Irrigation and nitrogen management of African (*Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt) and commercial ginger (*Zingiber officinale* Roscoe)

by

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DECLARATION

I, Auges Gatabazi declare that the research reported in this thesis is based on my original work and it has not been submitted to this or any other institution of higher education. This research is being submitted for the degree Doctor of Philosophy in Agronomy at the University of Pretoria. I further declare that all sources cited or quoted are indicated and acknowledged by a list of references.

SIGNATURES

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Mr A. Gatabazi

DATE: …….

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LIST OF ABBREVIATIONS OR ACRONYMS

ΔS	Change in soil water storage
AG	African ginger
AE	Agronomic efficiency
ASW	Available soil water
ATP	Adenosine 5$^{;}$ - triphosphate
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CG	Commercial ginger
CEC	Cations exchange capacity
CO_2	Carbon dioxide
CV	Coefficient of variation
CSUP	Carbon substrate utilisation profiles
CSU	Carbon source utilisation
DM	Dry matter
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
ET	Evapotranspiration
FAO	Food and Agriculture Organisation of the United Nations
FAOSTAT	Food and Agriculture Organisation of the United Nations Statistics
FC	Field capacity
FIpar	Fractional interceprion of photosynthetically active radiation
g	gram
I	Index
HI	Harvest index
HPO_4^{2-}	Hydrogen phosphate
K^+	Potassium cation
kg	kilogram
LAN	Limestone ammonium nitrate
LAI	Leaf area index

LSD	Least significant difference
MAD	Maximum allowable depletion
MT	Million tonnes
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
N	Nitrogen
NO_3^-	Ammonium nitrate
NUE	Nitrogen use efficiency
PAR	Photosynthetically active radiation
PAW	Plant available water
PO_4^{3-}	Phosphorus as oxyanions phosphorus
PWP	Permanent wilting point
R	Runoff
RCBD	Randomised complete block design
RH	Relative humidity
RH_{min}	Daily minimum relative humidity
RH_{max}	Daily maximum relative humidity
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
T	Treatment
Ta_{max}	Maximum air temperature
Ta_{min}	Minimum air temperature
µm	Micrometer
USA	United States of America
WUE	Water use efficiency
Y	Yield

IRRIGATION AND NITROGEN MANAGEMENT OF AFRICAN (*Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt) AND COMMERCIAL GINGER (*Zingiber officinale* Roscoe)

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ABSTRACT

Medicinal plants are important and valuable natural resources. South Africa is well-endowed with very diverse flora and fauna that include a considerable number of medicinal plant species. Most medicinal plants have gained popularity for the treatment or prevention of various ailments.

Ginger species (*Zingiber officinale* and *Siphonochilus aethiopicus)* are essential natural resources, which provide many useful products for use in food as a spice or as medicine. The two species contain beneficial secondary metabolites useful for treating many diseases and numerous digestive imbalances such as indigestion, vomiting, heartburn, diarrhoea and pregnancy-related nausea. However, the role of different agronomic practices such as irrigation, water stress and nutrient management are crucial for enhancing the yield and quality of ginger species. Due to climate change, rainfall is often less while more erratic, putting more pressure on irrigation resources in agriculture to sustain or even increase food production for a growing population. The major plant factors negatively affected by water limitations are plant

growth, quality and crop yield. The second most constraining factor in plant growth and quality is the lack of plant nutrients. Macronutrients, such as N, P and K are most important in plants to complete their life cycle and play a significant role in the growth and development of plants.

This study investigated the growth, yield and phytochemical profiling of two ginger species under different maximum allowable depletion levels of soil water content and nutrient management. The soil water study was conducted under a rain-shelter at the Experimental Farm on the Hillcrest campus of the University of Pretoria, South Africa. The experiment was laid out in a randomized complete block design (RCBD) with two factors (ginger species and water regimes) and three replicates per treatment. The two experimental factors included the two species of ginger (*commercial and African ginger*) and four water levels (irrigated as soon as 20-25% of available soil water (ASW) was depleted, 40-45% of ASW depleted, 60-65% of ASW depleted and 80-85% of ASW depleted).

Growth and development parameters such as height, leaf number and stem number were evaluated and varied between species and irrigation treatments. Irrigation treatment effects on plant growth and development were dependent on plant species. Leaf area index and fractional interception of photosynthetically active radiation (FIpar) values were higher in African ginger than commercial ginger in both seasons. Scanning electron microscopy images showed that both ginger species had more stomatal pores and open stomata under well-watered than stressed conditions. The study demonstrated that fresh and dry yields were higher for commercial ginger, compared to African ginger. The fresh and dry matter yields for severely water stressed plants were higher for commercial ginger than for African ginger. Water use efficiency in terms of fresh commercial ginger yield was highest for the moderately water-stressed treatment. The severely water-stressed irrigation regime (i.e. 80-85 MAD) resulted in higher production of total flavonoid content, phenolic content and increased antioxidant activity in both species.

The most important region of the soil is the rhizosphere, where the roots of the soil closely interact with microbes in metabolic processes for nutrient uptake, plant growth, and maintaining plant health. The root-associated microbiomes are structured in distinct compartments whose composition are affected by several factors, such as water regime, soil type and plant genotype. Although not a primary aim of this research, we were interested in the effect of the two ginger species and soil water level on microbial diversity and microbial enzymatic activity in the root zone, as it can have a significant impact on soil health. From the PCA and the dendrogram results related to carbon source utilisation, the African ginger treatments tended to group, except for AG60-65, while for commercial ginger the PCA results clustered three of the four treatments together, with CG40-45 being the out layer. In general, the African ginger treatments exhibited a slightly higher microbial richness and abundance, compared to the commercial ginger treatments. Soil microbial enzymatic activity, viz. microbial activity such as ß-glucosidase activity, alkaline phosphatase activity, acid phosphatase activity and urease activity were not significantly affected by species nor water level.

The plant nutrient study was conducted in a glasshouse and had six treatments involving two ginger species (African and commercial ginger) and three N fertilizer rates (0, 200 and 260 kg $ha^{-1}N$). The study demonstrated that the growth parameters (height, tiller and leaf number) of *Z. officinale* and *S. aethiopicus* were significantly affected by N fertilization rates. Commercial ginger exhibited higher measures of plant height, several tillers, chlorophyll content and leaf number than African ginger. Higher nitrogen levels increased the percentage open stomata in both ginger species. The fresh and dry yield of commercial ginger were higher, compared to African ginger.

The study established that ginger species vary in respect to morphological and physiological adaptive mechanisms, secondary metabolites produced and soil microbial diversity in response

to various water regimes. The adaptive mechanisms probably ensure the species survival under severe conditions and allow the species to produce relatively high yields and secondary metabolites. Detailed knowledge of these mechanisms for the two ginger species could be useful in the improvement of the plants for future breeding initiatives.

In conclusion, this study has produced much-needed information on the response of commercial and African ginger to irrigation and nitrogen management practices. This will promote the successful cultivation of endangered medicinal plant species facing extinction from over-harvesting pressure in their natural habitats, and thereby help conserve plant biodiversity.

Keywords: *African ginger, antioxidant, commercial ginger, flavonoid, irrigation, maximum allowable depletion levels, microbial diversity, nitrogen fertiliser levels.*

CHAPTER 1

GENERAL INTRODUCTION

1.1. BACKGROUND

Plants could be important sources of medicine and therapeutics for maintaining good health and conditions. South Africa is well-endowed with very diverse flora and fauna that include a considerable number of medicinal plant species (Chinsamy *et al.,* 2011). The majority of South Africans still rely on medicinal plants as they are considered safe, easily accessible and affordable, especially within rural communities. This is in contrast to the high cost of modern drugs, inaccessibility of modern health services and cultural acceptability of modern medicine (Elujoba *et al.,* 2006). Most medicinal plants, even today, are collected from the wild, and the continued over-harvesting and exploitation of many traditional medicinal plants have become a threat to the country's species diversity with a loss in the population of many species in their natural habitat. As a result, many medicinal plant species are endangered and extinct in the wild (Okigbo *et al.,* 2008).

Most medicinal plants have gained popularity for the treatment or prevention of a variety of aliments. Natural products have proven to be the most abundant source of medicinal compounds. Many drugs, including aspirin and morphine, have been derived from medicinal plant species (Rafieian-Kopaei, 2012). Conservation strategies, including cultivation of these medicinal plants, are urgently needed to ensure their availability to the industry as well as to the people associated with traditional medicine systems. Optimization of cultivation practices such as irrigation, integrated pest management, nutrient management and optimized plant densities can comprehensively contribute to meet the ever-increasing demand.

The family Zingiberaceae is well-known for its medicinal value and is distributed worldwide (Elujoba *et al.,* 2006). Ginger species *Zingiber officinale* and *Siphonochilus aethiopicus* are important natural resources, which provide many useful products as food ingredients, spices and medicines (Basak *et al.,* 2010; Makunga *et al.,* 2008). The two plants species contain beneficial secondary metabolites useful for treating many diseases and numerous digestive imbalances such as indigestion, vomiting, heartburn, diarrhoea and pregnancy-related nausea (Shukla & Singh, 2007). The Zingiberaceae members have also been used as antioxidants, and as anti-inflammatory and anti-coagulant agents and to lower cholesterol (Asif, 2015). Phenolic and flavonoid compounds have been described as markers of biotic and abiotic stress tolerance in plants. Abiotic stress induces oxidative damage to plant cells due to increasing noxious reactive oxygen species (ROS) in the chloroplasts. However, phenolics and flavonoids have been recognised to be involved in the relieve of oxidative stress caused by ROS (Yildz-Akta et al., 2009; Quan et al., 2016). Assessment and cultivation of these plant species are essential to demonstrate their potency as medicinal plants and potential to be conserved for utilization in several therapeutic formulations.

1.2 AIMS

The study aimed to investigate the growth, water use, yield, quality and water use efficiency of two ginger species under different maximum allowable depletion levels of soil water content and nutrient management.

1.3 OBJECTIVES OF THE STUDY

- To assess the growth and yield response of two ginger species to different soil water regimes.
- To determine ginger species quality (total flavonoid, phenolic and antioxidant contents) under different soil water regimes.

- To evaluate the effects of water regimes on soil microbial diversity for two ginger species.
- To investigate growth and yield of two ginger species in response to nitrogen level..
- To examine quality (total flavonoid, phenolic and antioxidant contents) of two ginger species in response to nitrogen level.

1.4 STRUCTURE OF THE THESIS

The thesis is structured as follows:

Chapter 1: This chapter contains the general introduction of the study, outlining the aim and objectives of the study.

Chapter 2: This section provides the literature review of the study, indicating the importance of the two ginger species, and the effects of agronomic production practices, water availability, nutrient uptake and secondary metabolites on the ginger species.

Chapter 3: This chapter demonstrates the growth and yield response of the two ginger species to four water levels.

Chapter 4: This chapter covers the effect of water stress on antioxidant properties and yield parameters of the two ginger species in response to harvest period.

Chapter 5: This chapter provides results on the effect of four water levels on soil microbial diversity properties for the two ginger species.

Chapter 6: This chapter describes growth and yield response of the two ginger species to N fertiliser levels.

Chapter 7: This chapter reports on the total flavonoid, phenolic and anti-oxidant contents in two ginger species in responses of nitrogen fertiliser levels.

Chapter 8: This chapter contains the general conclusion and rommendations of the study.

CHAPTER 2

LITERATURE REVIEW

2.1. OVERVIEW OF MEDICINAL PLANTS

Medicinal plants form an important part of the pharmaceutical, agriculture and food industries. Globally, many populations (mainly rural societies) still rely on medicinal plants for traditional treatment of numerous diseases (Busia, 2005). This is attributed to the high cost of modern drugs, inaccessibility of modern health services and cultural acceptability of traditional medicine (Maroyi, 2011). Knowledge on utilization, management and conservation of the variety of medicinal plants differ according to localities as many users have developed their way of medicinal plant collection (Phondani *et al*., 2014). Recently the emerging trend in research has been a worldwide intensification to investigate the biological activities of medicinal plants and their role in the treatment of diverse diseases. The proper medicinal use and value of a variety of plants are well known, but many still need to be explored (Fenell *et al.,* 2004). Africa is endowed with many medicinal plant species that are used to manufacture and treat many ailments (Farombi, 2003). Commercial ginger (*Zingiber officinale*) and African ginger (*Siphonochilus aethiopicus*) are members of the Zingiberaceae family regarded as important medicinal plants (Surh, 2002; van Wyk *et al.,* 1997). The Zingiberaceae is prominent family, well-known for its medicinal properties, distributed widely throughout the world as critical natural resources, which provide many useful products for use in food, and as spices and medicines.

2.2 BOTANICAL CLASSIFICATION AND USES OF THE TWO GINGER SPECIES

2.2.1. African ginger (*Siphonochilus aethiopicus*)

African ginger (*Siphonochilus aethiopicus*) is indigenous to South Africa, and the plant is distributed through different regions of Mpumalanga, KwaZulu-Natal and Limpopo provinces of

the Country (Hankey & Reynolds, 2002). The plant has numerous medicinal applications for various ailments including chest ailments, treating of malaria, headaches, asthma and colds (Opoku *et al.,* 2000). The enormous medicinal value of African ginger has resulted in the exploitation and over-exploitation of the species. It is estimated that approximately 2 tons of *S. aethiopicus* are traded annually in regions of KwaZulu-Natal province alone for R450.00 per kilogram (Mander, 1998). The plant has deciduous leaves that resemble *Zea mays* (Figure 2.1B) and cone-shaped rhizomes (Figure 2.1C). The plant also has a purplish flower a the yellowish spot in the middle, which only opens for one day (Figure 2.1A). African ginger is propagated vegetatively using rhizomes and is cultivated mainly and the warmer regions of South Africa. The rhizomes are harvested 6 to 10 months after planting, as the yield and quality of the rhizomes are then at their highest.

African ginger (*Siphonochilus aethiopicus*) has traditionally been used for the treatment of coughs, colds, asthma, headaches, pain, inflammation and malaria (van Wyk, 2008). The rhizome extracts contain anti-bacterial, anti-inflammatory and anti-malarial properties (Light *et al*., 2002; Gericke, 2001; Lategan *et al.,* 2009; Verotta & Rogers, 1997). The rhizomes and roots serve as a good source of spice, and to treat diarrhoea and stomach infections in East Africa (Burkill, 2000). In Nigeria, the rhizomes of *S. aethiopicus* serve as spice and flavour to enhance yam (Igoli *et al.,* 2012). Traditionally, a mixture of rhizomes and roots has been reported to be used to treat hysteria and relieve dysmenorrhoea (Igoli *et al.,* 2012). The plants also contain essential oils in the roots and rhizomes, which is used for its aroma and to flavour spices (Viljoen *et al.,* 2002). The essential oils are also in great demand in the pharmaceutical and food industries as a flavouring and food condiment. They are also used in liquors, concentrates in non-alcoholic drinks, and perfumery.

