannins content was significantly ($P<0.05$) affected by the interaction between irrigation and drying method in *A. cruentus* (Figure 6.3). Total gallotannins content were significantly higher in samples obtained from severe and medium drought stress conditions then sun dried (30/60% ETc x sun drying) compared with the other treatment combinations. Under water stress conditions tannins (gallatannins) are formed to deal with moisture stress (Ncube *et al*. 2013) and could accaount for results obaerved. Voster *et al*. (2007) reported that preservation through sun drying is a preferred option for rural households that have limited resources. Other treatment combinations that performed comparatively similar were samples from drought stress conditions subjected to oven or shade drying (30% ETc x oven/shade drying). Drought stressed plants subjected to shade and sun drying are cost effective treatments because less water is used for production and the drying methods require less resources. Overall, treatments combinations with sun drying had the highest gallotannins content than oven and shade drying while treatment a combination of drought stressed plants performed better than medium and well watered plants (Table 6.1). The results of gallotannin content were consistent with those of flavonoid and phenolic content indicating the possibility of producing *A. cruentus* under marginal areas and using inexpensive drying methods to preserve the quality of the produce.

Total gallotannins content in *V. unguiculata* was significantly affected by drying and irrigation independently (Table 6.1). Sun and shade drying had significantly higher gallotannins content compared to oven drying although the differences between sun and shade drying was not significant. Shade and sun dried samples have been reported to have the highest nutrient retention than oven dried samples (Joshi and Mehta 2010). Drought stressed and well watered plants had higher gallotannin content than medium stressed plants. Deficit irrigation has been reported to stimulate the biosynthesis of phytochemicals in plants and improves crop quality (Malejana *et al*. 2018). The ability of *V. unguiculata* in drought stress conditions to produce comparably similar gallotannin content with well watered plants indicates its adaptability under water limited conditions. Since limited water application and well watered plants produced similar results and hence application of limited water could be an economical viable option.

The independent influence of irrigation on total gallotannins content in *B.* vulgaris is presented in Table 6.1. Drought stressed plants had higher gallotannin content (10.20 mg/gDW) relative to medium stressed and well watered plants (9.5 and 9.8 mg/gDW respectively) in *B. vulgaris*. High phenolic productions are favoured by drought stress and high temperature (Ncube *et al*. 2012) and could accaount for the results obaerved. When plants are exposed to stress they produce secondary metabolites for protection against oxidative damage (Sarker and

Oba 2018; Malejana *et al*. 2018) and gallotannins are one such compounds serving this purpose. Natural drying (drying in the shade or in the sun) methods performed better than oven drying in all crops. These methods are still the most widely used because of the lower cost and affordability although it is difficult to control large quantities and achieve consistent quality standards. Drought stressed plants had better retention of nutrient quality compared to well-watered plants.

6.4 Conclusion

Drying methods and water regimes influenced flavonoid, phenolic and gallotannins content of all crops under study. *C. olitorius* grown under full irrigation and shade dried had higher total phenolic indicating that addition of water could lead to improved production. Medium irrigated and shade dried (60% ETc x shade drying) also retained better phenolic in *C. olitorius*. Total gallotannins content was retained in drought stressed plants subjected to all drying methods with shade and sun drying being economic due to use of less and inexpensive resources. Although flavonoids were not affected by irrigation and drying; application of less water and sun/shade drying is cost effective. *A. cruentus* grown under drought stress and shade dried retained better total phenolics, flavonoids and gallotannins content being cost effective by less water application and less resources for drying. In *V. unguiculata*, total phenolic content was high in plants grown under limited water and sun dried similar to well watered plants subjected to shade drying. Sun drying retained flavonoids and galatanins better than other treatments followed by shade drying. Drought stress retained better gallotanninn content similar to well conditions, therefore, drought stress treatments is economic as water is saved. In *Beta vulgaris*, total phenolic content was high in plants grown under limited water and sun dried similar to well watered plants subjected to shade drying. Shade drying retained flavonoids better than other treatments. Drought stress retained better gallotanninn content similar drought stress treatments is economic as water is saved. *V. unguiculata* and *C. olitorius* were comparable to

B. vulgaris in retention of bioactive compounds while *A. cruentus* was better that all crops under similar conditions. Household can adopt drying methods that promotes high retention of nutritional and sensory quality attributes and water regimes that are cost effective in preserving water. Sun and shade drying retained flavonoid, phenolic and gallotannins content better than oven drying in all crops and are thus better methods of drying. For shade drying there is need to regulate heat to avoid degradation of nutrients while in sun drying there is need to reduce exposure of vegetables to contaminants like dust and insects and direct ultraviolet. Drought stressed plants in most crops were better in terms of retention of flavonoid, phenolic and gallotannins content compared to well irrigated plants. This concurs with the notion that they are better adapted for marginal areas of production without compromising nutritional quality. The study indicate potential to manipulate preharvest factors such as water regimes or practise deficit irrigation inorder to optimise postharvest yield quality such as phenolic compounds. Similar studies in future should also include additional drying methods such as freeze, microwave and solar drying. Further studies are also needed to explore nutritional variation as a function of drying in different harvests, considering that leaves are harvested several times per season.

Acknowledgments

The authors acknowledge funding from the Department of Rural Development, National Research Foundation, Pretoria, South Africa and Agricultural Research Council – Vegetable and Ornamental Plants, South Africa.

Conflicts of Interest: The authors declare no conflict of interest.

References

Annandale, J.G., Stirzaker, R.J., Singels, A., Van Der Laan, M., Laker, M.C., 2011. Irrigation schedule research, South African experiences and future prospects. Water South Africa 37, 751–763.

Bankole, S.A., Osho, A., Joda, A.O., Enikuomehin, O.A., 2005. Effect of drying method on the quality and storability of 'egusi' melon seeds (*Colocynthis citrullus* L.). Afr. J. Biotechnol. 4, 799–803.

Bhila, T.E., Ratsaka, M.M., Kanengoni, A., Siebrits, F.K., 2010. Effect of sun drying on microbes in non-conventional agricultural by-products. S. Afr. J. Anim. Sci. 40, 484–487.

Faber, M., Oelofse, A., Van Jaarsveld, P.J., Wenhold, F.A.M., Van Rensburg, J.W.S., 2010. African leafy vegetables consumed by households in the Limpopo and KwaZulu-Natal provinces in South Africa. South Afr. J. Clin. Nutr. 23 (1), 30–38.

Fellows, P.J., 2009. Food Processing Technology, Principles and Practice, 3rd ed. CRC Press: Boca Raton, FL, USA; Woodhead Publishing Limited, Oxford, UK.

Gan, R.Y., Kong, K.W., Li, H.B., Wu, K., Ge, Y.Y., Chan, C.L., Shi, X.M., Corke, H., 2018. Separation, identification, and bioactivities of the main gallotannins of red sword bean (*Canavalia gladiata*) coats. Front. Chem. 6, 39. https://doi.org/10.3389/Fchem.2018.00039.

García-Calderón, M., Pons-Ferrer, T., Mrázova, A., Pal'ove-Balang, P., Vilková, M., Pérez-Delgado, C.M., Vega, J.M., Eliášová, A., Repčák, M., Márquez, A.J., Betti, M., 2015. Modulation of phenolic metabolism under stress conditions in a *Lotus japonicus* mutant lacking plastidic glutamine synthetase. Front. Plant Sci. 6, 760. https://doi.org/10.3389/fpls.2015.00760.

Joshi, P., Mehta, D., 2010. Effect of dehydration on the nutritive value of drumstick leaves. J. Metabolomics Syst. Biol. 1, 5–9.

Kaur, C., Kapoor, H.C., 2001. Antioxidants in fruits and vegetables - the illennium's health. Int. J. Food Sci. Technol. 36, 703–725.

Kaur, C., Kapoor, H.C., 2002. Antioxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol. 37, 153–161.

Mabhaudhi, T., Modi, A.T., Beletse, Y.G., 2013. Growth, phonological and yield responses of a Bambara groundnut (*Vigna subter-ranea* L. Verdc) landrace to imposed water stress: ll. Rain shelter conditions. Water Sa 39, 191–198.

Makkar, H.P.S., 1999. Quantification of Tannins in Tree Foliage: A Laboratory Manual for the FAO/IAEA Co-Ordinated Research Project on Use of Nuclear and Related Techniques to Develop Simple Tannin Assay for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on the Tanniniferous Tree Foliage; Joint FAO/ IAEA Division of Nuclear Techniques in Food and Agriculture: Vienna, Austria.

Malejane, D.N., Tinyani, P., Soundy, P., Sultanbawa, Y., Sivakumar, D., 2017. Deficit irrigation improves phenolic content and antioxidant activity in leafy lettuce varieties. Food Sci. Nutr. 6 (2), 334–341.

Mampholo, M.B., Sivakumar, D., Van Rensberg, W.J., 2015. Variation in bioactive compounds and quality parameters in different modified atmosphere packaging during postharvest storage of traditional leafy vegetables (*Amaranthus cruentus* L. and *Solanum retroflexum*). J. Food Qual. 38, 1745–4557.

MasarirambI, M.T., Mavuso, V., Songwe, V.D., Nkambule, T.P., Mhazo, N., 2010. Indigenous post-harvest handling and processing of traditional vegetables in Swaziland: a review. Afr. J. Agric. Res. 5, 3333–3341.

Maseko, I., Mabhaudhi, T., Tesfay, S., Araya, H.T., Fezzehazion, M., Duplooy, C.P.D., 2018. African leafy vegetables: a review of status, production and utilization in South Africa. Sustainability 10, 16.

Mdziniso, P., Hinds, M.J., Bellmer, D.D., Brown, B., Payton, M.E., 2006a. Physical quality and carotene content of solar dried green leafy and yellow succulent vegetables. Plant Foods Hum. Nutr. 61, 13–21.

Mdziniso, P., Hinds, M.J., Bellmer, D.D., Brown, B., Payton, M.E., 2006b. Physical quality and carotene content of solar-dried green leafy and yellow succulent vegetables. Plant Foods Hum. Nutr. 61 (1), 13–21.

Min, O.H., Carey, E.E., Rajashekar, C.B., 2010. Regulated water deficits improve phytochemical concentration in lettuce. J. Am. Soc. Hortic. Sci. 135, 223–229.

Misra, S., Maikhuri, R., Kala, C., Rao, K., Saxena, K., 2008. Wild leafy vegetables: a study of their subsistence dietetic support to the inhabitants of Nanda Devi Biosphere Reserve, India. J. Ethnobiol. Ethnomed. 4, 15.