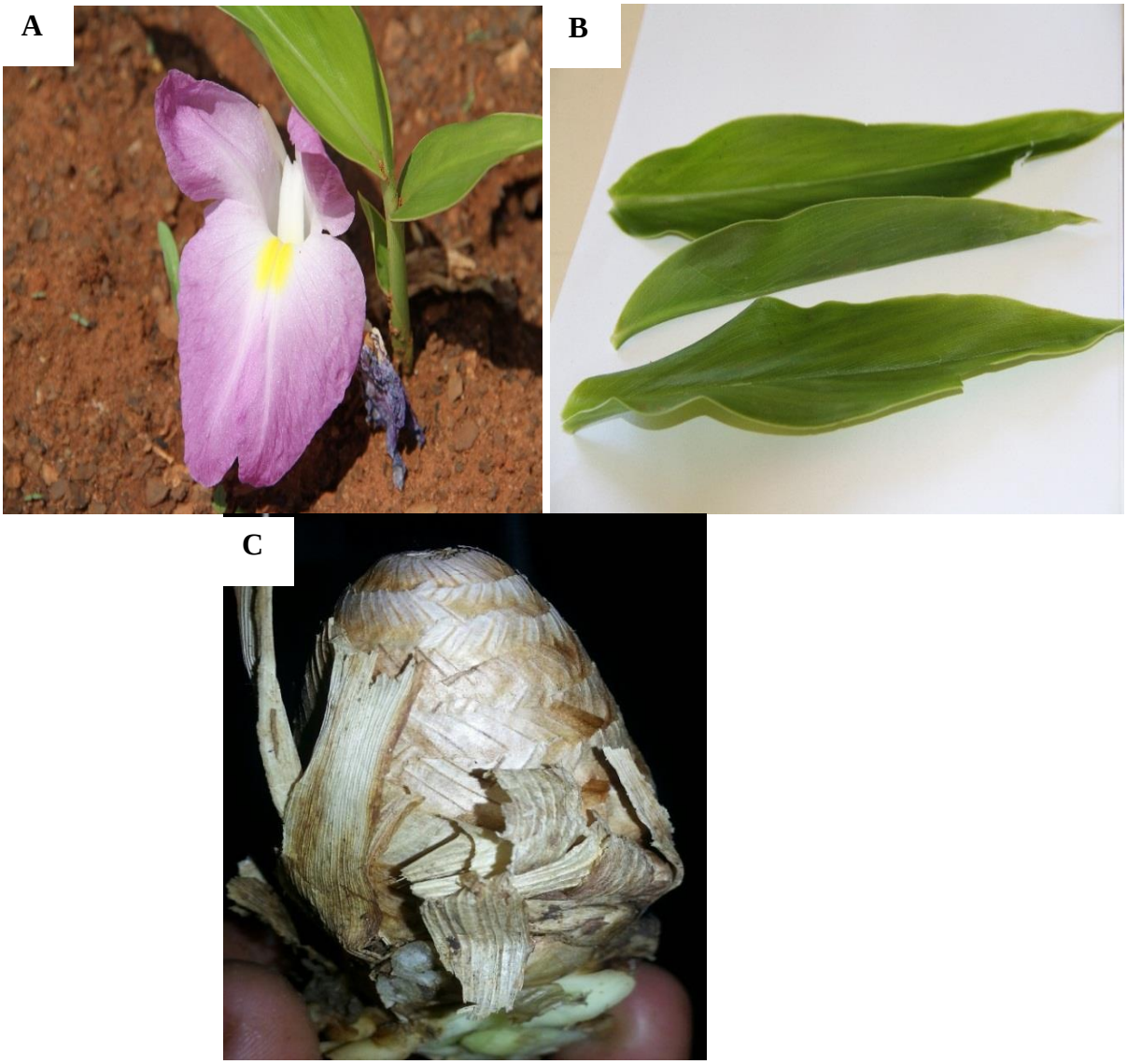


Figure 2-1: African ginger (A) flower, (B) leaves and (C) cone-shaped rhizome.

2.2.2. Commercial ginger (*Zingiber officinale*)

Commercial ginger (*Zingiber officinale*) is an erect plant that has many fibrous roots and a pseudo stem (shoots). The plant has deciduous leaves (Figure 2.2A), and rhizomes (Figure 2.2B) are borne underground. The fibrous roots of the plant have been reported to increase in plant growth (Malu *et al.*, 2009). Commercial ginger is propagated vegetatively from small pieces of rhizome (Babu

et al., 1992). The plant can be cultivated under irrigation, or rain-fed conditions and can be harvested from 6 to 10 months after planting for optimum yield and quality.

Commercial ginger is a horticultural plant, and it has been used as a medicinal plant, spice and daily dietary vegetable worldwide (Ravindaran *et al.*, 1994, Kackar, *et al.*, 1993; Singh *et al.*, 2015). The plant has the potential to be used as a cash crop and is widely used in the manufacturing industry as an ingredient for the development of numerous products (Kizhakkayil & Sasikumar, 2011). The plant has the potential to treat headaches, colds and nausea (Mishra *et al.*, 2012; Ravindaran *et al*., 1994). The medicinal potency of this species is attributed to its high antioxidant and phenolic compounds content (Karimi *et al*., 2010). The rhizomes are juicy with a mild taste and can be used in making tea infused with honey for flavouring (Agrahari *et al.,* 2015). The rhizomes also contain essential oils such as gingerol and zingiberene, which are known to prevent skin cancer and ovarian cancer (Ahmed *et al.*, 2012; Akram *et al.*, 2011). Previous studies reported on the potency of Z*. officinale's* anti-inflammatory, and anti-hyperglycemic properties, and its ability to stimulate appetite (Singab *et al.*, 2014). Dried rhizome powder is reported to contain chemicals such as proteins (10%), starch (40 to 60%), fibres (5%), fats (10%), inorganic material (6%), oleoresin (1 to 4%) and up to 10% of residual moisture (Verma & Bordia, 2001).

Figure 2-2: Image of commercial ginger tillers and rhizomes

2.3. GINGER SPECIES USED AND CURRENT PRODUCTION LEVELS WORLDWIDE

Commercial ginger (*Z. officinale*) is ranked the number one spice in the world, with cultivation estimated at 1 620 493 tonnes worldwide in 2012 (FAOSTART, 2010; FAO, 2012). India is the leading *Z. officinale* producer with about 655 000 t, followed by China with 450 686 t, Nepal (276 150 t), Indonesia (266 145 t), Thailand (164 406 t), Bangladesh (77 000 t), Japan (59 302 t), Nigeria (58 190 t), Cameroon (52 840 t) and the Philippines (27 197 t) (Figure 2.3). The total export of *Z. officinale* in 2009 was estimated at 491 408 t, with a monetary value equivalent to 406 million USD. China is regarded as the top *Z. officinale* exporter worldwide with a 69.3% share (FAOSTAT, 2010).

African ginger is grown in many African countries and is considered important in different regions of South Africa, Zimbabwe, Swaziland, Malawi, and Zambia (van Wyk *et al.*, 1997).

African ginger is a popular plant species that is widely used throughout South Africa. The plant is in danger of extinction due to over-harvesting (Manzini, 2005). Lack of appropriate cultivation skills of this plant has led to plant scarcity as it does not regrow after being harvested destructively

(Anon., 1998). As a result, the plant species face the risk of total extinction and is listed on the Red List of South African endangered plant species (Semenya & Maroyi, 2019).

African ginger is regarded as one of the most important medicinal plant species in South Africa. It is one of the most important medicinal species with a long history of traditional healer use and one of the most popular of all traditional medicinal plants in South Africa. However, much attention has been paid to the chemistry and biological activity of the plant due to the healing properties of the rhizomes and roots for a variety of ailments including coughs, colds, asthma, headache, candida, and malaria (Street & Prinsloo, 2012; Lauw *et al*., 2002). The plant has also gained popularity in Nigeria, where a variety which has purple flowers and white corolla tubes is grown. New areas for ginger species cultivation are increasing in areas of Ethiopia but mainly under small-scale production. The production of ginger species in different parts of Ethiopia covers an area of 381 ha with an annual production of 1869 t (FAO, 2007).

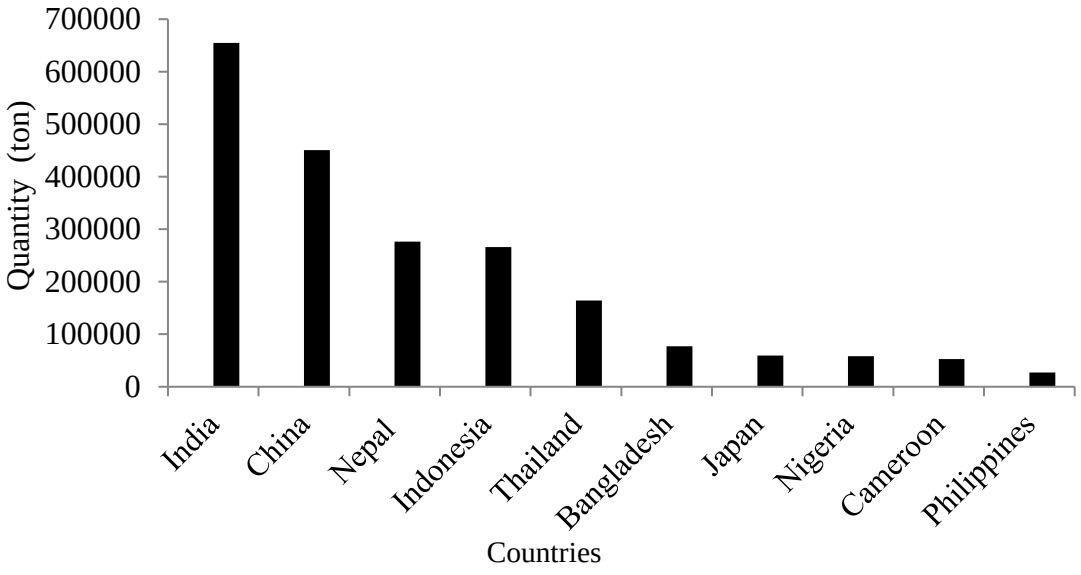


Figure 2-3: Top ten countries producing *Zingiber officinale* (FAOSTAT, 2010).

2.4. SECONDARY METABOLITES IN GINGER SPECIES

Commercial ginger has been reported as a plant rich in secondary metabolites, specifically flavonoids and phenolic compounds, indicating possible pharmacological activity (Ghasemzadeh *et al.*, 2010). The rhizomes are mostly preferred due to the higher content of flavonoids in the rhizomes compared to the tillers and leaves (Ghasemzadeh *et al*., 2012). According to Ghasemzadeh *et al*. (2010), flavonoids play an essential role as antioxidants by scavenging free superoxide radicals, with the potential to reduce cancer risk. Flavonoid compounds are very important in human health as well as in plant physiology, where they act as antimicrobial and preventing free radical damage to biological molecules such as proteins, lipids and DNA. (Ververidis *et al.*, 2007). The antioxidants from flavonoids play an important role in preventing free radical damage to biological molecules such as proteins, lipids and DNA. Damage to biological molecules can,, cause severe diseases such as cancer, diabetes, and cardiovascular problems (Atmani *et al.*, 2009). Furthermore, they are important in plant pigmentation, synthesised by phenylalanine (Havsteen, 2002). The composition of phytochemicals and flavouring content of *Z. officinale* ginger differ with the variety, type and agronomic practices (Agrahari *et al.*, 2015).

Zingiber officinale contains many sources of phenolic compounds, such as shangaol, gingerol and has antioxidant properties (Surh, 2003). Phenolic compounds play an important role in protecting plants against stress conditions, infections and ultraviolet radiation. Phenolics and flavonoids play an important functional role as free radical scavengers and quenchers of singlet oxygen formation. Due to their important roles in plant and human health, it would be useful to have a better understanding of flavonoid concentration and biological activities. That could indicate their importance as therapeutic agents and medicinal herbs (Ghasemzadeh & Ghasemzadeh, 2011). They are crucial for plant growth and reproduction, and are produced as a response to

environmental factors such as light, chilling and pollution (Valentine et al., 2003). Kreps et al. (2002) reported that high irradiation and cold stress can lead to elevated levels of flavonoids in plants.

Selmar & Kleinwachter. (2013) reported that medicinal plants grown under water deficiency conditions reveal much higher concentrations of secondary metabolite products compared with identical plants of the same species cultivated with ample water supply. Water shortage induces drought stress-related metabolic responses and, due to stomatal closure, the uptake of CO_2 decreases significantly. As a result, the consumption of reduction equivalents ($NADPH + H^+$) for CO_2 fixation via the Calvin cycle declines considerably, generating a large oxidative stress and an over supply of reduction equivalents. As a consequence, metabolic processes are shifted towards biosynthetic activities that consume reduction equivalents. Accordingly, the synthesis of reduced compounds, such as phenolics, flavonoids and antioxidants are enhanced. Due to the stress-related induction of superoxide dismutase (SOD) and ascorbate peroxidase (APX), superoxidase radicals are detoxified and thus production of large amounts of Reactive Oxgen Species (ROS) is prevented. The strong increase in the reduction potential (ratio of $NADPH + H^+$ to $NADP^+$) enhances, according to the law of mass action, the synthesis of highly reduced natural products (Figure 2.4).

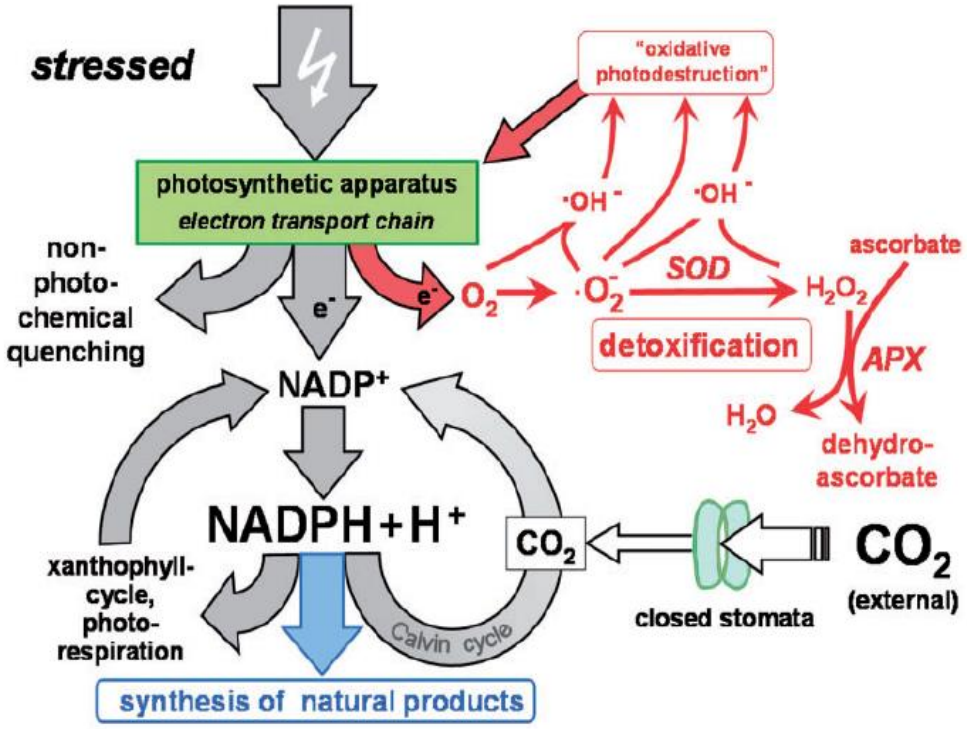


Figure 2-4: Mechanism of synthesis of secondary metabolites due to water stress (Selmar & Kleinwachter, 2013)

Flavonoids and phenolics are specific compounds that play the role of protecting humans, plants and animals against cell-damaging effects of free radical reactive oxygen species (ROS). The antioxidant and free radical imbalances may result in cellular damage and cause oxidative stress (Kukić *et al*., 2006). In the *Z. officinale* plant, antioxidant activities are found mainly in the rhizomes and leaves. It was found that increasing CO_2 concentrations in a plant may increase antioxidant activities (Ghasemzadeh & Jaafar, 2011). They also play a significant role in food preservation due to the free radicals formed when exposed to light, air and temperature (Ghasemzadeh & Jaafar, 2011). Antioxidants are carotenoids and are necessary for protecting organisms and cells against adverse effects of light and air.

Antioxidants and flavonoids are found in the chloroplast, and play a role as scavengers of singlet oxygen and stabilizers of the chloroplast outer envelope membrane. Antioxidants and flavonoids may effectively control key steps of cell growth and regulation of the development of the whole plant and individual organs (Giovanni et al., 2012). Most phenolic and flavonoid compounds, which are often accumulated in the vacuoles of plant cells, are glycosides (Ghasemzadeh & Ghasemzadeh, 2011).

Secondary metabolites are responsible for biological properties and antimicrobials in the treatment of different diseases (Silva & Fernandez, 2010). Bao *et al.* (2010) reported that *Z. officinale* inhibited bacterial colony growth and therefore has antimicrobial properties. The rhizomes of *Z. officinale* are also used as medicine and for pharmacological purposes, including anticoagulation, anticancer, and cardiovascular agents (Agrahari *et al.,* 2015). *Zingiber officinale* compounds can eliminate oral pathogens at a minimum bactericidal concentration (Park *et al.,* 2008). The chemical composition and antimicrobial activities of fresh and dry *Z. officinale* oil on *Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, Aspergillus niger, Saccharomyces cerevisiae, Trichoderma spp,* and *Pencillium* spp, have been investigated by Sasidharan & Menon (2010). Sasidharan & Menon (2010) also showed that *Z. officinale* oil could be used for the treatment of many bacterial and fungal diseases. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities by Habsah *et al.* (2000), also demonstrated antifungal activities against *Aspergillus ochraceous*. Similarly, Bellik (2014) reported antioxidant activity and antimicrobial potency of essential oleoresin oil of *Z. officinale* against pathogens, including *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus*

African ginger is an equally important medicinal plant that contains health-promoting compounds and functional properties which can prevent and treat several health conditions. Previous studies

have indicated that various extracts of African ginger possess a wide range of pharmacological properties such as antimicrobial, anti-inflammatory and anti-candida properties (Light *et al.,* 2002). Holzapfel *et al.* (2002) isolated chemical compounds that include phenolics and flavonoids type from the plant, while Viljoen *et al.* (2002) reported siphonochilone content of up to 0.2% of the dry weight of the *Siphonochilus* plant.

2.5 ROLE OF NUTRIENTS IN CROP PRODUCTION

Enhancing plant nutrients for increased crop production is a very important tool in agriculture and the foundation of sustainable food production (Bryan *et al.,* 2009). Fertiliser application either through the basal or foliar application is important for increasing plant yield and quality. Macronutrients, such as N, P and K are most important in plants to complete their life cycle and play a major role in the growth and development of plants. Nitrogen is taken up as ammonium (NH_4+) or nitrate (NO_3^-), phosphorus as oxyanions phosphorus (PO_4^{3-}) and K in the form of K^+ as a free cation (Maathuis, 2009). The three elements have been reported to increase growth, quality and yield of various plant species (Tripathi *et al.,* 2014). Nitrogen is a nutrient that mostly limits plant growth, as it plays a significant role in plant regulatory and metabolic processes (Hashmi *et al.,* 2010). Nitrogen regulates plant metabolic processes and is critical to synthesize amino acids, which are the building elements of protein, nucleotides, chlorophyll, and numerous other metabolites and cellular components (Nunes-Nesi *et al.,* 2010). The application of N fertilizers at the correct recommended rate and timing has the potential to improve growth and yield (Chaturvedi, 2006).

Biosynthensis of secondary metabolites in medicinal plants is strongly influenced by several factors such as environmental conditions, agricultural management, harvest time, water and nutrient availability. Among these different factors, nitrogen fertilizer plays a key role in

controlling the concentration of secondary metabolites (Sugiharto & Sugiyama, 1992). The lack of plant nutrients may cause alteration in flavonoid and antioxidant content and composition in several crops. For example Tavarini et al. (2015) reported that an optimum N rate of 150 kg ha^{-1} increased antioxidant content in *Stevia rebaudiana* Bertoni,

Phosphorus is essential for biological growth and development (Rodríguez & Fraga, 1999). It is a key macronutrient element that plays a role in controlling enzyme reactions in the metabolic pathways and is part of several important molecules such as phospholipids, adenosine triphosphate (ATP) and nucleic acid synthesis (Takehisa *et al.,* 2013). It plays a role in different metabolic processes, including energy generation, photosynthesis, glycolysis, membrane synthesis, redox reactions, and nitrogen fixation (Schachtma *et al.,* 1998). According to Hinsinger (2001), most plant species prefer P at the pH 4.5 to 5.0 in the form of dehydrogenise phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{2-}) although their occurrence in the soil is at low concentrations. The dissolution-precipitation of P bearing minerals, hydrolysis of organic matter and adsorption-desorption are main factors that control the amount of available P in the soil (Maseko & Dakora., 2013). Ullrich & Novacky (1990) reported that at a rate of 2 mm. h^{-1}, P moves simplistically from the root surface to the xylem.

Potassium is an important element in plant nutrition and is assimilated by roots in large quantities and later distributed to plant cells. Potassium plays a significant role in the metabolism processes such as photosynthesis, osmoregulation, maintenance of the plasma membrane of proteins and turgor driven movement (Gierth & Mäser, 2007). Due to plant stress factors such as light intensity, nutrient limitation, heat and drought, the damage to plants may catalyse reactive oxygen species (ROS). It is known that potassium can nutritionally reduce ROS production by lowering the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and maintain

photosynthetic electron transport (Cakmak, 2005). As a nutrient, it is needed for physiological processes, such as stress signalling, and translocation of photo assimilates needed for good crop quality (Römheld & Kirkby, 2010). According to Gairola *et al.* (2009), potassium increased chlorophyll content, leaf area index and percentage dry weight of Swiss chard. The deficit in nitrogen, phosphorus and potassium have a negative impact on general soil fertility levels, and this can reduce crop yields (Postma & Lych, 2011).

2.6 ROLE OF WATER IN CROP PRODUCTION

Global irrigation water demand has increased as irrigated agriculture plays an important role in increased food production (Boutrea, 2010). Irrigation water demand can be regarded as a derived demand for food, and this requires good management of existing water supplies in order to meet world food demand (Carruthers *et al.,* 1997). Wallance (2000) reported that agriculture is the largest user of fresh water. Approximately 10 to 30% of the available water, including ground water and rainwater, is used globally by plants for transpiration. Deficit irrigation through the application of water below full crop water requirement (evapotranspiration) can be used as a method of minimizing irrigation water use in the agriculture sector (Fereres & Soriano 2007). It is a strategy to prevent the soil water deficit falling below the threshold level of a particular soil and crop (Ritchie *et al.,* 1990). One way to managing irrigation is to measure soil water content. The available soil water content refers to the water in the soil profile, which can be used by plants (Hanson *et al.,* 2000). The objective of scheduling irrigation is to irrigate, especially during sensitive growth periods in order to maximised crop yield (Reddy & Reddy, 1993). Irrigation scheduling has the purpose to either partially or fully provide the required amount of water to increase crop quality and yield (Wiedenfed, 2004).

Water use efficiency is the ratio of biomass accumulation, expressed as carbon dioxide assimilation to water consumed by the plant expressed as transpiration or evapotranspiration over the growing season (Fischer, 2014). Thus, a reduction in evapotranspiration and runoff losses can significantly contribute to improving WUE (Fischer, 2014). This is especially of importance in arid and semi-arid areas where water resources are limited (Webber *et al.,* 2006; De Pascale *et al.,* 2011). Water limitations can cause major abiotic stresses, which may decrease plant growth, quality and yield of crops (Waraich *et al.,* 2011b). A study by Azhar *et al.* (2011) demonstrated that water stresses altered growth, physiology and secondary metabolites of Desi Ajwain (*Trachyspermum ammi* L.). Physiological parameters, such as transpiration and stomatal conductance, have been reported to decrease significantly with increasing water stress levels (Shao *et al.,* 2008). Decreasing of growth parameters such as plant height, flower yield, shoot weight and apigenin content of Chamomile (*Matricaria recutita* L.) have and impact on secondary metabolites due to drought stress (Baghaliana *et al.,* 2011). Water deficit decreased net photosynthetic rate, stomatal conductance and biomass production of *Glycyrrhiza uralensis;* however, water deficit did not affect root biomass (Li *et al.,* 2011). Jaafar *et al.* (2012) reported increased total phenolics, flavonoids and anthocyanin content under severe water stress of Kacip Fatimah (*Labisia pumile*). Previous reports by Ghasemzadeh *et al.* (2010a) and Mokgehle *et al.* (2017) showed an increase in total phenolic and flavonoid content in the leaves of *Labisia pumile* and African ginger, respectively. The increase in most of the secondary metabolites such as phenolics and flavonoids is due to fertiliser and water content in the plant. The increase in secondary metabolites with nitrogen application occurs because of the stimulating effect of nitrogen on leaf growth, which positively affects photosynthesis and secondary metabolite production.

Drought stress can result in an unbalance between the production of antioxidants and reactive oxygen species (ROS) and may decrease antioxidant content in the plant (Waraich *et al.,* 2011). Drought stress has a negative effect on the physiology and plant growth due to cell membrane shrinkage followed by plant cell death if the drought persists (Ashraf & Foolad, 2007). Drought stress may inhibit photosynthesis by affecting the opening and closing of stomata with damage to the photosynthetic apparatus and chlorophyll structure (Waraich *et al.,* 2011). Crop production is affected by many abiotic and biotic stresses. However, water deficit is the major abiotic factor that has a significant impact on crop yield (Valliyodan & Nguyen, 2006). Abiotic stress impacts mainly the metabolic and photosynthetic functioning of plant species, which result in reduced yield (Jaleel *et al.,* 2009). Drought decreases the rate of CO_2 assimilation and also change of stomatal conductance (Anjum *et al.,* 2011). Deficit irrigation, where crops are subjected to different water stress levels at different growth stages, may increase economic yield (Reddy & Reddy, 1993). Due to worldwide water resource limitations, partial root-zone drying (deficit irrigation) can be very beneficial in crop production (Shahanazari *et al.,* 2007). The mechanism is beneficial in optimizing or moderating crop yield by applying less water (Ali & Talukder, 2008). Deficit irrigation minimizes the adverse effects of drought on crop yield by maintaining the necessary water level for consumption (Panda *et al.,* 2003). Jain *et al.* (1997) revealed that applying water deficit should depend on crop growth stages. Management of water deficit irrigation is an important strategy for scarce water supplies and an important tool to achieve and minimised water use in agriculture (Fereres & Soriano, 2007). Mulu & Alamirew (2012) reported improved water use efficiency and water productivity without significantly reducing yield by deficit irrigation. Deficit irrigation is imperative for higher yields per unit of irrigation water applied (English & RaJa, 1996).

CHAPTER 3

EVALUATING GROWTH, YIELD, AND WATER USE EFFICIENCY OF AFRICAN AND COMMERCIAL GINGER SPECIES IN SOUTH AFRICA

ABSTRACT

Ginger species play an important economic role as medicinal plants, food flavourings, and dietary supplements. Products from ginger, including oil and fresh and dried rhizomes can be used to treat malaria, asthma, headaches, and act as anti-inflammatory and anti-microbial agents. The cultivation of wild plant species can alleviate the pressure from harvesting from the wild. Under cultivation, the significant constraints on crop yield and quality are water availability and plant nutrition. Therefore, the impact of water stress on commercial and African ginger was assessed in this rain shelter study. Irrigation treatments were based on the maximum allowable depletion (MAD) levels of plant available water in the root zone (T1: 20–25% MAD, the control; T2: 40–45% MAD; T3: 60–65% MAD; T4: 80–85% MAD). As water stress decreased, the plant height and number of stems per plant of both plant species were positively affected. The number of open stomata was higher for well-watered and less stressed treatments in both ginger species. Higher fresh and the dry rhizome yields were recorded for commercial ginger at all water treatments as compared to those from African ginger. In general, water use efficiency (WUE) of fresh and dry rhizome yield was higher for commercial ginger as compared to the indigenous African ginger, while moderately stressed treatments generally resulted in the highest WUE for both species.

Keywords: *Africa ginger, commercial ginger, growth, irrigation, water use efficiency, yield*

3.1 INTRODUCTION

South Africa is characterised by flora that is well known for its abundance in aromatic compounds, however, currently, about 2576 plant species are threatened with extinction due to over-harvesting from the wild (Louw *et al.*, 2002). This includes some species in the genus *Zingiber*, which belong to the family Zingiberaceae (Okigbo *et al*., 2008), African (*Siphonochilus aethiopicus*) and commercial ginger (*Zingiber officinale*) belonging to the family. This genus is one of the most abundant genera, comprising of perennial ornamentals and aromatic herbs that are cultivated for valuable medicines (Mander *et al.,* 2007). African and commercial gingers are considered to be important traditional medicinal plants in southern Africa, which are used for the treatment of a variety of human ailments (van Wyk, 2008). Products, including oil and fresh and dried rhizomes, can be used to treat malaria, asthma, headaches, and act as anti-inflammatory and anti-microbial agents (Okigbo *et al.*, 2009). The trade in traditional medicines in South Africa is estimated to be worth R2.9 billion per year (Mander, 1998). In South Africa, medicinal plants serve as a key in the rural industry and business incubators, with 771 species being harvested from the wild for sale. The production of commercial ginger is well documented in different parts of the world (Dubey *et al.,* 2004). In South Africa, African ginger was identified as one of the species with commercial production potential. It was estimated that 1.9 tonnes of African ginger, totalling about52,000 plants, is annually traded in South Africa and the current situation necessitates an alternative supply of medicinal plant material to meet the demand (van Wyk, 2008).

With climate change and the slow-growing capabilities of some wild medicinal species, the threat of extinction of these species is high, and it has become urgent for research to address the strategies of improving medicinal yield through improved cultivation technologies.

Water is one of the essential role players in crop production, which affect crop yield. Water availability can also affect the quality, and earlier reports have shown that drought stress can increase the concentration of secondary metabolites including ginger (Onder *et al*., 2005; Anjum *et al.,* 2011). The incidences of drought in many sub-Saharan growing areas due to the negative effects of climate change restrict growth and photosynthetic activities of crops (Magadza, 1994). Water shortage affects crops according to crop growth stages and physiological plant processes. Beside physiological responses, plants also undergo morphological alterations, causing changes in the distribution of assimilates, which can reduce vegetative growth and hinder the development of plant reproductive organs (Prasad *et al*., 2008). According to Galmés *et al*. (2007), the ability of plants to survive under varying stress conditions depends on growth stage, intensity and duration of water deficit, andplant species. Crop species growing under variable water supply, especially during yield formation stages, can still produce optimum yields with maximum water use efficiency (WUE) (Reddy & Redy, 1993). There is limited information available on the response of ginger species subjected to varying water stress regimes. Information regarding the plant's response to water stress regimes and drought tolerance mechanisms can help devise appropriate irrigation management strategies and be useful in breeding programmes for the selection of genotypes that can withstand extreme conditions.

Several studies have reported on the role of medicinal plants as agents with anti-microbial and anti-inflammatory properties, and some are used for treating various ailments. Ghasemzadeh *et al*. (2010b) indicated the medicinal potential of Malaysia ginger (Z. *officinale*) varieties through their high antioxidant activities, as well as the total contents of phenolic compounds and flavonoids. Additionally, numerous experimental studies on the growth and yield response of medicinal plants have been investigated. Wilson & Ovid (2008) assessed the growth and yield

responses of ginger (Z. *officinale* Roscoe), as affected by shade and fertilizer applications. Jasim *et al.* (2014) reported that plant growth promoting products had considerable effects on the growth of ginger. A field study on nitrogen and water management reported a higher leaf area index (LAI) under well-watered treatments for Perlargonium sidoides (Mofokeng *et al.*, 2015). Although several studies have reported on the medicinal potential of ginger species, yield, and plant response to growth regulators, there is still limited information available on the response of ginger to water supply, which may significantly affect growth, yield and quality. Furthermore, there is virtually no published information on the cultivation of African ginger. Therefore, the aim of this study was to examine the response of commercial and African ginger to water stress by evaluating their water use, growth, yield, and water use efficiency.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The experiment was conducted under a rain shelter at the Hatfield Experiential Farm of University of Pretoria, Pretoria, South Africa, Hillcrest campus. The experiment was completed during the 2015/2016 and 2016/2017 cropping seasons. The area is located at 25°45'S and 28°16'E with an elevation of 1350 m above sea level.

3.2.2 Soil analysis and fertilizer application

Soil samples were randomly taken from depths of 0-20 cm, 20-40 cm, 40-60 cm and 60-80 cm to assess chemical and physical properties (Tables 3.1 and 3.2). The soil of the experiment site was characterised as a sandy clay loam of the Hutton (Soil Classification Working Group, 1991) form with a clay content of 36% at 0-20 cm soil depth (Table 3.2). Based on the soil sample analysis, phosphorus was applied as basal application at planting at a rate of 170 kg P ha^{-1} (as Superphosphate 8.3%) while nitrogen and potassium were applied at rates of 220 kg ha^{-1} (LAN

28%) and 170 kg ha^{-1} (KCl 50%), respectively for both ginger species. The N and K were applied in a split application of equal amounts at 45 and 90 days after planting. Weather data was obtained from an automatic weather station at the Hatfield Experimental Farm (Table 3.3).