Modi, M., Modi, A.T., Hendriks, S., 2006. Potential role for wild vegetables in household food security, A preliminary case study in KwaZulu-Natal, South Africa. Afr. J. Food Agric. Nutr. Dev. 6 (1), 1684–5358.

Moyo, M., Amoo, S.O., Ncube, B., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2012. Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa. South Afr. J. Bot. 84, 65–71.

Mphahlele, R.R., Fawole, O.A., Makunga, N.P., Opara, U.L., 2016. Effect of drying on the bioactive compounds, antioxidant, antibacterial and antityrosinase activities of pomegranate peel. BMC Complement. Altern. Med. 16, 143.

Naczk, M., Shahidi, F., 2004. Extraction and analysis of phenolics in food. J. Chromatogr. A 1054, 95–111.

Ncube, B., Finnie, J.F., Van Staden, J., 2012. Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. South Afr. J. Bot. 82, 11–20.

Ncube, B., Finnie, J.F., Van Staden, J., 2013. Dissecting the stress metabolic alterations in in vitro *Cyrtanthus* regenerants. Plant Physiol. Biochem. 65, 102–110.

Ndhlala, A.R., Kasiyamhuru, A., Mupure, C., Chitindingu, K., Benhura, M.A., Muchuweti, M., 2007. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. Food Chem. 103, 82–87.

Nyathi, M.K., Van Halsema, G.E., Beletse, Y.G., Annandale, J.G., Struike, P.C., 2018. Nutritional water productivity of selected leafy vegetables. Agric. Water Manag. 209, 111–122.

Oelofse, A., Van Averbeke, W., 2012. Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods; WRC TT535/12; Water Research Commission: Pretoria, South Africa.

Rababah, T.M., Al-U'datt, M., Alhamad, M., Al-Mahasneh, M., Ereifej, K., Andrade, J., 2015. Effects of drying process on total phenolics, antioxidant activity and flavonoid contents of common Mediterranean herbs. Int. J. Agri. Biol. Eng. 8, 145–150.

Rajeswari, R., 2010. Dehydration of Green Leafy Vegetables and Its Effect on Quality.

Master's Thesis. Dharwad University of Agricultural Sciences, Dharwad- India.

Sarker, U., Oba, S., 2018. Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of Amaranthus leafy vegetable. BMC Plant Biol. 18, 258.

Schieber, A., Keller, P., Carle, R., 2001. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. J. Chromatogr. 910 (2), 265–273.

Smith, I.F., Eyzaguirre, P., 2007. African leafy vegetables: their role in the World Health Organization's global fruit and vegetables initiative. Plant Food 7, 3–5.

Van Averbeke, W., Juma, K.A., Tshikalange, T.E., 2007. The commodity systems of *Brassica rapa* L. subsp. *chinensis* and *Solanum* retroflexum Dun. In Vhembe, Limpopo Province, South Africa. Water South Afr. 33, 349–353.

Venskutonis, P.R., Kraujalis, P., 2013. Nutritional components of amaranth seeds and vegetables: a review on composition, properties, and uses. Compr. Rev. Food Sci.

Food Saf. 12, 381–412. https://doi.org/10.1111/1541-4337.12021.

Vorster, H.J., Van Rensburg, W.J., Venter, S.L., Van Zijl, J.J.B., 2005. (Re)-creating awareness of traditional leafy vegetables in communities. Proceedings of the Regional Workshop on African Leafy Vegetables for Improved Nutrition.

Voster, H.J., van Rensburg, J.W., Van Zijl, J.J.B., Venter, S.L., 2007. The importance of traditional leafy vegetables in South Africa. Afr. J. Food Agric. Nutr. Dev. 7, 1–13.

Zhang, M., Hettiarachchy, N.S., Horax, R., Chen, P., Over, K.F., 2009. Effect of maturity stages and drying methods on the retention of selected nutrients and phytochemicals in bitter melon (*Momordica charantia*) leaf. J. Food Sci. 74, C441–C448.

CHAPTER 7

Influence of postharvest packaging, temperature and storage time on the phenolic composition and antioxidant properties of *Corchorus olitorius*

I. Maseko[1*], T. Mabhaudhi[2], S. Tesfay[1], B. Ncube[3], V.G.P. Chimonyo[2], H. T. Araya[3], M. Fessehazion[3], A Ndhlala[3], C.P. Du Plooy[3]

[1]Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa;
[22]Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa;[3]Agricultural Research Council, Vegetable and Ornamental Plant Institute (ARC-VOPI), Private Bag X293, Pretoria 0001, South Africa;
*Correspondence: innocentmsk94@gmail.com,

Abstract

BACKGROUND: Production and utilisation of African leafy vegetables is hindered by lack of information on postharvest management. The objective of this study was to assess variation in nutrient content of *Corchorus olitorius* in response to packaging (non-perforated and perforated), temperature [room storage, refrigerated storage (4°C)] and retail storage (10°C) and storage time (2, 4, 6, 8, 10 days).
RESULTS: *Corchorus olitorius* samples from each treatment were analysed for total flavonoids, total phenolics, antioxidant activity, *β*-carotene and overall acceptance. Phenolic contents were high in treatment combination involving 4 and 10°C compared to room temperature while a decrease was observed with an increase in storage time. Flavonoid content increased significantly with time up to 6 days then declined. Total phenolic content was significantly higher in leaves kept at 4 and 10°C combined with non-perforated packaging and was not significantly different to those stored at 4°C combined with perforated packaging. Total phenolic content decreased as storage time increased with non-perforated packaging treatment combination performing better than perforated. As storage time increased combined with any temperature, phenolic content decreased, with 4 and 10°C treatment combinations performing better than room temperature treatments. Antioxidant activity and overall acceptance was improved in any treatment combination kept at 4 and 10°C compared to room temperature for both packagings as storage time increased.
CONCLUSION: *Corchorus olitorius* leaves stored at room temperature had a shelf life of 2 days, at 4°C of 8 days and 10°C for 10 days in non-perforated and perforated packaging. Results indicated that overall quality was maintained when leaves were stored at 10°C for 10 days and 4°C for 8 days using both types of packaging.

Keywords: bioactive compound, shelf life, yield

7.1 Introduction

Corchorus olitorius (Jute mallow) is one of the major African leafy vegetables (ALVs) growing naturally in South Africa with a potential for development into a commercial crop.[1,2] It belongs to the Tiliaceae family and is an erect annual herb that varies from 20 cm to approximately 1.5 m in height.[1,2] The stems are angular with simple oblong to lanceolate leaves that have serrated margins and distinct hair-like teeth at the base.[1,2] The crop contains high levels of iron, protein, calcium, thiamin, riboflavin, niacin, folate and dietary fibre and thus a good candidate in alleviating nutrient deficiencies.[3,4] Previous studies have shown that ALVs are rich sources of phenolic compounds and other phytochemicals with antioxidant properties that contribute to reducing oxidative damage.[5,6] Despite the abundance of *Corchorus olitorius* species, rich in nutrients and adapted to marginal production conditions, it is still considered a wild species and is not being cultivated in South Africa.[7] It is currently harvested and utilised from the wild and people in the northern regions of South Africa appreciate its sliminess more than those in the south regions and add bicarbonate of soda to reduce the sliminess when cooking.[8,9,10] One of the main challenges to its production and utilisation is seasonality and high perishability in its fresh form.[1] Considering its nutritional composition and potential for nutritional security, the possibility of storing the leaves and increasing its shelf life thus require urgent attention.

Corchorus olitorius would also potentially fetch good prices if there were innovative ways of presenting it in the market such as packaging which can attract the attention of consumers.[7] According to Matenge *et al*.[11] there is need to improve the image of ALVs in order to improve acceptability, preference and consumption by mostly younger consumers. At present, most ALV are simply uprooted or cut at the stems, sometimes washed, then tied into bunches and presented in the market. Proper packaging is essential for protecting ALVs against spoilage and microorganism decay, preserve their quality and provide convenience of handling. [12] Voster *et al*.[13] reported that even dried leafy vegetable products can be packaged to increase shelf life.

Temperature and storage conditions are some of the factors that influence the deterioration of harvested commodities.[14] Higher temperatures accelerate physiological deterioration and quality loss. Nyaura *et al*.[15] reported an 88% decline in ascorbic acid content in amaranth vegetable when kept at room temperature after four days of storage while the lowest loss was observed at 5°C (55% loss) after 23 days of storage. Based on this study, shelf life extension and nutrient preservation of vegetable amaranth can be achieved through storage at 5°C. Storage time and temperature has been reported to significantly impact on shelf life and quality of vegetable produce.[16] It is very important to have sufficiently long shelf life of produce while maintaining good nutritional quality for its intended consumers.[17] Fresh green leaves of *Amaranthus* reportedly lost 85% of β-carotene due to inappropriate storage conditions.[18] Thus, it is of great importance to establish the appropriate storage time and temperature to maintain the quality of vegetables.[19]

A study conducted on okra (*Abelmoschus esculentus* L. Moench) on various storage temperatures (4°C, 8.5°C, 13°C and room temperature) showed that marketable pods were from the 13°C storage temperature in non-perforated and perforated packaging.[20,21] Thompson[22] further reported that optimal storage temperatures range between 13℃ and 18℃ for okra. Despite these promising results, it is difficult to extrapolate these recommendations because the composition and concentration of phytochemicals are influenced by various factors such as crop species, cultural practices, geographic origin, climatic conditions, postharvest storage conditions and postharvest processing procedures.[23] Recent studies conducted in South Africa on *A. cruentus* and *S. retroflexum*[24] and on *B. chinensis* [12] indicate that modified packaging and storage at 10°C can reduce postharvest losses and retain the overall quality and bioactive compounds on the retailer's shelf during marketing. South Africa has a high diversity of ALVs that are available for consumption that include *C. olitorius.* However, literature information is very scanty on the effects of combined factors such as packaging, temperature and storage time on the changes in chemical propersties of *olitorius*. This study, therefore, was conducted with the aim of determining the effect of postharvest packaging, temperature and storage time on the total phenolic content, flavonoid, antioxidant properties and marketability of *C.olitorius*.

7.2 Materials and methods

7.2.1 Plant material and growth conditions

Corchorus olitorius was grown in an open field trial at the Agricultural Research Council (ARC) - Vegetable and Ornamental Plant (VOP), Roodeplaat, Pretoria (25°35' S; 28°21' E; 1164 masl), under full irrigation during 2015/2016 summer season. The crop was irrigated three times a week to meet crop water requirements and fertiliser was applied according to soil analysis results done at the Agricultural Research Council–Institute for Soil, Climate and Water (ARC–ISCW), Acardia, Pretoria. Leaf growth was monitored throughout the growing period and harvested during the early morning for the trial. The leaves were harvested at six (6) weeks after transplanting (WATP) and packed in an upright position in clean plastic crates and immediately transported to the storage facility (100 m from the harvesting site) for packaging and storage. Each treatment had 3 replicates, each containing approximately 200 g of fresh leaves.

7.2.2 Packaging and storage

Approximately 200 g of fresh leaves per replication were packaged separately in two types of biorientated polypropylene packages, perforated (micro perforations) and non-perforated (according to the supplier) obtained from Knilam Packaging (Pty) Ltd (Cape Town, South Africa). This packaging is used by vegetable retailers across South Africa. The thickness of the bags was 35 µm (size 40 cm × 18 cm), and sealed with a heat sealer in order to create suitable internal atmospheres. The packed produce were then stored at 4°C, 10°C and room temperature for 2, 4, 6, 8 and 10 days. The 10°C storage temperature chosen for this study is representative of the retail display market conditions in South Africa [24] while 4°C is the standard temperature used in commercial retail stores in Johannesburg, Gauteng Province, South Africa. [25] The temperatures of 4°C, and 10°C were attained using refrigerators while room temperature (approx temperature range 22°C-30°C) was obtained by placing the leaves on tables in the open at room temperature that was well-lit during the day and dark during the evening.

7.2.3 Sample preparation

Leaf samples were removed after 2, 4, 6, 8 and 10 days from different storage temperature conditions and the changes with respect to quality and bioactive compounds in the leaves were investigated. Fresh leaf samples were separately oven dried at 50°C for 48 h. Dried plant materials were ground into fine powder and extracted (1:20 w/v) with 50% aqueous methanol in an ultrasonic bath for 1 h. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The extracts were concentrated under pressure using a rotary evaporator at 30°C and completely dried under a stream of air. The extracts were stored in airtight vials at 10°C until needed for various analysis. Fresh extracts of 50% aqueous methanol were used in the phytochemical analysis.

7.2.4 Determination of total phenolics and flavonoids

Dried samples of 2 g were extracted with 20 ml of 50% (v/v) aqueous methanol by sonication on ice for 20 min. Whatman No. 1 filter paper was used to filter extracts under vacuum. Folin Ciocalteu (Folin C) assay as described by Makkar [26] with slight modifications was used to dertemine amounts of total phenolic compounds in plant samples. Fifty microlitres of each extract were transferred into test tubes then 950 µl of distilled water were added followed by 1 N Folin C reagent (500 µl) and 2% sodium carbonate (2.5 ml). The test mixtures were incubated under room temperature for 40 min. The absorbance was read at 725 nm using a UV-vis spectrophotometer (Varian Cary 50, Australia) against a blank consisting of aqueous methanol instead of the extract. Total phenolic concentrations were expressed in mg gallic acid equivalents (GAE) per g dry weight (DW). Total flavonoid content was determined following the vanillin assay of Makkar [26] and expressed as µg catechin equivalents (CTE) per g DW. Extracts (50 µl) were made up to 1 ml with methanol in test tubes before adding 2.5 ml methanolic-HCl (95:5, v/v) and 2.5 ml vanillin reagent (1 g 100 ml^{-1} acetic acid). Similar preparations of a blank that contained methanol instead of plant extracts were made. Absorbance was read at 500 nm using a UV-vis spectrophotometer after 20 min at room temperature.

7.2.4 β-Carotene-linoleic acid model system (CLAMS)

The delay or inhibition of *β*-carotene and linoleic acid oxidation was measured according to the method described by Amarowicz *et al.* [27] with slight modifications. The antioxidant assay measures the ability of a test solution to prevent or minimize the coupled oxidation of *β*-carotene and linoleic acid in an emulsified aqueous system. In the reaction, the emulsion loses its orange colour due to the reaction with radicals, but this process can be inhibited by antioxidants.

β-Carotene (10 mg) was dissolved in 5 ml chloroform in a brown Schott bottle. The excess chloroform was evaporated under vacuum, leaving a thin film of *β*-carotene near to dryness. Linoleic acid (200 µl) and Tween 20 (2 ml) were immediately added to the thin film of *β*-carotene and mixed with aerated distilled water (497.8 ml), giving a final *β*-carotene concentration of 20 µg/ml. The mixture was further saturated with oxygen by vigorous agitation to form an orange coloured emulsion. The emulsion (4.8 ml) was dispensed into test tubes to which the sample extracts (200 µl of 6.25 mg/ml) were added, giving a final concentration of 250 µg/ml in the reaction mixtures. Absorbance for each reaction was immediately ($t = 0$) measured at 470 nm and incubated at 50°C, with the absorbance of each reaction mixture being measured every 30 min for 180 min. Tween 20 solution was used to blank the spectrophotometer. The negative control consisted of 50% methanol in place of the sample. The rate of *β*-carotene bleaching was calculated using the following formula:

$$\text{Rate of bleaching (R)} = \left\{\ln\left(\frac{A_{t=0}}{A_{t=t}}\right)\right\} \times \frac{1}{t}$$

Where $A_{t=0}$ is the absorbance of the emulsion at 0 min; and $A_{t=t}$ is the absorbance at time t (90 min; any point on the curve can be used for the calculation). The calculated average rates were used to determine the antioxidant activity (ANT) of the respective samples, and expressed as percentage of inhibition of the rate of *β*-carotene bleaching using the formula:

$$\% \text{ ANT} = \left(\frac{R_{control} - R_{sample}}{R_{control}}\right) \times 100$$

Where $R_{control}$ and R_{sample} represent the respective average *β*-carotene bleaching rates for the control and test samples, respectively. Antioxidant activity was further expressed as the oxidation rate ratio (ORR) based on the equation:

$$\text{ORR} = \frac{R_{sample}}{R_{control}}$$

7.2.5 Ferric-reducing power Assay (FRAP)

The ferric reducing power of the *Corchorus* extracts were determined based on the method by Lim *et al.*[28] with slight modifications. Each resuspended sample extract (50 µl) at 6.25 mg/ml and the positive control (BHT dissolved in methanol) was added to a 96 well microtiter plate in triplicate and two-fold serially diluted down the wells of the plate. To each well, 40 µl potassium phosphate buffer (0.2 M, pH 7.2) and 40 µl potassium ferricyanide (1% in phosphate buffer, w/v) were added. The microtiter plate was covered with foil and incubated at 50°C for 20 min. After the incubation period, 40 µl trichloroacetic acid (10% in phosphate buffer, w/v), 150 µl distilled water and 50 µl $FeCl_3$ (0.1% in phosphate buffer, w/v) were added. The microtiter plate was re-covered with foil and incubated at room temperature for 30 min. The ferric-reducing power assay involves the reduction of the Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) form. Absorbance of the formed Fe^{2+} was measured at 630 nm using a microtitre plate reader (Opsys MRTM, Dynex Technologies Inc., Palm City, FL, USA). The ferric-reducing power of the cultivar extracts and ascorbic acid were expressed graphically by plotting absorbance against concentration. The assay was repeated twice.

7.2.6 Overall evaluation

Overall acceptance evaluation was carried according Mampholo et al. (2013; 2015).[24,12] The panellists were asked to assess the overall acceptance of the fresh product at each storage interval after opening the bags at 2 day intervals. The leaves were individually scored according to a structured hedonic scale from 1 to 10 (10–9 excellent, no defects; 8–7 good; 6–5 fair, with acceptable marketability; 4–3 poor; 2–1 inedible).

7.2.7 Statistical analysis

All assays were done in triplicate, and the results reported as mean ± standard deviation (SE). One-way and two-way analysis of variance (ANOVA) were used to find differences among and between treatment combinations and significantly different mean values were separated using the Duncan multiple range test (DMRT). ($P \leq 0.05$). Data computations were done using SPSS for Windows (IBM SPSS, version 25.0, Chicago, IL, USA).

7.3 Results and discussion

7.3.1 Total flavonoid content

Consumption of indigenous leafy vegetables such as *C. olitorius* can contribute to beneficial flavonoids in diets. Flavonoids possess several biological effects such as anti-inflammatory, antioxidant and anticarcinogenic activities.[29] Total flavonoid content results of *C.os olitorius* are presented in Table 1. The interactions between storage time, temperature and packaging were not significant. However, storage duration as an individual factor significantly ($p<0.05$) affected flavonoid content (Table 7.1). Flavonoid levels increased from 2 days (1.156 mg/g DW) with increase in storage duration up to 6 days (1.457 mg/g DW) then started to decline. Results indicate that *Corchorus olitorius* can be stored up to 6 days to preserve flavonoids with further storage exhibiting a decline. The possible explanation to results observed could be that flavonoids degrade with storage while lower flavonoid content in shorter storage days could imply that flavonoids are synthesided from other phenolics into flavonoids. Decrease in flavonoid content with storage time has been previously reported in other studies, such as Mampholo *et al.*[24] in *A. cruentus*, Baltacıoğlu [30] in rowanberry fruit and Raya *et al.* [31] in *Clinacanthus nutans*.

Table 7.1. Flavonoid content of *C. olitorius* grown under full irrigation and stored at different storage conditions

Storage period (days)	Mean CE mg/g DW) ±Std. Dev
2 days	1.156 ±0.181
4 days	1.238 ±0.226
6 days	1.457 ±0. 358
8 days	1.315 ±0.729
10 days	1.215 ±0.269
Temperature	
Room Temperature	1.340±0.549
10°C	1.251±0.365
4°C	1.111±0.288
Packaging	
Non-perforated	1.229±0.375
Perforated	1.243±0.375

Values expressed as catechin equivalents (CE) per gram of sample dry weight. Mean values with different letters are significantly different ($p < 0.05$; $n = 3$)

7.3.2 Total phenolic content

Phenolic compounds have become a measurable indicator of nutritional quality of food because of their antioxidant properties[32] which provides protection against reactive oxygen species in the body.[33] Total phenolic content responded strongly ($p<0.05$) to the interaction effect of packaging and temperature (Figure 7.1). Phenolic contents were significantly higher in *Corchorus* leaves kept at 4°C in perforated packaging (80 mg/g DW) and at 10°C (75 mg/g DW) in non-perforated packaging (Figure 7.1). Lower total phenolics were recorded in leaves kept at room temperature in both perforated and non-perforated packaging which was also not significantly different from those kept at 10°C under perforated packaging. With the exception of samples stored at 4°C, all samples stored in non-perforated packaging had higher phenolic content than those stored in perforated packaging. Generally, on average, irrespective of the packaging type, the observed characteristic trend in this study was a decrease in phenolic content with an increase in storage temperature. High temperature could have led to accelerated metabolic processes leading to the degradation of phenolic compounds in the stored leaves. Padda and Picha [34] and Albert *et al*.[35] reported that a relationship exist between temperature and levels of phenolic compounds in plant tissues, in which lower temperatures are associated with higher levels of phenolic compounds. In this study, high storage temperature reduce phenolic composition while low temperature regardless of the packaging was able to retain nutritional value.