Table 3.1: Chemical properties of different soil layers of the experimental field site

Chemical properties	Soil depth (cm)			
	0-20	20-40	40-60	60-80
pH(H_2O)	6.3	6.6	6.8	6.9
EC (mS/m^{-1})	64	66	28	26
CEC (cmol (+) kg^{-1})	14.7	10.2	9.7	10.7
Organic matter content (%)	0.9	0.8	0.7	0.7
Total N (mg kg^{-1})	19.6	14.6	14.3	13
Available P (mg kg^{-1})	44.6	43.8	4.3	2.3
Available K (cmol (+) kg^{-1})	0.95	0.12	0.09	0.07

NH_4OAc extractable cations; Bray-1 (P); Kjeldahl method

Table 3.2: Physical properties of different soil layers of the experimental field site

Soil depth (cm)	Particle size (%)			Bulk density (g cm^{-3})	FC weight (%)	PWP weight (%)
	Clay	Silt	Sand			
0-20	36.0	2.0	62.0	1.1	0.280	0.159
20-40	36.0	8.0	56.0	1.1	0.302	0.182
40-60	46.0	4.0	50.0	1.0	0.326	0.206
60-80	44.0	8.0	48.0	1.0	0.331	0.213

FC: Soil moisture at field capacity. PWP: Permanent Wilting Point

Table 3.3: Monthly mean weather data measured at the Hatfield Experimental Farm during the two growing seasons

Month	Temperature (^{o}C)		Relative Humidity (%)		Solar radiation (MJ m^{-2})	Wind speed (m s^{-1})	Daily ETo (mm)	Total rainfall (mm)
2015/2016	Max	Min	Max	Min				
November	31.3	15.3	56.0	9.1	19.7	2.3	6.4	22.2
December	32.9	15.3	62.	12.9	19.7	1.9	6.1	51.7
January	30.3	17.7	66.0	21.1	18.0	1.8	5.3	43.6
February	32.1	17.9	65.7	17.8	18.6	1.6	5.4	22.1
March	29.2	16.2	66.9	21.6	15.1	1.2	3.9	16.5
April	27.7	12.7	68.6	18.3	14.4	1.4	3.7	0.6
May	22.3	8.4	69.8	20.4	11.1	1.4	2.7	1.6
June	20.6	5.8	68.5	20.4	10.4	1.2	2.3	0.4
July	20.7	5.8	61.9	12.9	10.9	1.4	2.7	0.1
August	20.2	4.3	66.9	10.2	12.9	1.6	3.2	0
2016/2017								
November	28.7	15.7	68.5	22.4	15.4	2.0	4.8	25.4
December	29.7	16.9	69.3	22.8	17.5	1.9	5.2	62.1
January	28.6	16.6	71.8	26.7	17.0	1.8	4.8	66.3
February	27.9	17.1	71.1	30.5	14.1	1.8	4.2	32.1
March	29.0	14.4	70.2	17.7	15.8	1.7	4.4	22.4
April	31.8	15.3	62.9	12.5	15.0	2.3	4.0	12.9
May	20.2	5.8	84.8	30.7	24.2	1.1	4.1	3.1
June	21.3	5.8	87.2	30.9	21.0	1.5	3.2	1.5
July	22.0	5.9	83.1	28.1	19.2	1.5	3.2	0.5
August	25.1	7.8	70.2	23.7	19.6	2.1	4.2	0

Max: maximum, Min: minimum, ETo: daily reference evapotranspiration

3.2.3 Experimental layout and treatments

The experiment was laid out in a randomized complete block design (RCBD) with two ginger species, four irrigation regimes and three replicate blocks. Each experimental plot was 4 m^2 (2.0 m × 2.0 m) in size, with eight plant rows per plot, and a 1 m spaces between the blocks. The spacing between plants rows was 0.25 m and 0.25 m within rows, giving a plant population of 64 plants per 4 m^2 (160 000 plants ha^{-1}), and the two ginger species used were commercial (*Zingiber officinale*) and African ginger (*Siphonochilus aethiopicus*). The water treatments were as follows.

- Irrigation treatment 1 (T1- Control): soil refilled to field capacity when 20-25% of available soil water (ASW) was depleted.
- Irrigation treatment 2 (T2): Refilled to field capacity when 40-45% of ASW was depleted.
- Irrigation treatment 3 (T3): Refilled to field capacity when 60-65% of ASW was depleted.
- Irrigation treatment 4 (T4): Refilled to field capacity when 80-85% of ASW was depleted.

Table 3.4: Ginger species and irrigation treatment combinations used in the experiment

No	Treatment	Description
1	CG + T1	Commercial ginger + 20-25% MAD
2	CG + T2	Commercial ginger + 40-45% MAD
3	CG + T3	Commercial ginger + 60-65% MAD
4	CG + T4	Commercial ginger + 80-85% MAD
5	AG + T1	African ginger + 20-25% MAD
6	AG + T2	African ginger + 40-45% MAD
7	AG + T3	African ginger + 60-65% MAD
8	AG + T4	African ginger + 80-85% MAD

MAD: Maximum allowable depletion, AG: African ginger, CG: Commercial Ginger

3.2.4 Planting material collection and trial establishment

Commercial and African ginger rhizomes were acquired from Fortuna Company and Agricultural Research Council (ARC) in South Africa, respectively. The rhizomes of both plant species were stored for three months at room temperature (±25 °C) until they were transplanted into the field. The obtained ginger rhizome sizes ranged from 20 to 30 g and were planted by hand at a depth of 7 cm. General pest and disease management practices were followed when necessary. Weed management was done manually throughout the growing season. During trial establishment, the plots were irrigated by using overhead sprinkler irrigation for 1 hour. Thereafter, all plots were irrigated to field capacity, every time the predetermined soil water deficit per treatment was reached.

3.2.5 Soil water monitoring and irrigation scheduling

A 503DR CPN Hydro Probe neutron water meter (Campbell Pacific Nuclear, California, USA) that was calibrated for the experimental site was used to measure the soil water content. Access tubes were installed in the middle of each plot and readings were taken at 0.2 m intervals to a depth of 0.8 m.

Irrigation scheduling was based on the measured depletion of available soil water (ASW). The ASW was considered as the difference between root zone water storage at field capacity and the permanent wilting point. The effective root zone of ginger plants was estimated to be 0.40 m, and although water contents to a depth of 0.8 m were recorded, irrigation was based on ASW depletion of the effective root zone. The depleted percentage of ASW (θd) was calculated using Equation (1) (Panda *et al.*, 2003).

$$Depletion\ of\ available\ soil\ water = 100\frac{1}{n}\sum_{1}^{n}\frac{FC_i - \theta_i}{FC_i - PWP_i} \qquad (1)$$

where n is the number of layers of the actual rooting depth used in the soil water content measurement, *FCi* is the soil water content at field capacity for ith layer, θ_i is the soil water content in the ith layer, and *PWPi* is the soil water content at permanent wilting point on volume basis.

3.2.6 Growth parameters

Data on growth was collected on a monthly basis, and eight plants were randomly selected and tagged as data plants. Parameters, including plant height, number of stems per plant, and number of leaves per plant were measured every month from 100 days after planting to 240 days after planting.

Leaf area index and canopy interception of photosynthetically active radiation (PAR) were measured once a month while using a ceptometer (Accupar model LP-80, Decagon Devices Inc., Pullman, WA, USA). PAR measurements were collected from above and below the canopy. In order to calculate the fractional interception (FI) of PAR, the ratio between the above canopy and below canopy PAR measurement was used, as indicated in Equation (2) (Fan *et al.*, 2014).

$$FIPAR = 1 - (\frac{PAR\ below\ canopy}{PAR\ above\ canopy}) \qquad (2)$$

3.2.7 Yield parameters

Data on yield parameters was collected at 240 days after planting. The plants from each plot were carefully dug up, and rhizomes were separated from leaves and stems. Fresh rhizome mass was measured directly after harvest, and dry matter yield was determined after cutting into thinner slices to facilitate good drying, leaves and stems were oven dried at 50 °C for 72 hours.

3.2.8 Water use and water use efficiency

Crop water use or evapotranspiration (ET) was determined by using the soil water balance of equation (Tesfaye *et al.,* 2006), as follows:

$$ET = I + P - D - R - \Delta S \qquad (3)$$

Where, ET refers to crop water use/crop evapotranspiration (mm), I represents irrigation (mm), P represents precipitation (mm), D refers to drainage (mm), R is the surface runoff (mm), and ΔS is the change in soil water storage between planting and harvest (mm). Runoff and drainage were assumed to be negligible, as irrigation was carefully managed to prevent over-irrigation or runoff and rain was excluded by the rain shelter.

Water-use efficiency (WUE) was calculated as yield divided by the volume of irrigation water used, using the following equation (Tesfaye *et al*., 2006);

$$WUE = \frac{Y}{\sum ET} \qquad (4)$$

where WUE is the water use efficiency in kg ha^{-1} mm^{-1}, Y represents the yield in kg ha^{-1}, and ∑ET is the total seasonal water use in mm.

3.2.9 Scanning electron microscopy (SEM) parameters

The response of the plants to irrigation regimes in terms of stomata number and the number of open stomata were collected and analysed from scanning electron microscope images, following the method that was described by Eiasu *et al.* (2007), with slight modifications. A leaf sample (10 mm × 10 mm) was cut from the third leaf from the top of the plant and then fixed in 3% w/v aqueous solution glutaraldehyde (0.05 M phosphate buffer at pH 7.0) immediately after cutting. The samples were then thoroughly rinsed with distilled water, and the procedure was repeated three times. Thereafter, the samples were post-fixed in osmium tetraoxide (1% m/v) and incubated for two hours. Samples were further dehydrated into a series of ethanol concentrations of 30, 50,

70, 90, and 100% (m/v) for 15 min and then dried in a critical point drying apparatus (Bio-Rad E300, Watford, UK). The dried samples were mounted on copper stubs and coated with gold in a vacuum coating unit (Polaron E5200C, Watford, UK). The samples were then observed under a JSM 840 scanning electron microscope (JEOL, Tokyo, Japan) at 350 × magnifications. Total number and number of open stomata were counted at 350× magnification.

3.2.10 Statistical analysis

The data was subjected to analysis of variance by using Statistical Analysis System software (SAS) version 9.4 (Cary, N.C., U.S.A). The means were separated using Tukey's multiple range test at 5% probability. Correlations among the parameters were constructed using Microsoft Excel 2010.

3.3 RESULTS AND DISCUSSION

3.3.1 Soil water used

The amounts of water that were used by each treatment of two ginger species during the 2015/2016 and 2016/2017 growing seasons are presented in Table 3.5. The data showed that there were significant differences among water treatments, but the ginger species did not significantly differ in water use for the same treatments within a specific season. The results are clear that, for both commercial and African ginger, the well-watered treatment (20–25% MAD) showed significantly higher water use when compared to other treatments in both years. Water use significantly decreased with an increase in water stress level for both species in the first season (2015/2016; Table 3.5). The obtained data indicate that both ginger species showed no significant differences in water use between treatments 40–45%, 60–65%, and 80–85% MAD during the second season (2016/2017; Table 3.5). Soil water uptake can vary widely with the season, management practices, location, and crop varieties. During 2016/2017, substantially more water was depleted for all of the stress treatments, as compared to 2015/2016. This could be explained by more rapid canopy

development early in the 2016/2017 season (higher FI values when water treatments commenced; Figure 3.5C, D), which resulted in higher total water use amounts.

As expected, when the irrigation interval became longer (higher MAD percentage), the top soil layer dried out more, the proportion of water that is taken up from the deeper soil layers (0.4–0.80 m) increased, and less water in total was taken up (Zhang *et al.*, 2004).

Table 3.5: Total water use of two ginger species subjected to different irrigation regimes over two growing seasons.

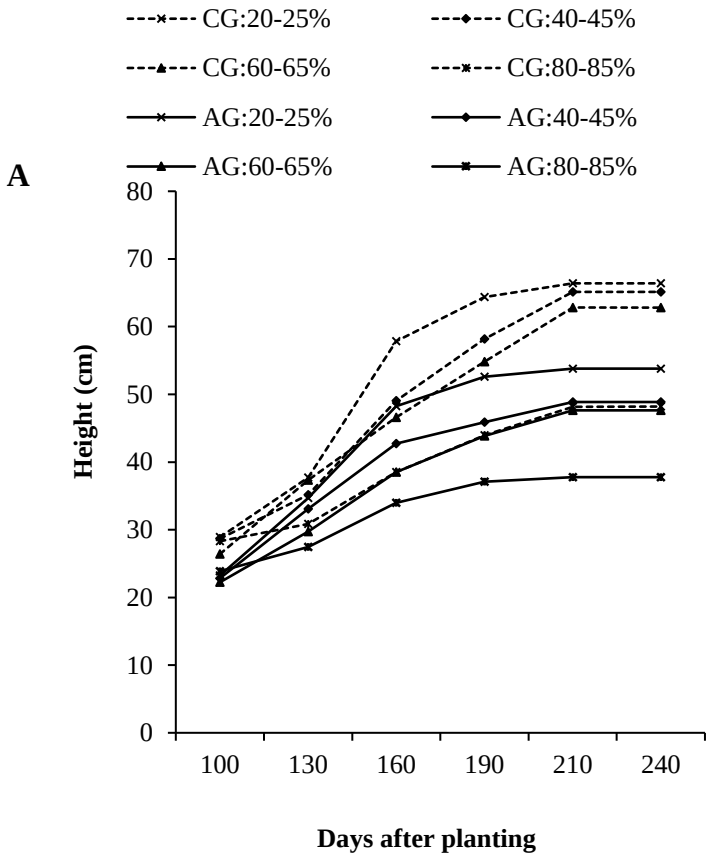
Ginger species	Water use (mm)		
	MAD (%)	2015/2016	2016/2017
CG	20-25	469[a]	549[a]
	40-45	284[b]	362[bc]
	60-65	176[c]	344[bc]
	80-85	109[d]	329[c]
AG	20-25	462[a]	543[a]
	40-45	275[b]	381[b]
	60-65	183[c]	363[bc]
	80-85	117[d]	341[bc]
LSD	------	25.6	48.1

CG: Commercial ginger, AG: Africa ginger. MAD: maximum allowable depletion of plant available soil water. Values in a column with the same letter are not significantly different from each other at $p<0.05$.

3.3.2 Plant height

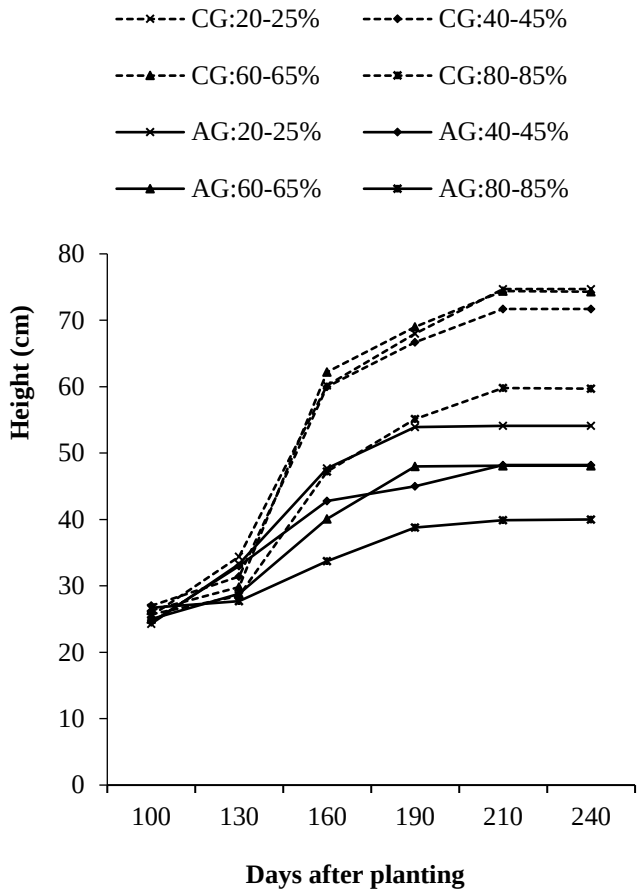
The results showed significant differences between the treatments in plant height during the two cropping seasons in response to the different irrigation regimes for both ginger species (Figure 3.1A). African ginger showed statistically significant differences in plant height for treatments AG: 20–25%, MAD, and AG: 60–65% MAD (Figure 3.1B). The significant differences manifested from 100 days after planting to 240 days after planting for the first cropping season, while for the second seasons, the significant differences were revealed from 160 days after planting to 240 days after planting.