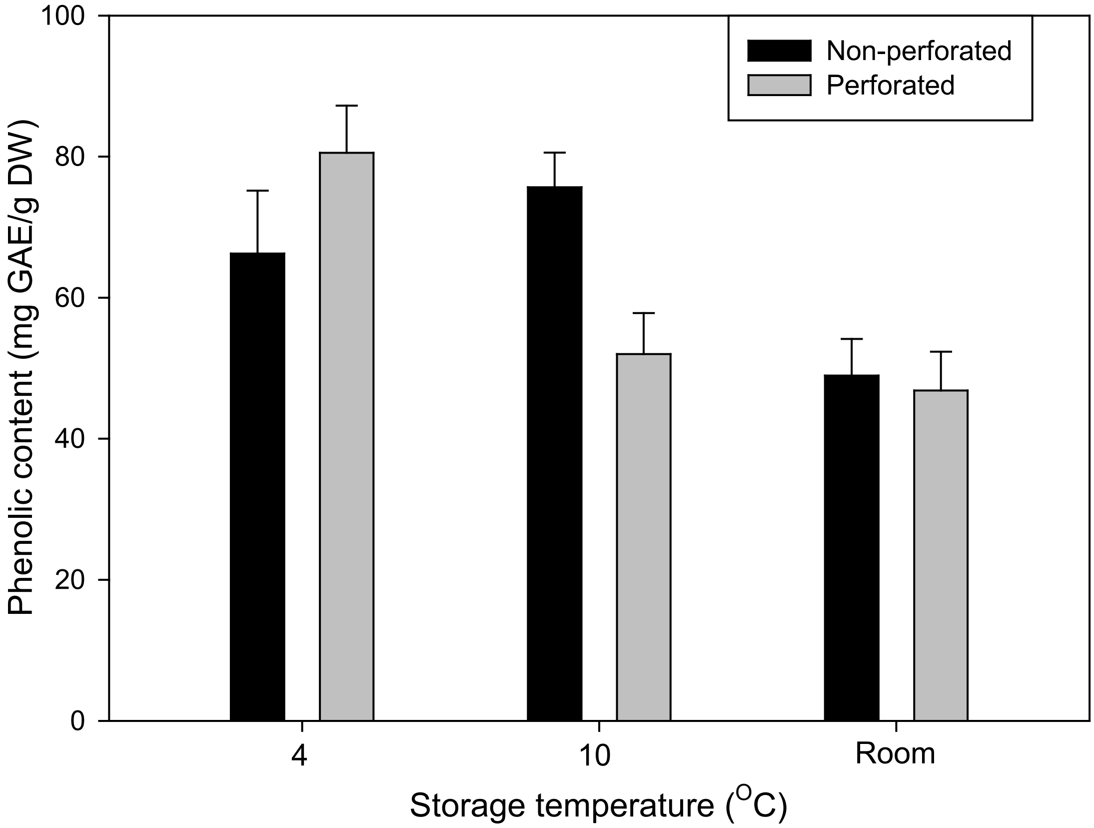


Figure 7.1. Interaction effect of packaging and temperature on total phenolic content of *C. olitorius*

Total phenolic content in *C. olitorius* were also significantly affected by storage time and packaging (Figure 7.2). Total phenolic content decreased significantly ($p<0.05$) as storage time increased for all packaging treatments. The longer the leaves were stored the higher the loss of nutritive values for both packaging. However, any treatment combination of storage duration and non perforated packaging perfomed better that perforated treatment combinations. The results concurs with Mampholo *et al.*[24] who reported a decrease in total phenolic content with increase in storage time across various packaging treatments in *A. cruentus* and *S. retroflexum*. Fawole and Opara[36] reported that degradation of total phenolic is related to enzymatic oxidation of polyphenol oxidase and peroxidase over time. Furthermore, in all storage durations, samples stored in non-perforated packaging maintained higher levels of phenolic content than those in perforated packaging except for 8 days of storage (Figure 2). However, Heyes[37]found that non-perforated packaging failed to retain flavour and aroma in comparison to perforated packaging. Phenolic compounds are responsible for flavour and colour in fruits and vegetables.[24] Varying perforation of packaging has significant effect in retention of phenolic compounds in *Corchorus*. If farmers decide to store *C. olitorius*, non-perforated packaging may be of use because degradation of phenolic compounds is reduced as compared to perforated packaging.

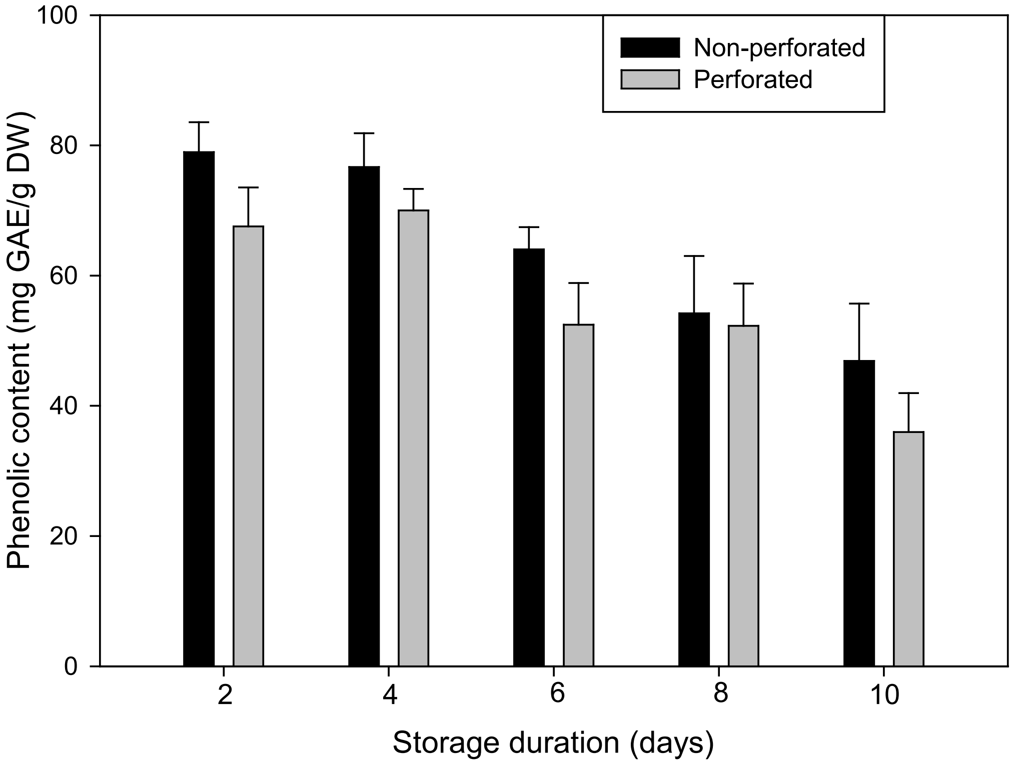


Figure 7.2. Interaction effect of storage duration and packaging type on the total phenolic levels of *C. olitorius*

Postharvest storage time and temperature has been reported to influence antioxidant activity and total phenolic content.[38] Similarly, total phenolic content in *C. olitorius* was affected by storage time and temperature in the current study (Figure 3). Phenolic content for leaves kept at 4°C increased significantly from 2 days (78 mg/g DW) to 4 days (137 mg/g DW) then declined from 6 days (74 mg/g DW), 8 days (39 mg/g DW) up to 10 days (22 mg/g DW). Increase in phenolic content for the first few days asserts to the fact that low temperatures maintain quality better than higher temperatures. Lowering of phenolics by low temperature (4°C) as storage duration increased might have been due to chilling injury at low temperatures for a prolonged period of time.[21] Leaves kept at 10°C showed an alternating decrease and increase as storage time increased up to 10 days, but the changes were not as drastic compared to room temperature and 4°C. For leaves kept at room temperature, phenolic content decreased from 2 days (71 mg/g DW) to 4 days (28 mg/g DW) followed by an increase from 6 days (71 mg/g DW) to 8 days (71 mg/g DW) then a decline at 10 days (71 mg/g DW). Temperature plays a pivotal role in the shelf life and quality of stored vegetable produce. Drastic decline of phenolic during room temperature storage (4 days) may be attributed to breakdown of phenolics at high temperatures, while a sudden increase in phenolics may be due to formation of phenolics from the breakdown of other substances with increase in storage time.

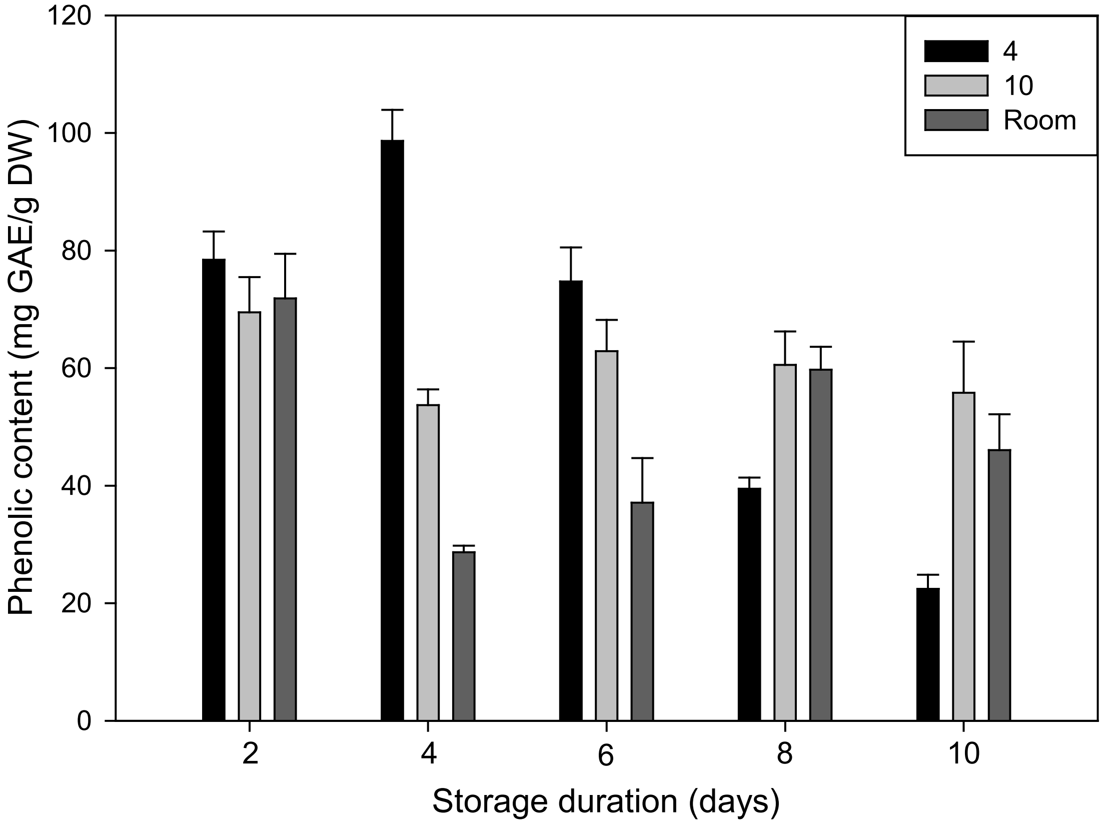
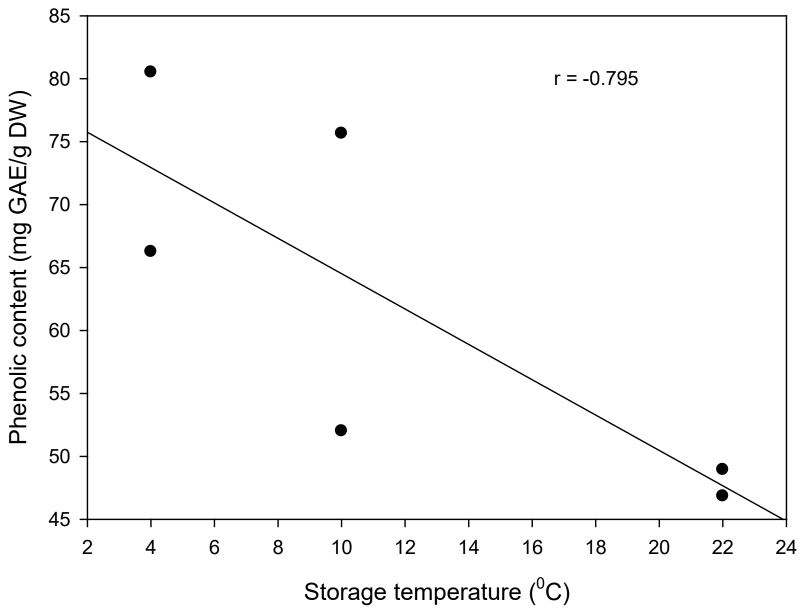
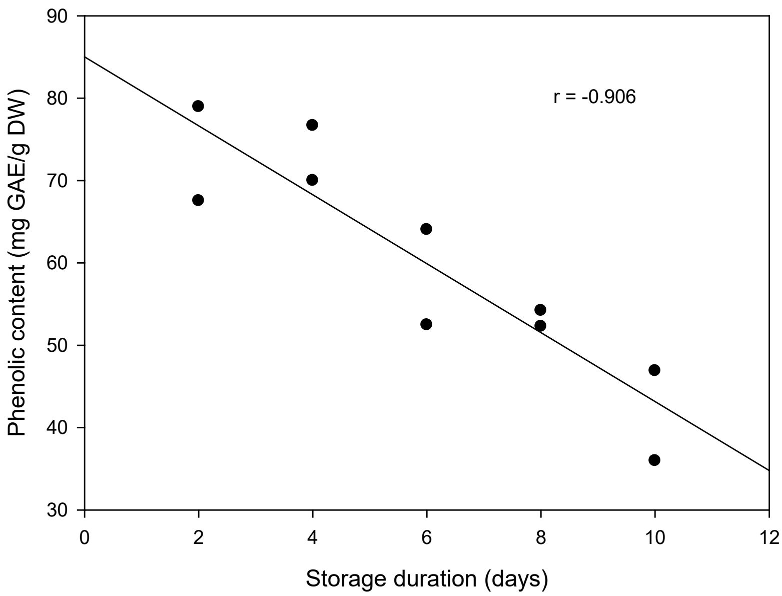


Figure 7.3. Interaction effect of storage duration and storage condition on total phenolic content of *C. olitorius*

A correlation analysis between total phenolics and storage conditions (temperature and storage duration) revealed significant strong negative relationships with coefficient (r) values of -0.795 to -0.906 (Figure 4a-b) for temperature and storage duration respectively. Increasing temperature resulted in a decrease in phenolics under storage. Similarity, Prabhu and Barrett[20] concluded that to minimise qualitative and nutritive losses, consumers should store leafy green vegetables such as *C. tora* and *C. tridens* at 4°C after harvest.



(a)



(b)

Figure 7.4. Correlation between storage duration and temperature on total phenolic content of *C. olitorius*

Similarly, storage duration showed a negative correlation on phenolic content (R = -0.906). Keeping *C. olitorius* for longer resulted in reduced phenolic content. Previous findings have shown higher total phenolic content in lettuce during early days of storage.[39,40]

7.3.3 Antioxidant activity

The results of the delay in *β*-carotene bleaching, recorded as antioxidant activity (ANT %) and oxidation rate ratio (ORR), calculated on the basis of the rate of *β*-carotene bleaching at time = 60 min are shown in Tables 7.2 and 7.3. Antioxidant activity (ANT) was significantly ($p<0.05$) influenced by the interaction between packaging and temperature in *Corchorus olitorius* samples (Table 7.2). The highest ANT (%) was recorded in leaves kept at 4 and 10°C in non-perforated packaging which was similar to those kept at room temperature in perforated packaging. Perforated packaging retained better nutritional quality than non-perforated packaging.[37] The results obtained in this study may be a reflection of the variation influenced by perforation treatment.[12] Lower ORR values denote better antioxidant potentials therefore leaves in non-perforated packaging kept at 4°C and 10°C exhibited the most antioxidant activity which were statistically similar to those kept in perforated packaging at room temperature (Figure 7.4).

Table 7.2. Interaction effect of packaging and temperature on antioxidant activity (AA % and ORR) of *Corchorus olitorius* as determined by β-carotene-linoleic acid model system

Treatment	**(ANT %) ±Std. Dev**	**ORR±Std. Dev**
Non-Perforated x 4°C	70.25±8.758	0.297±0.087
Non-Perforated x10°C	71.33±17.804	0.287±0.178
Non-Perforated x room temperature	67.15±7.624	0.329±0.076
Perforated x 4°C	65.76±6.790	0.342±0.067
Perforated x 10°C	65.58±13.801	0.344±0.138
Perforated x room temperature	71.28±13.722	0.287±0.137

Antioxidant activity was significant ($P<0.05$) in response to storage time and temperature in *C. olitorius* (Table 3). The highest ANT (%) was from leaves kept at 10 °C for 8 days compared to all treatments followed by 4 °C for 8 days, room temperature for 8 days and room temperature for 4 days (Table 7.3). Higher ANT (%) in leaves kept at room temperature may be due to decomposition. Overall acceptance results presented later shows that leaves kept at room temperature were only marketable for 2 days hence it shows they can still be utilised for other things. Generally, ANT (%) increased from 2, 4, 6 up 8 days then declined at 10 days, a trend similar to that observed in phenolic compounds. Similarly, ORR was significantly lower (good activity) in all treatment combinations that had significantly higher ANT (%) (Table 3). The lowest ORR was obtained when leaves were kept at 10°C for 8 days as with the ANT (%) shown in Table 3. Lower ORR values, denote better antioxidant potentials. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage.[41] Storage of vegetables and fruits is often associated with loss of antioxidant compounds.[42] Decrease in antioxidant capacity with prolonged storage may be due to the O_2 promoted oxidation of the constitutive phenolic compounds and vitamin C.[43] Low temperature could decrease the rate of biochemical processes inleaves, thus maintening antioxidant agents. Postharvest storage time and temperature influences antioxidant activity and total phenolic content.[38] The decrease in the levels of antioxidants during storage was also reported in other leafy vegetables.[12,24] Losses of different bioactive compounds can be minimal if optimal storage time and temperature can be established so that the product's shelf-life can be increased.

Table 7.3. Interaction effect of storage and temperature on Antioxidant activity (AA % and ORR) of *Corchorus olitorius* as determined by β-carotene-linoleic acid model system

Treatment	**(ANT %) ±Std. Dev**	**ORR±Std. Dev**
2 days x 4°C	68±9.654	0.319±0.096
2 days x 10°C	60±5.016	0.404±0.050
2 days x room temperature	62±5.160	0.375±0.051
4 days x 4°C	69±5.054	0.312±0.050
4 days x 10°C	65±9.958	0.351±0.099
4 days x room temperature	71±12.034	0.293±0.120
6 days x 4°C	61±5.390	0.390±0.053
6 days x 10°C	61±6.878	0.390±0.068
6 days x room temperature	69±8.967	0.307±0.089
8 days x 4°C	77±6.400	0.231±0.064
8 days x 10°C	95±2.780	0.047±0.027
8 days x room temperature	75±19.108	0.249±0.191
10 days x T4°C	68±3.444	0.324±0.034
10 days x 10°C	68±18.568	0.322±0.185
10 days x room temperature	68±5.169	0.325±0.051

The abilities of storage duration, packaging and temperature to reduce Fe^{3+} complexes in solution are presented in Figure 7.5.