The results showed an increase in plant height from 135 to 235 days after planting for the treatments of both ginger species. Taller plants were observed for all of the commercial ginger treatments when compared to those of African ginger in both seasons. For both ginger species, plant height at low to moderate water stress resulted in similar plant heights, while the severely stressed plants were stunted most. An earlier study on *Satureja hortensis* L. also reported decreased plant height in response to highly water-stressed conditions (Wilson & Ovid, 2008). Mofokeng *et al.* (2015) also reported that the plant height of *Pelargonium sidoides* DC was reduced under severely stressed conditions as compared to the well-watered treatments. Mabhaudhi *et al.* (2011) reported similar results for Bambara groundnut.



CG: Commercial ginger; AG: African ginger. LSD_{100} = 4.46; LSD_{130} = 5.67; LSD_{160} = 7.55; LSD_{190} = 3.79; LSD_{210} = 4.0; and, LSD_{240} = 3.0.

B



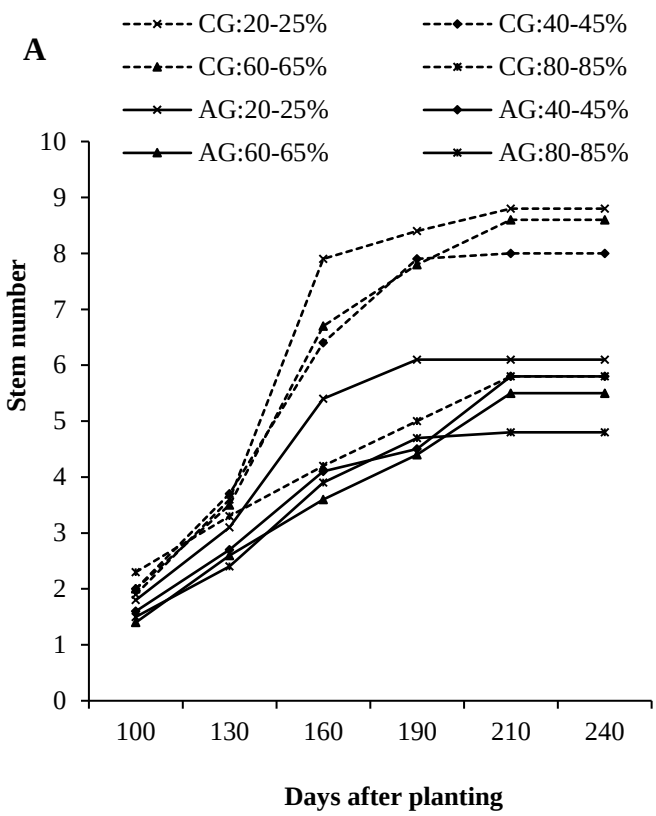
CG: Commercial ginger; AG: African ginger. LSD_{100} = 3.86; LSD_{130} = 2.8; LSD_{160} = 12.42; LSD_{190} = 12.71; LSD_{210} = 14.25; and, LSD_{240} = 14.23.

Figure 3-1: Plant height of two ginger species in response to different water regimes during the (A) 2015/2016 and (B) 2016/2017 cropping seasons.

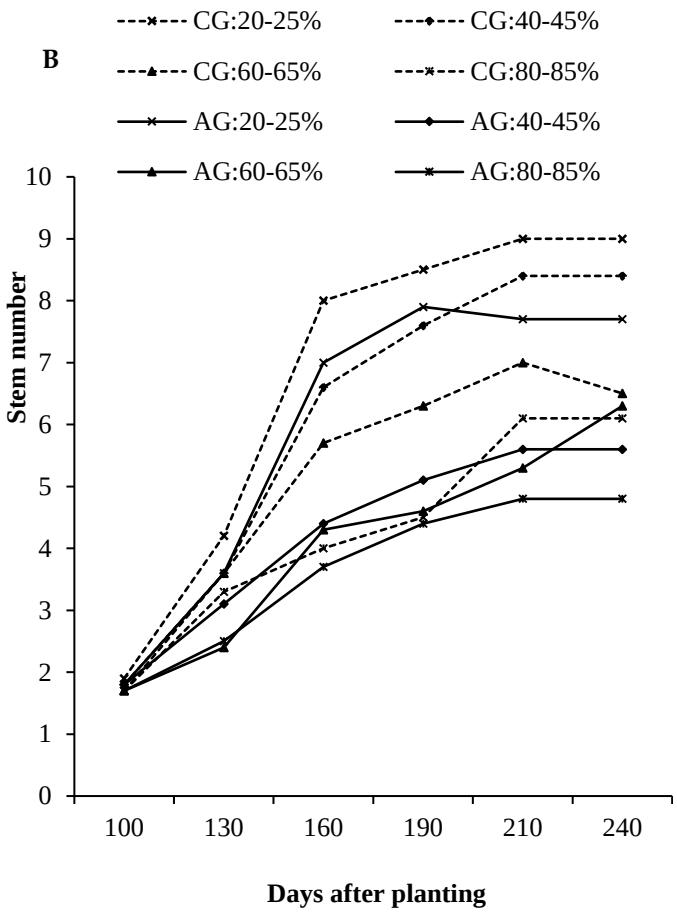
3.3.3 Stems per plant

Figure 3.2A, B represents the response of a number of stems per plant to different water regimes over two cropping seasons for African and commercial ginger. The results indicate that there were significant differences between the stem numbers for different treatments. During 2015/2016 treatments CG 20–25% MAD, CG 40–45% MAD, and CG 60–65% MAD showed significantly higher stem numbers from 130 to 240 days after planting (Figure 3.2A). It is notable that the three treatments of commercial ginger representing well-watered and intermediate water regimes did

not result in any reduction in stem numbers. The higher stem numbers for these three treatments (CG 20–25% MAD, CG 40–45% MAD, and CG 60–65% MAD) was apparent, as the availability of soil water improved plant growth. Since water plays an essential role in the physiological processes of plants (Mofokeng *et al.*, 2015), it can be deduced that the well-watered treatments enabled commercial ginger to maintain its normal physiological functions. Treatment CG 80–85% MAD showed the lowest number of stems as compared to other treatments, irrespective of the ginger species (Figure 3.1A). Jyotsna *et al.* (2012) obtained similar results followed by reported water stress decreasing the growth and plant biomass of *Salvia officinalis*. When severe water stress (treatment 80–85% MAD) was applied, the stem number declined more for both ginger species during both the 2015/2016 and 2016/2017 cropping seasons (Figure 3.2A, B), suggesting a strong effect on the development of the plant. Additionally, the first sign of water stress can often be detected by a reduction in cell turgesans and thereby a reduction in cell growth, especially in the stems and leaves.



CG: Commercial ginger; AG: African ginger. Lsd_{100} = 1.26; LSD_{130} = 0.88; LSD_{160} = 1.15; LSD_{190} = 1.58; LSD_{210} = 2.47; and, LSD_{240} = 2.47.



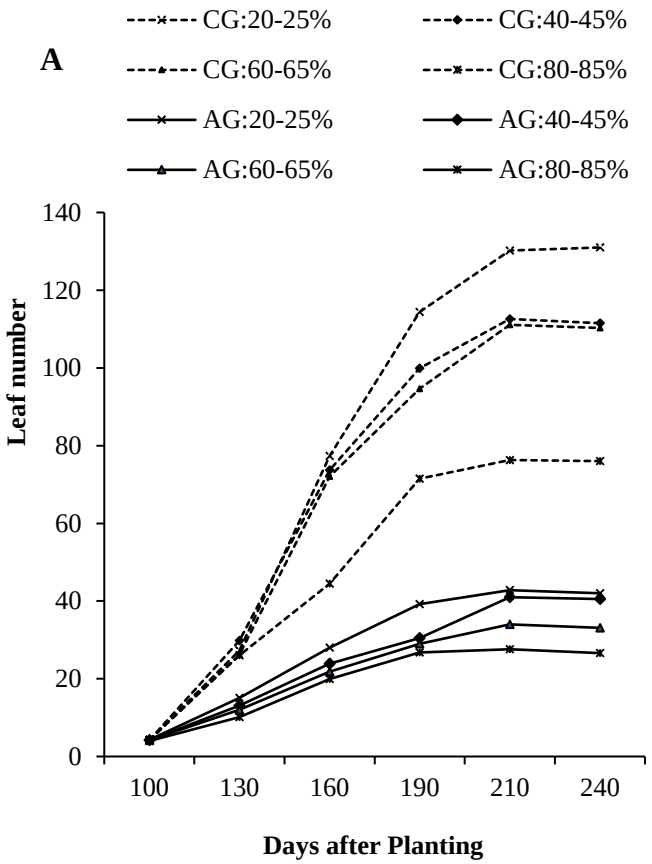
CG: Commercial ginger; AG: African ginger. LSD_{100} = 0.39; LSD_{130} = 0.34; LSD_{160} = 1.37; LSD_{190} = 1.96; LSD_{210} = 1.80; and, LSD_{240} = 1.71.

Figure 3-2: Number of stems per plant of two ginger species in response to different water regimes during the (A) 2015/2016 and (B) 2016/2017 cropping seasons.

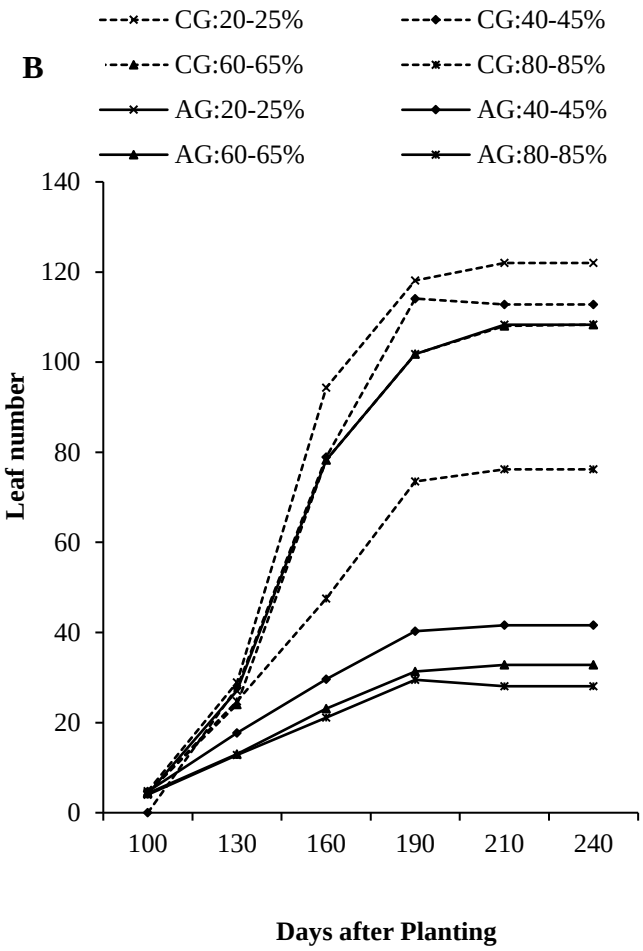
3.3.4. Number of Leaves

The results in Figure 3.3A, B showed the number of leaves per plant for commercial and African ginger, as affected by different irrigation regimes during the 2015/2016 and 2016/2017 cropping season. The total number of leaves was much higher in the treatment of CG 20–25% MAD compared to other treatments during the 2015/2016 and 2016/2017 seasons (Figure 3.3A and B). The increases in the total number of leaves were more pronounced for well-watered and intermediate treatments (CG 20–25% MAD, CG 40–45% MAD, and CG 60–65% MAD) than

severe stressed treatment (AG 80–85% MAD) of African ginger for 2015/2016 and 2016/2017 season. The severe stressed treatment (AG 80–85% MAD) that showed the least number of leaves during both seasons may limit the development of the plants. The relatively low supply of water to the stressed treatments resulted in poor leaf formation and stunted growth. The severely stressed treatment (CG 80–85% MAD) resulted in an intermediate number of leaves, which agrees with the findings on the response of water on the essential oil of oregano as reported on by Said-AlAhl *et al.* (2009). This result differs from the findings that were reported by Mofokeng *et al.* (2015). We postulate that this result may have been due to insufficient time of exposure to the severely stressed conditions.



CG: Commercial ginger; AG: African ginger. $LSD_{100} = 0.45$; $LSD_{130} = 4.03$; $LSD_{160} = 3.19$; $LSD_{190} = 6.52$; $LSD_{210} = 29.19$; and, $LSD_{240} = 29.35$.



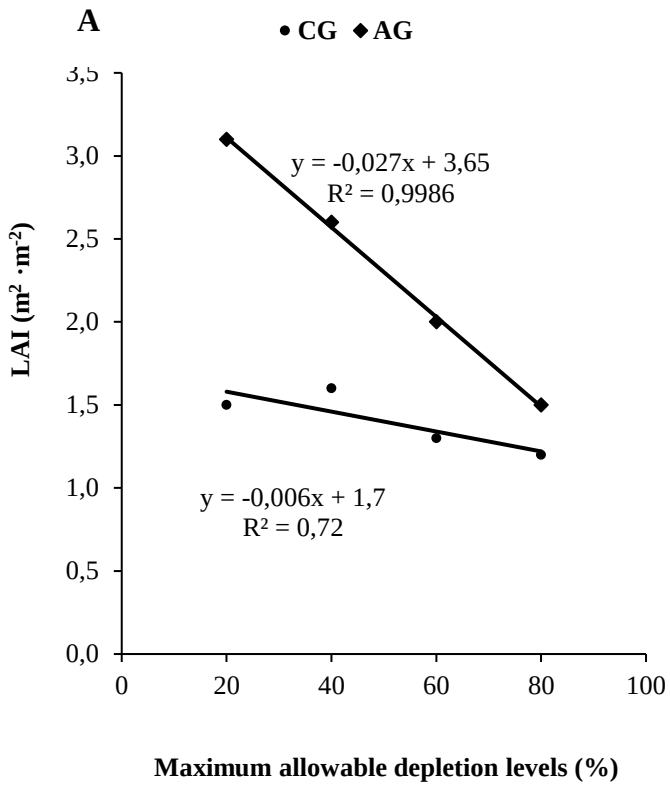
CG: Commercial ginger; AG: African ginger. LSD_{100} = 0.74; LSD_{130} = 2.51; LSD_{160} = 4.87; LSD_{190} = 10.46; LSD_{210} = 14.7; and, LSD_{240} = 14.6.

Figure 3-3: Number of leaves per plant of two ginger species in response to different water regimes during the (A) 2015/2016 and (B) 2016/2017 cropping seasons.

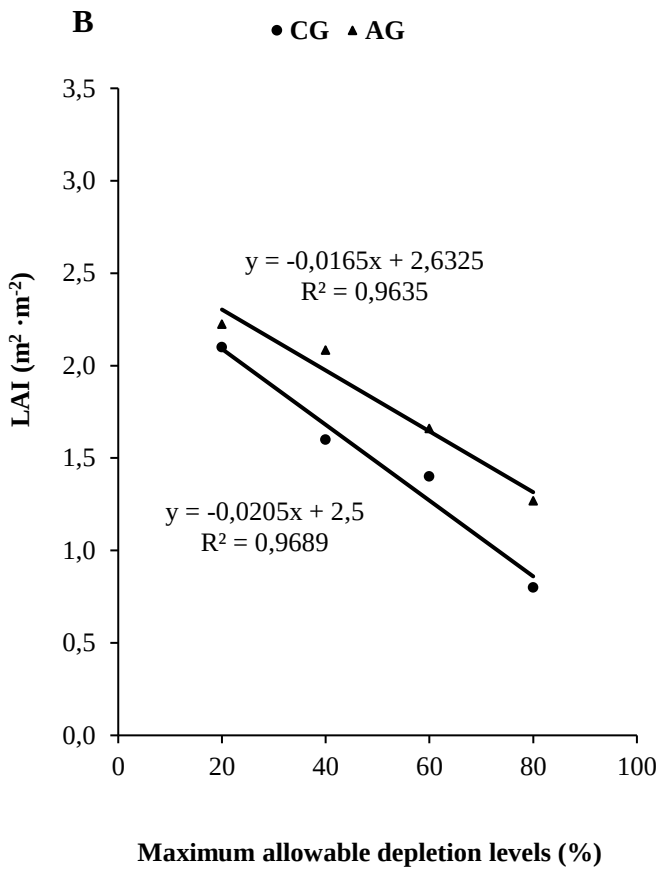
3.3.5. Leaf Area Index

The application of different irrigation regimes resulted in significantly different peak LAI values between treatments (Figure 3.4A, B). In 2015/2016, the leaf area index declined more under water stress conditions for African ginger than commercial ginger. In both seasons, the control treatment for African ginger species recorded the highest LAI of 3.1–2.2 m^2 m^{-2}, followed by the moderately stressed treatment (AG 40–45% MAD) with 2.63–2.0 m^2 m^{-2}. The severely stressed treatment of

commercial ginger (CG 80–85% MAD) had the lowest LAI values of 0.8–1.2 $m^2 m^{-2}$. The lower LAI value under stress is a result of fewer and smaller leaves and a well-documented response to drought for many crops. The leaf area index responds to physiological processes, such as leaf expansion and photosynthesis, which are essential in dry matter production (Blum, 2005). A previous study on the growth of *Pelargonium sidoides* in response to water and nitrogen level also revealed a decline in leaf area with water stress. Similarly, Acreche *et al.* (2009) recorded the highest LAI for well-watered as compared to the stressed treatments of commercial ginger.



CG: Commercial ginger; AG: African ginger. Lsd = 0.93.



CG: Commercial ginger; AG: African ginger. LSD = 0.53.

Figure 3-4: Peak Leaf Area Index (LAI) values of two ginger species in response to different water regimes during the (A) 2015/2016 and (B) 2016/2017 cropping seasons.

3.3.6 Fractional Interception of Photosynthetically Active Radiation (FI_{PAR})

The fractionally intercepted photosynthetically active radiation (FI_{PAR}) markedly varied between different irrigation regimes and the two ginger species (Figure 3.5A–D). The FI_{PAR} for the well-watered treatment (20–25% MAD) of both African ginger and commercial ginger generally showed the highest FI_{PAR} when compared to the other water treatments, while the severely stressed treatment (80–85% MAD) showed the lowest FI_{PAR} values. In most instances, commercial ginger recorded lower FI_{PAR}, values than African ginger at the same water treatments. The lower FI_{PAR} values for severely stressed treatments (CG 80–85% MAD) are likely associated with the smaller

canopy of these plants. The higher FI_{PAR} that was reported for African ginger as compared to commercial ginger is associated with the higher LAI recorded for African ginger in this study (Figure 3.4A, B).

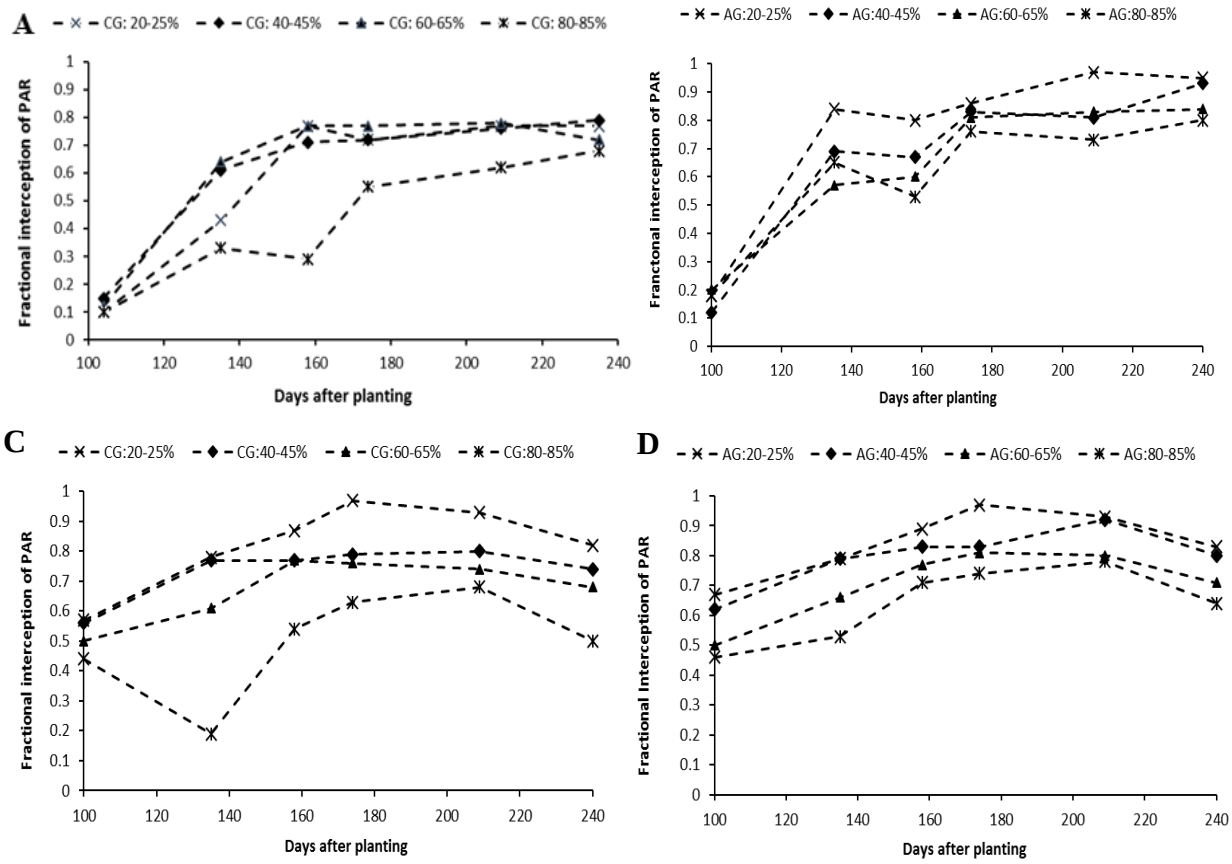


Figure 3-5: Fractional interception of photosynthetically active radiation (FI_{PAR}) of two ginger species in response to different water regimes during the (A) CG: 2015/2016, (B) AG: 2015/2016, (C) CG: 2016/2017; and, (D) AG: 2016/2017 cropping seasons.

3.3.7 Scanning Electron Microscopy (SEM) and Stomata

Open stomata analyses describing the response of two ginger species, as affected by different irrigation regimes, are presented in Figures 3.6 and 3.7. Stomatal conductance of the well-watered treatments was always significantly higher than that of the stressed treatments in most cases. The

results show that the control treatment for commercial ginger had a total of 60 and 43 stomata per unit area in the two cropping seasons, respectively, while 95% of stomata were open in both of the cropping seasons. The moderately stressed treatment (CG + 40–45% MAD) recorded 43.2% and 89.1% open stomata, respectively, in the two cropping seasons (Table 3.6). The control (unstressed treatment) and moderately stressed treatments had a higher number and higher percentage open stomata as compared to other commercial ginger treatments. For African ginger, the percentage of open stomata was higher for the control (CG + 20–25% MAD) when compared to the rest of the treatments. The severely water-stressed treatment (CG 80–85% MAD) showed the lowest percentage of open stomata.

The closing of stomata is associated with the inhibition of plant growth under drought stress, which may negatively affect crop yield (Waraich *et al.*, 2011). Although treatment AG 60–65% MAD recorded a higher total stomata number than other treatments of African ginger, less of the stomata were open, as compared to the control. The results indicate a trend of stressed treatments, resulting in lower stomata numbers per unit area and lower percentage open stomata in both ginger species (Table 3.6; Figures 3.6 and 3.7). African ginger generally showed more open stomata than commercial ginger. The opening and closing of stomata is regulated by the integration of environmental signals and endogenous hormonal stimuli (Daszkowska-Golec & Szarejko, 2013). In general, African ginger will lose more water through the open stomata under stress conditions than commercial ginger. This shows that African ginger has a less effective mechanism in preventing water losses through transpiration under drought than commercial ginger.

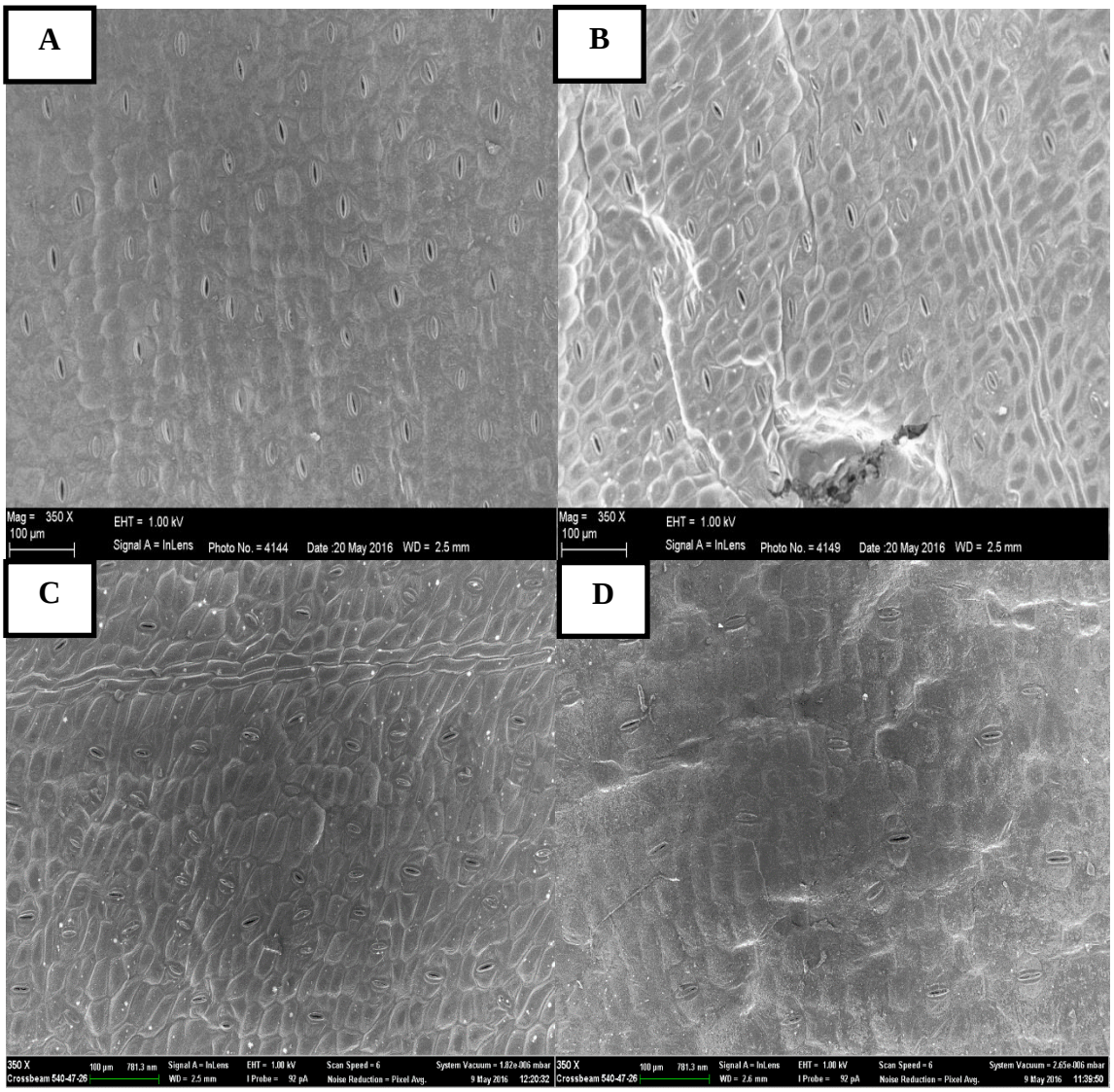


Figure 3-6: Scanning Electron Microscopy (SEM) images of open stomata pores of commercial ginger in response to different irrigation regimes during cropping season 2015/2016. (A) CG-20–25% MAD; (B) CG-40–45%MAD; (C) CG-60–65% MAD; and, (D) CG-80–85% MAD.

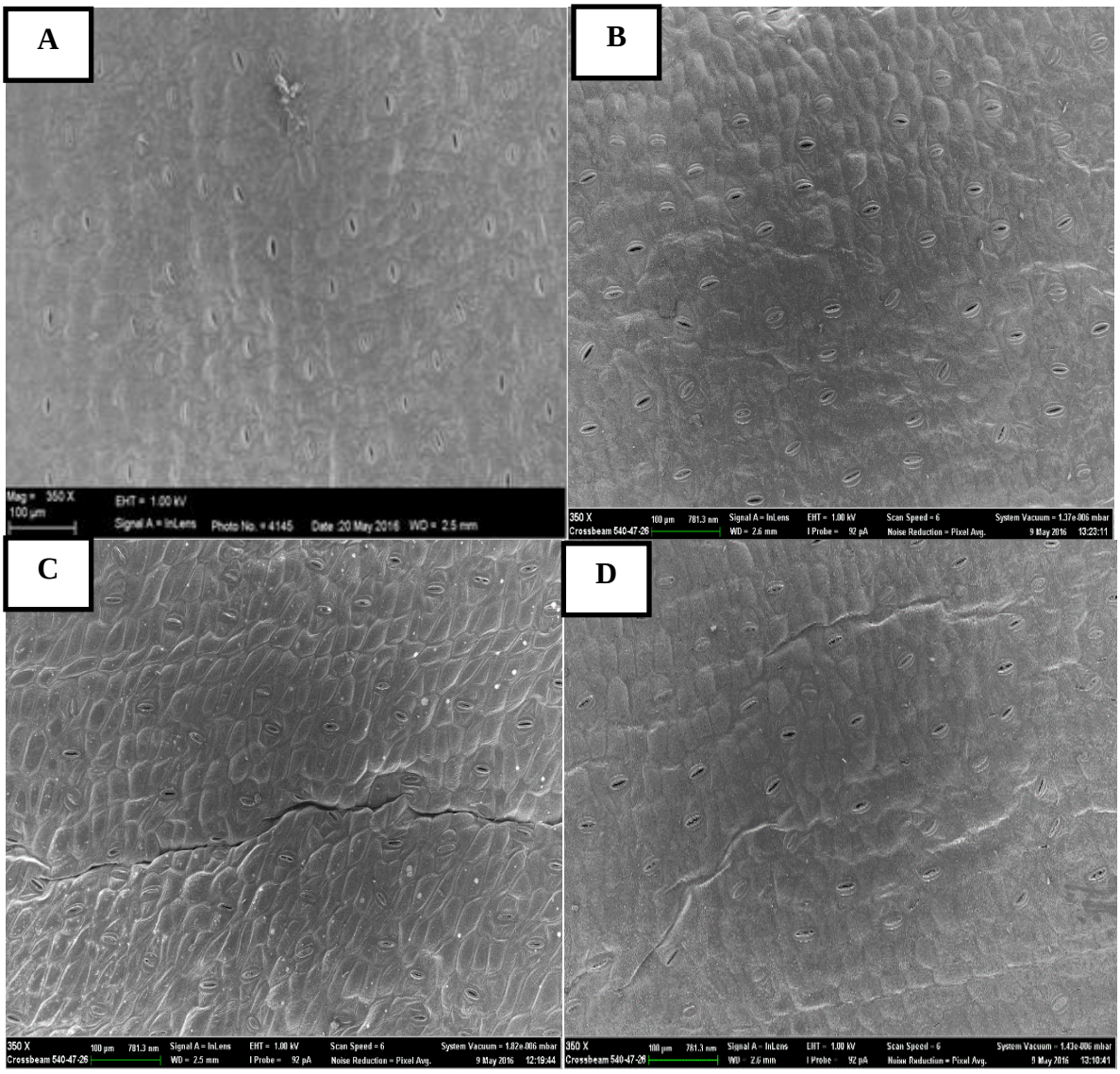


Figure 3-7: Scanning Electron Microscopy (SEM) images of open stomata of African ginger in response to different irrigation regimes during the cropping season 2015/2016. (**A**) AG-20–25% MAD; (**B**) AG-40–45% MAD; (**C**) AG-60–65% MAD; and, (**D**) AG-80–85% MAD.

Table 3.6: Total number of stomata and open stomata per 10 mm^2 in two ginger species as affected by different irrigation levels in the 2015/2016 and 2016/2017 seasons.