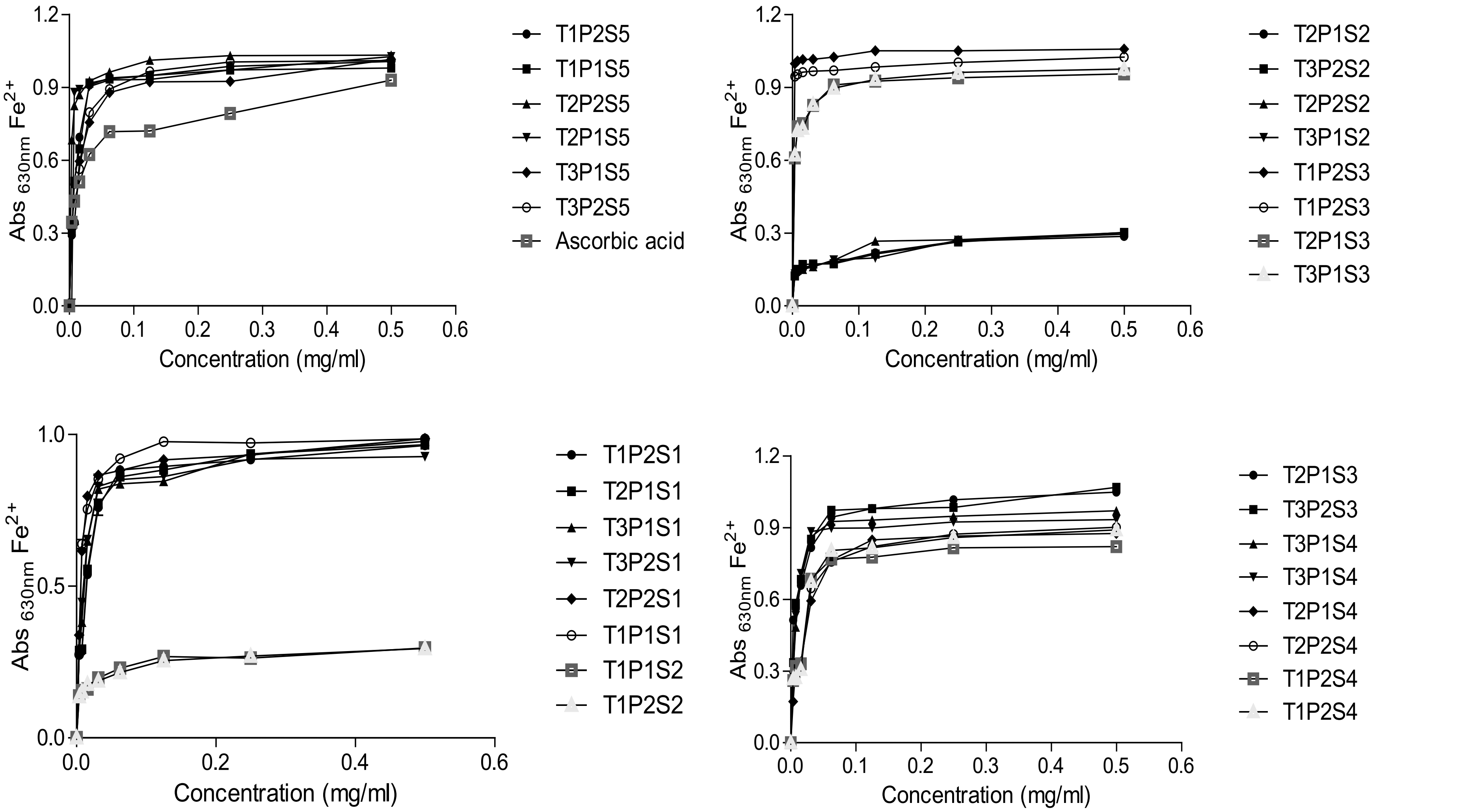


Figure **7**.5. Ferric reducing activity of *C. olitorius* as influenced by storage duration, packaging and temperature. n = 3. *S1 = 2 days; S2 = 4 days; S3 = 6 days; S4 = 10 days. *T1 = 4 °C; T2= 10 °C; T3 = room temperature. * P1 = Perforated; P2 = Non Perforated

Figure 7.5 was split into various sections for visibility of individual lines representing different factors. Reducing activity increased with increase in the concentration of all the treatment combinations. There were differences in the reduction power, with 4°C x perforated x 4 days, 4°C x non-perforated x 4 days, room temperature x perforated x 6 days and 4°C x non-perforated x 8 days performing as the least reducing agent. The treatment combinations with 10°C x perforated x 10 days, room temperature x non-perforated x 6 days, 4°C x perforated x 2 days, 4°C x non-perforated x 2 days, 10°C x non-perforated x 2 days, 10°C x perforated x 2 days, 4°C x non-perforated x 6 days and 4°C x perforated x 6 days were strong antioxidants (reductants) and exhibited higher activities compared to ascorbic acid used as a reference compound. The reducing activity of bioactive extracts is directly associated with antioxidant activity as the reduction of the Fe^{3+} complex is brought about by the donation of electrons.[44] Treatment combination with 4°C and 10°C had better antioxidant capacity compared to treatment combinations that were stored at room temperature. For storage durations of 2, 6 and 8 days, the highest antioxidant was obtained when leaves were kept at 4°C in both perforated and non-perforated packagings. At 10 days of storage time, FRAP showed higher antioxidant activity for treatment leaves kept at 10°C while leaves kept at room temperature had the lowest antioxidant activity. Decline in antioxidant activity during storage time has also been reported in other leafy vegetables.[12, 24]

7.3.4 Overall acceptance evaluation

In leafy vegetables, consumers seek visual quality (based on appearance) attributes which include freshness, uniformity of size, shape and typical colour, and free of defects.[12, 24] These quality attributes were evaluated in the current study as they are the first point of contact between the product and consumers. There was a significant ($P<0.001$) increase in overall acceptance score in response to the interaction effect of packaging, temperature and storage in *Corchorus olitorius* leaves (Figure 7.6). The treatments that acted as strong oxidants (Figure 7.5) such as 4°C x perforated x 2 days, 4°C x non-perforated x 2 days, 10°C x non-perforated x 2 days and10°C x perforated x 2 days were also observed to have the highest overall acceptance score (Figure 7.6).

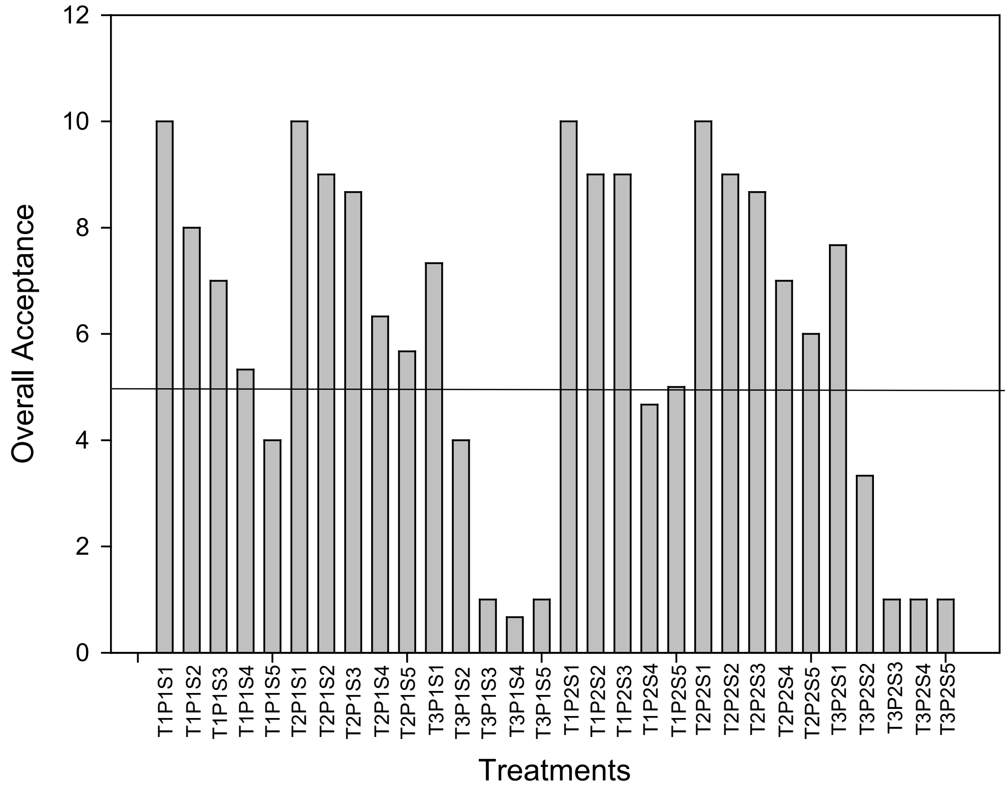


Figure **7**.6. Interaction effect of storage duration, packaging and temperature on overall quality of *C. olitorius*. n = 3. *S1 = 2days; S2 = 4days; S3 = 6days; S4 = 8days, S5 = 10days *T1 = 4 °C; T2= 10 °C; T3 = room temperature. * P1 = Perforated; P2 = Non Perforated

Overall acceptance score was observed to decrease with increase in storage time and temperature for the leaf samples subjected to different types of packaging (Figure 7.6). Leaves packed with either perforated or non-perforated plastic and stored at 4°C retained their marketability (score 5-10) for 8 days while leaves packed with perforated/non-perforated packaging and stored at 10°C remained marketable (score 6-10) for up to 10 days. Leaves packed in perforated/non-perforated packaging and stored at room temperature remained marketable for only 2 days (Figure 7.6). At 4 days of storage the leaves had turned brown as shown in Figure 7.7 and this coincided with drastic decline in phenolics during room temperature storage (4 days) shown in Figure 3 earlier. After 4 days of storage at room temperature some of the nutritional quality measured (ANT % and ORR in Table 3) started to increase possibly due to formation/conversion of phenolics from one form to the other. This could mean formation of other secondary metabolites which could be beneficial thus indicating posibility of using decaying leaves for other functions such as animal feeds etc. Mampholo *et al*.[24] reported similar findings on *A. cruentus* and S. *retroflexum* while Prabhu and Barrett[20] reported similar results on *C. tora* and *C.tridens.* The results of this study also concurs with those of Ngure *et al*.[21] who reported that Okra (*Abelmoschus esculentus* L. Moench) lose quality within two days under room temperature conditions leading to severe postharvest losses. Possible short postharvest life at room temperatures is because temperature facilitates respiration and other metabolic degradation of the product leading to loss of quality.