Ginger Species	Depletion (%)	2015/2016			2016/2017		
		Total Stomata	Open Stomata	Open Stomata %	Total Stomata	Open Stomata	Open Stomata %
CG	20–25	60	57	95.0	43	40	93.0
	40–45	74	32	43.2	37	33	89.1
	60–65	49	24	48.9	18	6	33.3
	80–85	21	11	52.3	12	7	58.3
AG	20–25	53	48	90.5	42	39	90.8
	40–45	57	45	78.9	42	36	85.7
	60–65	65	43	66.1	30	25	83.3
	80–85	47	32	68.0	30	7	23.3

CG: Commercial ginger; AG: African ginger.

3.3.8 Fresh and Dry Rhizome Yield

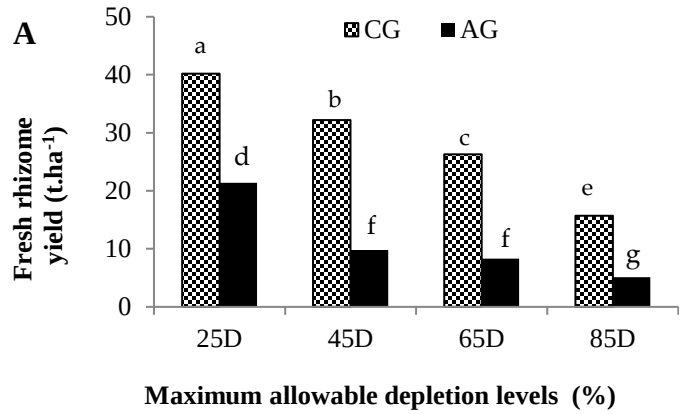
The fresh rhizome yield showed significant variations ($p < 0.05$) between the two ginger species and different irrigation regimes (Figure 3.8A, B). The highest fresh rhizome yield was recorded for commercial ginger under well-watered conditions (CG + 20–25% MAD and CG + 40–45% MAD) for both of the cropping seasons (Figure 3.8A, B). Total fresh rhizome yield for commercial ginger increased from 15.7 to 43.5 t ha^{-1} as the water stress level decreased (Figure 3.8A, B). Well-watered plants also showed increased yield for African ginger, when compared to stress treatments. This was due to higher values of growth parameters, such as leaf number, stem number, LAI, and FI_{PAR} for the control treatments. However, stressed treatments reduced crop canopy, closed stomata, and inconsequently resulted in lower photosynthesis, which eventually culminated in lower storage organ yield for both ginger species.

The differences in rhizome yield could mainly be related to the number of rhizomes per plant for the different species and water treatments. This emphasizes the importance of proper irrigation management to maximise yield at any given crop evapotranspiration level (Bekele & Tilahun,

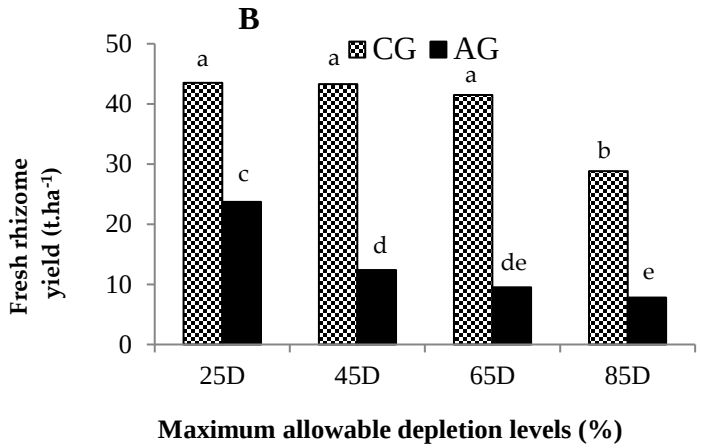
2007). The effect of water depletion level on species under water-stressed conditions showed that the maximum available depletion of less than 45% manifested in no differences in rhizome yield. An increase in the depletion level to 65% and higher resulted in a reduction of fresh rhizome yield. It is worth noting that the yield decrease in case of commercial ginger was gradual according to the water stress treatment, but in the case of African ginger, rhizome yield decreased almost half-fold when the MAD was raised from 20–25 to 40–45%. During the second growing season, even when the MAD increased up to 80–85% MAD, the yield of commercial ginger was only reduced by about one-quarter, but in the case of African ginger, even mild stress of 40–45% MAD reduced rhizome yield by half in both seasons. This confirms that African ginger is more susceptible to drought than commercial ginger. Similar results were reported for other drought-sensitive crops, such as potato (Onder *et al.*, 2005; Kirda, 2002).

Similar to fresh rhizome yield, varying soil water contents at different irrigation treatments also had significant effects on dry rhizome yield (Figure 3.9A, B). Commercial ginger is sold either fresh for household consumption or dry as a flavourant in the food industry, while African ginger is used in the manufacturing of various medicinal products, for which dry rhizomes are usually used. The dry rhizome yield of AG seemed to be more consistent across seasons than CG. The total dry rhizome yields for commercial ginger ranged from 3.1–4.2 to 8.1–8.7 t ha^{-1} for treatments 80–85% MAD and 20–25% MAD during the two cropping seasons, respectively. Figures 3.9A, B indicates that the dry rhizome yield for African ginger followed a decreasing trend from the well-watered treatment to the severely stressed treatment for both of the cropping seasons. Earlier studies on an onion crop also reported higher yields for the well-watered treatments as compared to the stressed treatments (Mulu & Alamirew, 2012). Severely stressed plants resulted in the lowest dry rhizome yield for both commercial and African ginger (Figure 3.9A, B). Reddy and Reddy

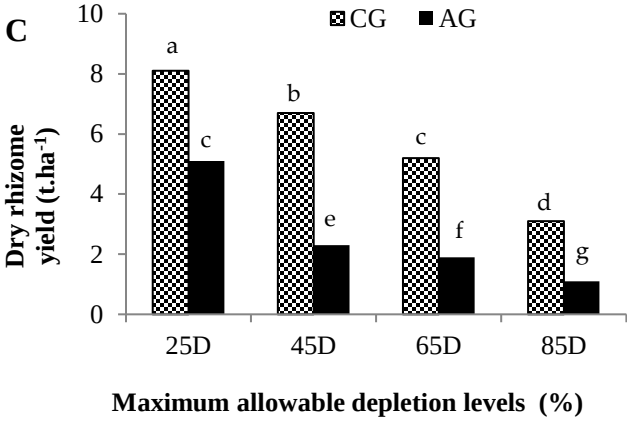
reported similar results indicating a reduction in the yield of peanuts with 80% depletion of available soil water. The higher yield for the control treatments in both of the ginger species was likely due to the observed increase in the number of leaves, which increased leaf area and photosynthesis capacity, as compared to the stressed treatments. A study of different irrigation regimes on the growth and yield of potato similarly revealed that total fresh and marketable tuber yield increased with an increasing amount of irrigation (Kirda, 2002).



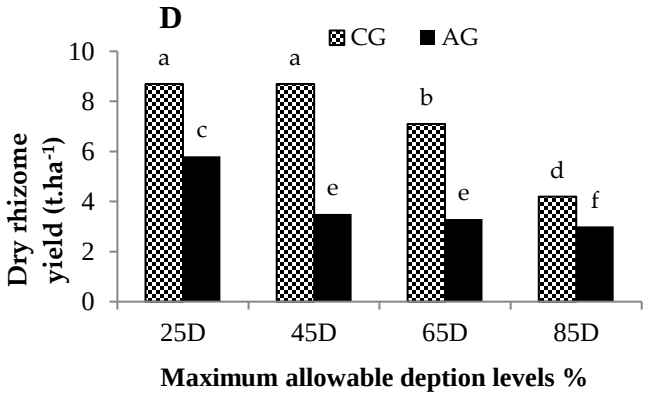
CG: Commercial ginger; AG: African ginger. LSD = 2.4



CG: Commercial ginger; AG: African ginger. LSD = 4.6



CG: Commercial ginger; AG: African ginger. LSD = 0.3



CG: Commercial ginger; AG: African ginger. LSD = 0.2

Figure 3-8: Fresh and dry rhizome yield (t ha^{-1}) of African and commercial ginger species in response to irrigation regimes during the (A and C) 2015/2016, and (B and D) 2016/2017 cropping seasons. Bars with the same letter are not significantly different at $p \leq 0.05$.

3.3.9 Water Use Efficiency

Irrigation water use efficiency (WUE) is the relation between yield and water use, and it was calculated as the yield divided by the volume of water used. Table 3.7 lists the irrigation water use efficiencies for the different treatments. The maximum WUE values for commercial ginger were 150 and 121 $kg \cdot ha^{-1}$ mm^{-1} for the stressed treatment (CG-60–65% MAD) in the two cropping seasons, respectively. Higher WUE values for moderately stressed treatments as compared to the well-watered treatment is common (Mulu & Alamirew, 2012) and can be explained by the fact that the 40–45% and 60–65% MAD treatments used substantially less water, but they did not produce much lower yields than the control.

Water use efficiency for African ginger was significantly lower in both of the cropping seasons when compared to commercial ginger (Table 3.7). For African ginger, there was a little variation in WUE between treatments, but the general trend was that the control and less stressed treatments had higher WUEs, which was opposite to the trend that was observed for commercial ginger. This is explained by the drastic drop in rhizome yield with the increase in water stress (an indication of drought sensitivity), while the corresponding decreases in water use was more gradual.

Table 3.7: Water use efficiency of commercial and African ginger in the 2015/2016 and 2016/2017 cropping seasons.

		2015/2016		2016/2017	
		Fresh	Dry	Fresh	Dry
Species	Depletion	WUE	WUE	WUE	WUE
	%	(kg·ha^{-1} mm^{-1})	(kg·ha^{-1} mm^{-1})	(kg·ha^{-1} mm^{-1})	(kg·ha^{-1} mm^{-1})
CG	20–25	85.8 ^{c}	17.3 ^{c}	79.2 ^{b}	15.9 ^{c}
	40–45	113.5 ^{b}	23.8 ^{b}	119.4 ^{a}	24.1 ^{a}
	60–65	150.0 ^{a}	30.0 ^{a}	122.8 ^{a}	21.2 ^{b}
	80–85	144.7 ^{a}	28.9 ^{a}	87.8 ^{b}	12.9 ^{d}
AG	20–25	46.4 ^{d}	11.2 ^{d}	43.7 ^{c}	10.7 ^{e}
	40–45	35.8 ^{d}	8.6 ^{d}	33.6 ^{cd}	9.2 ^{e}
	60–65	45.8 ^{d}	10.5 ^{d}	26.2 ^{d}	9.2 ^{e}
	80–85	44.5 ^{d}	9.9 ^{d}	23.1 ^{d}	8.9 ^{e}
LSd		**15.5**	**2.8**	**16.5**	**1.9**

CG: Commercial ginger; AG: African ginger. Values in a column followed by the same letter, do not differ significantly from each other ($p<0.05$)

3.4. CONCLUSIONS

Our study has provided much-needed information regarding the tolerance of ginger species to different levels of water stress. The amount of water use was higher under well-watered treatments as compared to stress treatments for both African ginger and commercial ginger. We have shown that growth and development parameter traits, such as height, leaf number, and stem number significantly varied between different species and irrigation treatments. Irrigation treatment effects on plant growth and development parameters depended upon plant species. The maximum LAI and FI_{PAR} values of African ginger were higher than those of commercial ginger, and this was reflected in both of the seasons. This was even though African ginger had fewer leaves, which thus reflects the bigger size of individual leaves of African ginger as compared to commercial ginger. Scanning electron microscopy images showed that both of the ginger species had more stomatal pores and open stomata under well-watered than stressed conditions. African ginger had a higher percentage of open stomata in all stressed treatments as compared to commercial ginger,

suggesting limited stomatal control to prevent excessive transpiration losses under severe drought conditions.

Commercial ginger showed higher plant height, stem number, and leaf number, while African ginger had higher leaf area index and FI_{PAR} when compared to commercial ginger. Fresh and dry rhizome yields were higher for commercial ginger as compared to African ginger in both seasons. Severely water stressed fresh and dry rhizome yields of commercial ginger were more affected than that of African ginger. Water use efficiency in terms of fresh commercial ginger yield was the highest at moderate water stress (CG-60–65% MAD). For dry rhizome yield, the water use efficiency was highest for either CG-60–65% or CG-40–45% MAD. Based on the results of our research, we can conclude that water stress had a striking effect on the growth, yield, and WUE of ginger species. Finally, the study showed that severe water stress reduced ginger rhizome yield in both species. However, commercial ginger seems to be less sensitive to water stress than African ginger.

CHAPTER 4

EFFECT OF WATER STRESS AND TIME OF HARVESTING ON YIELD, PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF TWO GINGER SPECIES

ABSTRACT

In South Africa, two species from the Zingereberaceae family, commercial ginger (*Zingiber officinale* Roscoe) and African ginger (*Siphonochilus aethiopicus*) play a significant role as medicinal plants. Water stress is known to affect the phenolic and antioxidant content of plants. The present study was conducted to determine the effect of irrigation regime and the harvest period of the two ginger species on yield, phenolic content and antioxidant properties. A field experiment was conducted at the Experiment Farm of the University of Pretoria, during the 2015/2016 cropping season. Four levels of water availability (20-25%, 40-45%, 60-65% and 80-85% maximum allowable depletion (MAD) of available soil water) and two ginger species were used. The well-watered control (20-25% MAD) resulted in a gradual increase in rhizome yield throughout the harvesting period for both plant species. Water use was higher in the treatments exposed to lower levels of water stress (20-25% MAD and 40-45% MAD) for commercial ginger compared to the moderate and severely stressed treatments. The severely stressed treatment (80-85% MAD) resulted in a slight decrease of rhizome yield, but a significant increase in total flavonoid and phenolic content of the rhizomes for both species as compared to the leaves and tillers. The total antioxidant content was highest for the severely stressed treatments, compared to well-watered and moderately stressed treatments. Rhizomes were higher in total phenolics, flavonoids and antioxidant content in comparison to tillers and leaves.

Keywords: *Antioxidant, flavonoid, phenolics, maximum available depletion level, rhizome yield and water use efficiency.*

4.1. INTRODUCTION

Commercial ginger (*Zingiber officinale* Roscoe) and African ginger (*Siphonochilus aethiopicus*) belong to the family Zingiberaceae. Traditionally, both plant species have been used for the treatment of many ailments ranging from malaria, asthma, headaches to chest ailments (van Wyk, 2008). The health-promoting properties of both ginger species are attributed to its rich phytochemistry (Butt & Sultant, 2011). The roots and rhizomes of the plant species contain high levels of anti-inflammatory and antioxidant components (Cai *et al*., 2004). Ghasemzadeh *et al.* (2010) reported that different ginger plant parts such as rhizomes, stems and leaves differ in levels of flavonoid, phenolic and antioxidant compounds. Furthermore, the two species contain volatile organic compounds, including sesquiterpene and monoterpenoid hydrocarbons, as well as non-volatile pungent compounds, including gingerols, shogaols and zingerone (Shukla & Singh, 2007). Both non-volatile and volatile components provide the distinct aroma and taste to the ginger species.

Water stress is one of the most important environmental stresses that affect plant growth and has a significant impact on physiological and biochemical processes, affecting almost all plant functions (Shao *et al.,* 2008). This form of abiotic stress has an immediate impact on water status and affects physiological parameters such as water potential, relative water content, stomatal reactions and photosynthesis. Furthermore, severe water stress has an impact on the roots and shoots of the plant, which results in a reduction in leaf surface area (Hsiao & Xu, 2000). Plants can develop adaptation mechanisms by altering their cellular metabolism to overcome environmental stresses (Kazan, 2013). Water stress leads to increased oxidative stress and inhibits the photochemical activities as a result of higher leakage of electrons towards O_2 during photosynthetic and respiratory processes in the plant cell (Sharma *et al*., 2012). The ability of

different plant species to cope under water-stressed conditions might be related to their ability to scavenge reactive oxygen species (ROS) by enhancing the activities of the antioxidant enzymes during a water loss. In environmental stress conditions such as drought, high activities of antioxidant enzymes are important for plants to tolerate stresses (Gill & Tuteja, 2010). The effect of water stress on the primary and secondary metabolites of plants has been evaluated in several plant species (Apel & Hirt, 2004). The concentration of various secondary metabolites is strongly affected by growing conditions and has an impact on the metabolic pathways responsible for the accumulation of the related natural products. The significant relationship between enhanced antioxidant enzyme activities and increased resistance to environmental stress depends on the stage of development and duration of stress (Bajguz & Hayat, 2009). Antioxidants and phenolics play an important role as oxygen scavengers and are involved in protecting human, animal and plant cells against the damaging effects of free radical reactive oxygen species, (ROS) (Gülçin, et al., 2002; Kukic et al., 2006).