Figure 7.7**.** *Corchorus* leaves kept at room temperature for 2 and 4 days after storage

Leaves packed at 4°C started showing some chilling injury at 6 days which increased with time up to 8 days. The treatment combination that had leaves kept at 10°C did not show leaf chilling injury even after 10 days of storage. These result coroborate with that of Tulio *et al*.[3] who reported that *Corchorus* leaves are sensitive to chilling injury manifested as browning symptoms at low storage temperatures (8°C and lower). *Corchorus* have been reported to have a longer storage life at 8°C than with the other storage temperatures, and the shelf life was 8 days which is closer to the findings of the current study.[3] *Corchorus* is chill injury-damaged when stored at low temperatures showing surface and internal discoloration (browning), pitting and water soaked areas. Browning, a symptom of chilling injury of Jute mallow, developed at lower temperature than 5°C.[3] Similarly, low storage temperature caused chilling injury in okra

(*Abelmoschus esculentus* L. Moench) across all the packaging methods tested.[21] Crops which are susceptible to chilling injury often have a short storage life as low temperatures cannot be used to slow deterioration and pathogen growth. The primary cause of chilling injury is thought to be damage to plant cell membranes.[21]

The present study shows that storing *Corchorus* leaves at 10°C for 10 days using either of the perforated or non-perforated packaging has better shelf life than storage of 4°C regardless of the packaging types**.** The results of the present study support the findings of Mampholo *et al*.[12,24] who reported 10°C as optimal condition for keeping freshness of leafy vegetables. Leaf browning was observed on the second day on any treatment combination that had leaves kept at room temperature and the severity of browning increased with increasing storage time as shown in Figure 7.7. Consumer perceives the greenness of leaves as a quality attribute in leafy vegetables therefore; browning will reduce the market value of the crop. Leaf browning somewhat indicate the end of the shelf life of a product. There was no off odour in leaves kept at 4°C and 10°C storage temperature. Leaves in non-perforated/perforated packaging and stored at room temperature had off odour after 2 days of storage. There was a gradual increase in weight loss with increase in storage duration and temperature (not presented). Highest weight loss was observed at room temperature with minimum weight loss observed at 4°C and 10°C in non-perforated and perforated packaging. Weight loss was due to loss of water from the leaves due to metabolic activities. Apart from temperature, water loss from fresh produce also causes deterioration through wilting and shrivelling, loss of textural quality (softening, flaccidity, limpness, loss of crispness and juiciness) and nutritional quality.[22]

7.4 Conclusion

Development of postharvest handling techniques for perishable products such as *C. olitorius* will lead to their successful utilisation and commercialisation. The deterioration of flavonoids, total phenolics, antioxidant activity and overall acceptance was minimal in treatment combination of 4°C/10°C compared to room temperature for both packaging as storage duration increased. *C. olitorius* leaves stored at room temperature had a shelf life of 2 days, at 4°C of 8 days and 10°C for 10 days in non-perforated and perforated packaging. Since the current study indicates that *Corchorus olitorius* can only be stored for 2 days at room temperatures, small holder farmers who do not have access to refrigeration and packaging can resort to various types of indigenous drying methods. The overall quality was maintained when leaves were stored at 10°C for 10 days and 4°C for 8 days using both types of packaging which are promising conditions for extending the shelf life of *C. olitorius.* Further studies needs to be conducted on other low cost packaging and also explore various effects of packaging perforations.Furthemore studies should be conducted to ascertain the compounds formed when leaves have lost their shelf life, there is a posibility that new fomed substance can be of benefits to humans.

Acknowledgments

The authors acknowledge Knilam Packaging (Pty) Ltd, Cape Town, South Africa for providing the packaging materials and the South African department of Rural Development for funding.

Reference

1. Oelofse A and van Averbeke W, *Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods*; WRC TT535/12; Water Research Commission: Pretoria, South Africa (2012).
2. Van Rensburg WSJ, van Averbeke W, Slabbert R, Faber M, van Jaarsveld P, van Heerden I and Oelofse A, African leafy vegetables in South Africa. *Water SA*. 33: 317-326. (2007).
3. Tulio AZ, Ose K, Chachin K and Ueda Y, Effects of storage temperatures on the postharvest quality of jute leaves (*Corchorus olitorius* L.). *Postharvest Biol. Technol* 26:329-338 (2002).
4. Ogunrinde AT and Fasinmirin JT, Soil moisture distribution pattern and yield of jute mallow (*Corchorus olitorius* L.) under three different soil fertility management. Proceedings of the Environmental Management Conference, Federal University of Agriculture, Abeokuta, Nigeria. 373-381 (2011).
5. Uusiku NP, Oelofse A, Duodu KG, Bester MJ and Faber M, Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: a review. *J. Food Compos. Anal* 23: 499–509 (2010).

6. Kaur C and Kapoor HC, Anti-oxidant activity and total phenolic content of some Asian vegetables. *Inter. J. Food Sci and Technol* 37: 153–161. (2002).
7. Maseko I, Mabhaudhi T, Tesfay S, Araya HT, Fezzehazion M and Plooy CPD African Leafy Vegetables: a review of status, production and utilization in South Africa. *Sust* 10: 16 (2018).
8. Schippers RR, African Indigenous Vegetables. An Overview of the Cultivated Species (revised edn.).[CD Rom]. Natural Resources Institute, Chatham, U.K. 245 pp. (2002)
9. Van Wyk B and Gericke N, People's Plants. A Guide to Useful Plants of Southern Africa. Briza Publications: Pretoria, South Africa, 352 pp. (2000).
10. Fox FW and Norwood Young ME, Food from the Veld: Edible Wild Plants of Southern Africa. Delta Books, Johannesburg, South Africa. 399 pp. (1982).
11. Matenge STP, van der Merwe D, de Beer H, Bosman MJC and Kruger A, Consumers' beliefs on indigenous and traditional foods and acceptance of products made with cow pea leaves. *Afr. J. Agric. Res 7*: 2243–2254. (2012).
12. Mampholo MB, Sivakumar D, Beukes M and Van Rensberg WJ, Effect of modified atmosphere packaging on the quality and bioactive compounds of Chinese cabbage (*Brasicca rapa* L. ssp. *chinensis*). *J. Sci. Food Agric. 93:* (2013).
13. Vorster HJ, Jansen van Rensburg WJ, Venter SL and van Zijl JJB, (Re)-creating awareness of traditional leafy vegetables in communities. In proceedings of the Regional Workshop on African Leafy Vegetables for Improved Nutrition, IPGRI, Nairobi, Kenya, 6–9 December 2005.
14. Kader AA, The return on investment in postharvest technology for assuring quality and safety of horticultural crops. *J. Agric. Invest 4*: 45–52 (2006).
15. Nyaura JA, Sila DN and Owino WO, Postharvest stability of vegetable amaranth (*Amaranthus dubius*) combined with low temperature and modified atmospheric packaging. *Food Sci. Qual. Manag 30*: 66–72. (2014).
16. Kader AA and Rolle RS, The role of post-harvest management in assuring the quality and safety of horticultural produce. FAO *Agr. Serv.* Bul. 152. 9 Ct. <http://www.fao.org/3/ a-y5431e.pdf/>.(2014).
17. Ahvenainen R, New approaches in improving the shelf life of minimally processed fruit and vegetables. *Trends Food Sci. Technol* 7:179–187 (1996)
18. Faber M, Phungula MAS, Venter SL, Dhanat MA and Benade AJS, Home gardens focusing on the production of yellow and dark green leafy vegetables increase the serum retinol concentrations of 2–5-year-old children in South Africa. *Am. J. Clin. Nutr 76*: 1048–1054. (2002).
19. Moretti CL, Manual de processamento minimo de frutas e hortalicas. 1st ed. Embrapa Hortalicas, Rio de Janeiro, Brasil. (2007).

20. Prabhu S and Barrett DM, Effects of storage condition and domestic cooking on the quality and nutrient content of African leafy vegetables (*Cassia tora* and *Corchorus tridens*). *J Sci Food Agric* 89:1709–1721 (2009).
21. Ngure JW, Joseph N, Aguyoh G and Goaquiong L, Interactive effects of packaging and storage temperatures on the shelf-life of okra. ARPN *J. Agric. Bio. Sci.*, 4(3): 43-52 (2009).
22. Thompson AK, Post-harvest technology of fruits and vegetables. Hartnolls Ltd. Bodmin Cornwell, Great Britain (1996).
23. Goncalves B, Landbo AK, Knudsen D, Silva AP and Moutinho-Pereira J Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *J. Agric. Food Chem*. 52:523–30 (2004).
24. Mampholo MB, Sivakumar D, van Rensberg WJ, Variation in bioactive compounds and quality parameters in different modified atmosphere packaging during postharvest storage of traditional leafy vegetables (*Amaranthus cruentus L*. and *Solanum retroflexum*). *J. Food Qual* 38: 1745–4557 (2015).
25. Mudau AR, Nkomo MM, Soundy P, Araya HT, Ngezimana W and Mudau FN, Influence of postharvest storage temperature and duration on quality of baby spinach. *HortTechnology 25*: 665–670 (2015).
26. Makkar HPS, Quantification of Tannins in Tree Foliage: A Laboratory Manual for the FAO/IAEA Co-Ordinated Research Project on Use of Nuclear and Related Techniques to Develop Simple Tannin Assay for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on the Tanniniferous Tree Foliage; Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture: Vienna, Austria (1999).
27. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B and Weil JA, Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem 84*: 551–562. (2004).
28. Lim TY, Lim YY and Yule CM, Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. *Food Chem 114*, 594–599 (2009).
29. Middleton Jr E, Kandaswami C and Theoharides TC, The effects of plant flavonoids on mammalian cells: Implication for inflammations, heart disease and cancer. *Pharmacological Reviews* 52, 673–751. (2008).
30. Baltacıoğlu C, Velioğlu S and Karacabey E, Changes in total phenolic and flavonoid contents of rowanberry fruit during postharvest storage. *J Food Qual* 34: 278–283 (2011).
31. Raya KB, Ahmad SH, Farhana SF, Mohammad M and Tajidin NE, Changes in phytochemical contents in different parts of *Clinacanthus nutans lindau* due to storage duration. *Bragantia*74(4):445-52 (2015).

32. Mertz C, Gancel AL, Gunata Z, Alter P, Dhuique-Mayer C, Vaillant F, Perez AN, Ruales J and Brat P, Phenolic compounds, carotenoids and antioxidant activity of three tropical fruits. *J. Food Compos Anal* 22: 381–387 (2009).
33. Podsędek A, Natural antioxidants and antioxidant activity of *Brassica* vegetables: a review. LWT- *Food Sci Technol* 40: 1–11. (2007).
34. Padda MS and Picha DH, Effect of low temperature storage on phenolic composition and antioxidant activity of sweet potatoes. *Postharvest Biol Tec* 47: 176-180 (2008).
35. Albert A, Sareedenchai V, Heller W, Seidlitz HK and Zidorn C Temperature is the key to altitudinal variation of phenolics in *Arnicamontana L.* cv. Arbo. *Oecologia* 160: 1-8. (2009).
36. Fawole OA and Opara UL, Effects of storage temperature and duration on physiological responses of pomegranate fruit. *Ind. Crop Prod*, 47, 300– 309 (2013).
37. Heyes J, Parsley. New Zealand institute for crop and food research, Palmerston North. www.ba.ars.usda.gov/hb66/102/parsley.pdf (2004).
38. Lamikanra O, Fresh-cut Fruits and Vegetables: science, Technology, and Market. CRC PressI Llc; Florida, USA (2002).
39. Chisari MA, Todaro RN, Barbagallo and Spagna G, Salinity effects on enzymatic browning and antioxidant capacity of fresh-cut baby Romaine lettuce (*Lactuca sativa*). *Food Chem* 119(4): 1502-1506 (2010).
40. Ferrante AL, Martinetti and Maggiore T, Biochemical changes in cut vs. intact lamb's lettuce (*Valerianella olitoria*) leaves during storage. *Int. J. Food Sci. Tech* 44(5): 1050-1056. (2009).
41. Navarro J, Flores P, Garrido C and Martinez V, Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem* 96: 66–73 (2006).
42. Hounsome N, Hounsome B, Tomos D and Edwards-Jones G, Changes in antioxidant compounds in white cabbage during winter storage. *Postharvest Biol. Technol* 52: 173-179 (2009).
43. Stewart D, Oparka J, Johnstone C, Iannetta PPM and Davies HV, Effect of Modified Packaging (MAP) on Soft Fruit Quality. In Annual Report of the Scottish Crop Research Institute (pp. 119–124). Invergowrie, Dundee, Scotland: (1999).
44. Ndhlala AR, Mulaudzi R, Ncube B, Abdelgadir H, du Plooy CP and van Staden J, Antioxidant, antimicrobial and phytochemical variations in thirteen *Moringa oleifera* cultivars. *Molecules* 19:480–494 (2014).

CHAPTER 8

8.1 General discussion

The literature review on Chapter 2 in the form of published review article provided evidence of low utilisation and production of African leafy vegetables (ALVs) in South Africa due to lack of sound agronomic practices, innovative processing and value-adding techniques. Furthermore, limited funding and lack of coordinated research were identified as some of the factors contributing to the slow uptake commercialisation of ALVs. Involvement of stakeholders such as government, private sectors, NGOs, research and academic institutions can generate valuable and extensive production information that can lead to successful commercialization of ALVs.

In Chapters 3 and 4, the agronomic field studies indicated that ALVs respondend positively to irrigation water application under varying growing conditions. The result for *A. cruentus* was consistent for both field and controlled environments. Most measured parameters were negatively affected by water stress and these results concured with other similar studies that reports *A. cruentus* as a less stress tolerant crop (Neluheni et al., 2007). In *V. unguiculata* CCI, plant height, yield and trace elements were not affected by drought stress under field conditions (Chapter 3). Fresh fresh mass was reduced under rain shelter conditions and other measured parameters showed a trend of increase with increase in water application up to 60% ETc with no further increase observed beyond this point (Chapter 4). General results were consistent for *V. unguiculata* even under rain shelter environment. *Corchorus olitorius* yield was reduced by water stress under rain shelter (Chapter 4) although it was not significantly affected under field conditions (Chapter 5). The possible explanation to such variation could be due to variation in degree of stress due to periodic rain water additions under field conditions. The results indicate the potential of production of *C. olitorius* in marginal areas although under extreme moisture stress irrigation would be needed to improve yield.

In the field experiment (Chapter 3) the following elements were high in *A. cruentus*: Na and Mn (drought stress-30% ETc), K (medium stress-60% ETc), Ca and P (full irrigation-100% ETc) while no significant differences were observed in *C. olitorius*. In *V. unguiculata*: Mn was high under drought stress conditions. The results were consistent with those of biomass yield. For example in *C. olitorius*, all measured parameters in the field including yield and nutrient quality were not affected by irrigation regimes. Therefore, application of 30% ETc would be more economic in *C. olitorius* since drought stress did not affect biomass yield quality (size, shape, colour, and freshness) and nutritional quality (micro and macronutrients).

Under severe drought conditions, the following were high: Ca in *B. vulgaris*, Ca and Mg in *A. cruentus* and *C. olitorius*. Under medium stress, the following were high: Na, K and Zn (*A. cruentus)*, Zn (*C. olitorius*), P and K (*V. unguiculata)*, Na and Zn (*B. vulgaris)*. The alternating high and low nutrient elements recorded between the most severe water stress (30% ETc) and medium stress (60 ETc) treatments across all crops in this study indicate that although the crops can be grown under drought conditions, irrigation can improve production in some of these vegetables. Similarly, these results mirror those obtained on biomass yield under rain shelter conditions. In addition, the response of ALVs to varying regimes in terms of macro and micro-nutrients under field conditions were similar to field results reported in Chapter 3. Generally, the results were consistent both under the field and rain shelter conditions in terms of nutritional quality hence most crops which produced high yield in the field also had better nutritional quality.

The levels of nutrients in vegetables is reported to be influenced by stages of plant development (Khader and Rama 2003; Modi et al., 2006). However, there is limited information on mineral content at different stages of maturity (Khader and Rama, 2003). In Chapter 5, a follow-up of how mineral content varied with harvest was conducted. Leaf Fe, Zn, Mn, Mg and Ca contents increased as time of harvesting increased from 6 weeks to 8 weeks, with no further change when crops were harvested at 10 weeks in *A. cruentus*, *V. unguiculata* and *B. vulgaris.* In *C. olitorius* on the other hand Fe, Zn, Mn, Mg and Na were higher when the crop was harvested at its early stages (6 weeks) compared to late harvesting (8 and 10 weeks). Apart from plant age or harvesting techniques, there are other factors that can affect nutritional quality such as fertiliser application or soil fertility, environment temperature, plant type, and the production techniques used (Chweya et al., 1997; Nnamani et al., 2009). Information on when the plant has high nutritional value could serve as a cost cutting measure especially for plants harvested numerous times. For example, according to the current study, there will be a need to fine-tune fertisation programme for *C. olitorius* since nutritional content decreases disproportionately with increase in harvests as the plant grows. To make sound recommendations on this aspect, other quality parameters need to be investigated and plant growth analysis monitored from the early days of plant establishment to ascertain the best time/plant age with high nutritional yield.

Postharvest studies (Chapter 6 and 7) provided evidence that certain postharvest management practices can retain nutrient and lead to increased shelf life than others. *Corchorus olitorius* leaves stored at room temperature had a shelf life of 2 days, while 8 days at 4°C and 10 days at 10°C in non-perforated and perforated packaging. Plant phenolics were studied because they are the most widely distributed secondary metabolites that provide strong protective effects against diseases such as cancer, arthritis, emphysema, retinopathy, neuro-degenerative cardiovascular diseases, atherosclerosis and cataracts. The way these bio active compounds respond to stress can be manipulated to ensure quality. The types of packaging used in the current study are costly for smallholder farmers and this necessitates also exploring low cost packaging. Some resource-constrained farmers who cannot afford the electricity expenses and high quality packaging can utilise low cost packaging. Resource poor households who cannot afford packaging can still resort to other means of increasing shelf life such as drying. Natural drying methods such as shade and sun drying from limited water or medium water irrigation conditions proved to increase shelf life of the studied ALVs better than oven drying. The three ALVs studied can grow under severe and medium drought stress and be sun- or shade-dried without significantly compromising major nutritional quality compared to *B. vulgaris* production which was limited by water availability. Phenolic compounds (flavonoids, tannins, phenolic, gallotannins) are produced in high concentrations under suboptimal conditions hence these condition could be altered to optimise production of these compunds. There is potential to optimise preharvest factors such as water or practise deficit irrigation to optimise phenolic compounds. Regulation of water levels and stress duration that favours optimum biomass yield without compromising accumulation of biocompounds provides an opportunity for future research studies on nutritional water productivity.

8.2 Conclusions

The following conclusions can be drawn from this study:

- A review of literature showed that increased research on production, nutrition, processing and marketing still requires attention as it hinders utilisation of ALVs.
- *Amaranthus cruentus* and *B. vulgaris* biomass yield and nutritional quality were reduced due to water stress in both field and rain shelter conditions. Using 60% ETc is suitable for production of *A. cruentus* and *B. vulgaris* var. *cicla. Corchorus olitorius* and *V. unguiculata* were not affected by water stress under field conditions. Use of 30% ETc is recommended for *V. unguiculata* and *C. olitorius* under field conditions.
- In terms of nutritional quality, results were alternatingly higher between the most severe water stress (30% ETc) and medium stress (60 ETc) treatments in all crops. Leaf Fe, Zn, Mn, Mg and Ca increased as time of harvesting increased from 6 to 8 weeks and remained the same after 10 weeks while in *C. olitorius*, Fe, Zn, Mn, Mg and Na were higher when harvested at early harvest (6 weeks) than other harvestings.
- *Corchurus olitorius* phenolic composition and antioxidant properties were affected by postharvest packaging, temperature and storage time. *Corchorus olitorius* leaves can be stored at room temperature for 2 days, or 8 days at 4°C and 10 days at 10°C in non-perforated and perforated packagings.
- Water stressed and medium stressed plants which were shade- and sun-dried retained better gallotannin, phenolic and flavonoid content than treatment combinations that were oven-dried with varying water regimes.

8.3 Recommendations

The following recommendations may be made, based on observations made during the study:

- Owing to plants being exposed to a multiplicity of environmental factors in their growing environment, there is need to conduct more research on the interaction effect of agronomic factors such as water, fertiliser and plant populations for ALVs under study for both field and controlled environments.
- To draw wide-ranging recommendations, it is essential that these experiments be conducted on multiple sites (different regions) over an extended period of time to consider effect of seasonal changes or one can use modelling studies.
- There is need to conduct more research on various drying methods which can be utilised by commercial farmers e.g. freeze drying, solar drying. Various nutritional parameters such as macro and microelements should be tested for various drying methods.
- Further research is required for both commercial and low cost packaging, different storage conditions and temperature of various species of ALVs. Nutritional variation due to packaging, storage and temperature should also be tested for each harvest since ALVs are harvested many times per season.

References

Chweya JA, Nzava AM. 1997. Cat's whiskers. *Cleome gynandra* L. promoting the conservation and use of underutilized and neglected crops. Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute.

Khader V, Rama S. 2003. Effect of maturity on macromineral content of selected leafy vegetables. *Asia Pacific Journal of Clinical Nutrition* 12(1): 45-49.

Modi M, Modi AT, Hendriks S. 2006. Potential role for wild vegetables in household food security: A preliminary case study in KwaZulu-Natal, South Africa. *African Journal of Food, Agriculture, Nutrition and Development* 6(1):1-13.

Neluheni K, du Plooy CP, Mayaba N. 2007. Yield response of leafy amaranths to different irrigation regimes. *African Crop Science Conference Proceedings 8: 1619–1623.*

Nesamvuni C, Steyn NP, Potgieter MJ. 2001. Nutritional value of wild, leafy vegetables consumed by the Vhavhenda. *South African Journal of Science 97*: 51–54.