Scientific and precise determinations for medicinal value, active principles and antioxidant properties have been examined in different plant species. However, information regarding the effect of variable factors such as plant part and water stress on phytochemical content and related antioxidant activity in ginger species is very limited. The present work was aimed to investigate the effect of water regime and date of harvesting on the yield, composition of phenolic, flavonoid and antioxidant content in rhizomes, tillers and leaves of commercial (*Zingiber officinale* Roscoe) and African ginger (*Siphonochilus aethiopicus*).

4.2 MATERIALS AND METHODS

In this chapter, the material produced in chapter three was used for further processing. The site, plant material, experiments and water use were describes in chapter three sections 3.2.1 to 3.2.5.

4.2.1 Leaf area index (LAI)

Leaf area index (LAI): Two plants in each plot were harvested once a month five to eight months after planting. Leaf area index was measured using an LI 3100 belt driven leaf area meter (Li-Cor, Lincohn, Nebraska (USA). Leaf area index (LAI) was calculated from the leaf area (LA) and harvested land area (H_{LA}) Equation (4.1).

$$LAI = \frac{LA(m^2)}{H_{LA(m^2)}} \qquad (1)$$

4.2.2 Yield analysis

Yield parameters such as fresh and dry mass of the rhizomes, leaves and tillers were assessed. Harvesting was done in monthly intervals, starting at five months after planting, with the last harvest at eight months after planting. Rhizomes, tillers and leaves were harvested from eight plants per treatment, the fresh mass determined and then oven dried (Economy oven, 620 Digital, Labotec) at 50 °C until a constant mass was obtained.

4.2.3 Determination of total phenolic content

The total phenolic content of the crude extract was determined using Folin–Ciocalteu reagents with analytical grade Gallic acid as the standard (mg GAE g^{-1} dry weight basis). About 1 mL of extract was mixed with 1 mL Folin–Ciocalteu phenol reagent. After 5 min, 7.5% sodium carbonate (2 mL) was added to the mixture. After being kept in total darkness for 2 hours to complete the reaction, the absorbance was measured at 750 nm using a spectrophotometer. Total phenolic content amounts of samples were calculated using the Gallic acid calibration curve. The results were expressed as mg Gallic acid g^{-1} of dry plant matter (Ghasemzadeh *et al.,* 2010).

4.2.4 Determination of total flavonoid content

The aluminium chloride ($AlCl_3$) colorimetric method was used to estimate total flavonoid content as described by Sultana *et al.* (2009). Extracts of each plant part (rhizome, leaf and tiller) of each ginger species (0.1 mL) were diluted with deionized water (0.3 mL) followed by 0.3 mL of 5 % sodium nitrite ($NaNO_2$). After 5 minutes, $AlCl_3$ (10%) was added at 25 ^oC and left for another 5 minutes, where after 0.2 mL NaOH (1.0 M) was added. The reaction mixture was diluted with 1 mL of deionized water and allowed to stand for 5 minutes. Absorbance was measured at 510 nm in triplicates using a spectrophotometer. For each sample, three readings were taken to get the average results, and a standard curve was plotted using quercetin as a standard (mg of QE g^{-1} dry mass basis).

4.2.5 Determination of total antioxidant activities using FRAP assay.

The ferric reducing power assay (FRAP) of each sample was determined according to the method described by Alothman *et al.* (2009). The reducing antioxidant power of the extract was measured spectrophotometrically. The two positive controls Butylated hydroxytoluene (BHT) and ascorbic acid were expressed by graphically plotting absorbance against concentration. The reaction mixture was performed and incubated in the dark at room temperature for 30 min. Samples for the assay was prepared in triplicate and repeated twice. The absorbance was read at 630 nm using a microtiter plate reader (ELISA, Microplate Reader, California, USA).

4.2.6 Data analysis

All data was subjected to analysis of variance using statistical analysis system software. GraphPad Prism version 5.00 for Windows (GraphPad Software Inc., San Diego, CA) was used for the construction of graphs. The treatment means were separated ($p \leq 0.05$) using Tukey honest significant difference test.

4.3 RESULTS AND DISCUSSION

4.3.1 Soil water used

The amounts of water used by each treatment during the growing season are presented in Table 4.1. The results show that commercial ginger tended to use more water at well-watered treatments (CG: 20-25% MAD and CG: 40-45% MAD) compared to African ginger. Water use were slightly increased with the moderate and severely stressed treatments of (60-65% MAD and (80-85%MAD) for African ginger compared to commercial ginger (Table 4.1). Water use was thus higher in control treatments for both species, compared to the other treatments. Soil water depletion levels in plant species can differ between soil types, season and irrigation system used. Soil water uptake could also vary widely with management practices, location and crop varieties. According to Zhang *et al.* (2004), as the irrigation interval became longer (higher MAD percentage), the top soil layer dries out more, and the proportion of water taken up from the deeper soil layers increases.

Table 4.1: Total water use during the growing season of two ginger species subjected to different irrigation regimes.

Ginger species	Water use (mm)		
	MAD (%)	2015/2016	2016/2017
CG	20-25	469^{a}	549^{a}
	40-45	284^{b}	362^{bc}
	60-65	176^{c}	344^{bc}
	80-85	109^{d}	329^{c}
AG	20-25	462^{a}	543^{a}
	40-45	275^{b}	381^{b}
	60-65	183^{c}	363^{bc}
	80-85	117^{d}	341^{bc}
LSD	------	25.6	48.1

CG: Commercial ginger, AG: Africa ginger. MAD: maximum allowable depletion of plant available soil water.

4.3.2 Effect of water stress and time of harvest on LAI of commercial ginger (CG) and African ginger (AG).

Leaf area index (LAI) is a growth parameter related to physiological compounds such as photosynthesis and fresh and dry mater production. The results show a significant interaction between irrigation regimes and ginger species on LAI harvest date and cropping seasons (Table 4.2). For both seasons and all harvest dates, the LAI of Africa ginger was higher compared to commercial ginger. African ginger LAI at all harvest dates was higher as compared to those of commercial ginger.Even though commercial ginger has a higher number of leaves and stems compared to African ginger, it revealed a higher LAI than commercial ginger. The results are clear that well water-watered treatment of 20-25% shows higher LAI with African and commercial ginger species. In the 2015/2016 season, the LAI tended to decrease with time (five>six>seven>eight) while in the 2016/2017 season the LAI only showed a decline at eight months after plant. This was due to the low mean maximum and minimum temperature and solar radiation in the second season that could have sustained and even increase LAI until seven months after planting.

Severely and moderate water stress treatments decreased LAI in both ginger species and cropping seasons. The decreasing in LAI for 60-65% MAD and 80-85% MAD treatments could be attributed to accelerated senescence of leaves under water stress (Munne *et al.,* 2004). The senescence of leaves and decrease in turgor are the first signs of water shortage, which further results in cell development and crop growth (Alishah *et al.,* 2006).

Table 4.2: The mean LAI yield of commercial ginger (CG) and African ginger (AG) in response to water stress and time of harvesting.

Ginger	MAD	LAI				LAI			
		2015/2016				2016/2017			
	%	Five	Six	Seven	Eight	Five	Six	Seven	Eight
CG	20-25	3.54^{a}	2.64^{abc}	2.89^{a}	2.21^{ab}	2.75^{abc}	2.91^{abc}	2.77^{abc}	2.24^{a}
	40-45	3.07^{abc}	2.08^{bc}	2.53^{ab}	2.05^{ab}	3.20^{ab}	2.59^{bc}	3.05^{ab}	1.95^{b}
	60-65	3.04^{abc}	1.93^{bc}	2.00^{ab}	1.56^{ab}	2.04^{cde}	2.09^{cd}	1.69^{cd}	1.66^{cd}
	80-85	1.48^{c}	1.57^{c}	1.56^{b}	0.96^{b}	1.13^{e}	1.35^{d}	1.16^{d}	1.38^{e}
AG	20-25	3.89^{a}	3.51^{a}	2.93^{a}	2.37^{a}	3.49^{a}	3.76^{a}	3.62^{a}	2.40^{a}
	40-45	3.86^{a}	3.35^{a}	2.82^{a}	2.14^{ab}	3.31^{a}	3.64^{a}	3.21^{ab}	2.21^{a}
	60-65	3.30^{ab}	2.98^{ab}	2.12^{ab}	2.03^{ab}	2.20^{bcd}	3.10^{ab}	2.03^{bcd}	1.86^{bc}
	80-85	1.72^{bc}	1.62^{c}	1.66^{b}	1.07^{b}	1.38^{de}	1.59^{d}	1.27^{d}	1.54^{de}
CV	----	18.8	16.28	15.07	24.96	14.88	12.42	18.04	4.35
LSD	----	1.62	1.15	1.00	1.29	1.04	0.94	1.22	0.23

Means values followed by the same letter in the same column were not significantly different at $p<0.05$ according to Tukey's test

4.3.3 Effect of water stress on rhizome, tiller and leaf yield of two ginger species

There were significant interactions between irrigation regimes and ginger species for fresh and dry rhizome (Figures 4.1 and 4.4), tiller (Figures 4.2 and 4.5) and leaf (Figures 4.3 and 4.6) yields. The results showed a gradual increase in fresh rhizome yield of the well-watered control (20-25% MAD) from five to eight months after harvesting for both plant species (Figure 4.1). The fresh (38.3 versus 21.5 $t.ha^{-1}$) and dry (8.8 versus 5.3 $t.ha^{-1}$) rhizome yields at eight months after planting were higher for commercial ginger as compared to African ginger for the well-watered control (20-25% MAD). In general, stressed treatments recorded the lowest yields across all months, although differences were not always significant. This could be due to the closing of stomata under water stressed conditions. The closing of stomata is associated with the inhibition of plant growth under drought stress, which can negatively affect crop yield. The yield reduction could also be due to the decreasing of growth parameters such as stems per plant, number of leaves and leaf area index. The decrease in fresh and dry rhizome yield for 60-65% MAD and the severely stressed 80-85% MAD treatment can also be attributed to accelerated senescence and shedding of leaves under water stress (Munne-Bosch & Alegre, 2004). Furthermore, water stress has been reported to reduce plant growth and affect various physiological and biochemical processes significant to plants (Bahreininejad *et al.,* 2013). According to Shao *et al.* (2008), the ability of plants to survive under stressed conditions depends on plant species, growth stage, duration and intensity of water deficit.

The findings of this study are consistent with previous reports that showed that fresh and dry yields were progressively reduced by increasing stress conditions for different plant species (Khalid, 2006; Khalil & Ismael, 2010; Anyum *et al.,* 2011). The results revealed that water stress treatments (60-65% MAD and 80-85% MAD) recorded the lowest tiller (Figure 4.2) and leaf yields (Figure 4.3) for both commercial and African ginger (although all differences were not significant). The

reduction in fresh and dry yield of leaves and tillers under water stress conditions (60-65% MAD and 80-85% MAD) can be attributed to crop canopy and biomass reduction, which impacted negatively on yield (Yuan *et al.,* 2003). Previous results on *Ocimum basilicum* were consistent with the findings of this study (Khalid, 2006). Significantly higher fresh and dry yield of rhizomes, leaves and tillers of both species were recorded for the well-watered treatments throughout the different harvests. This supports the findings of Cifre *et al.* (2005), who found that the increase in yield was directly associated with an increase in the amount of water irrigated. Earlier reports examined the impact of four levels of MAD (25, 40, 55 and 70%) on potato and showed increased growth and yield of potato at 25 and 40% MAD (Eiasu *et al.,* 2007). Comparing the results of the current irrigation treatment with the control confirms increased growth, while the other treatments decreased the yield.

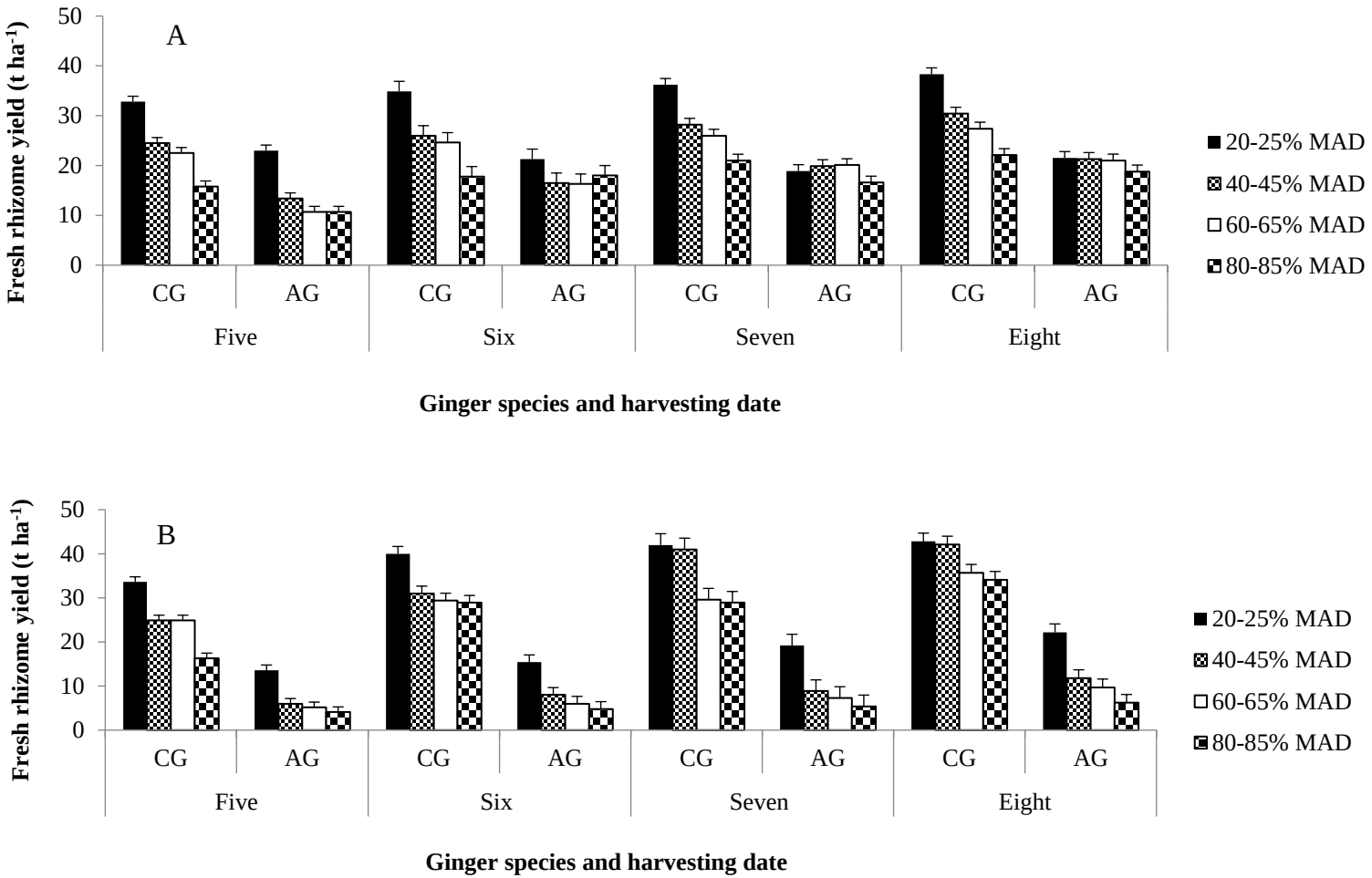


Figure 4-1: Fresh rhizome yield (t.ha^{-1}) of commercial ginger (CG) and African ginger (AG) at different harvest times (five to eight months after planting) in response to four water regimes during (A) 2015/2016 and (B) 2016/2017 cropping seasons.

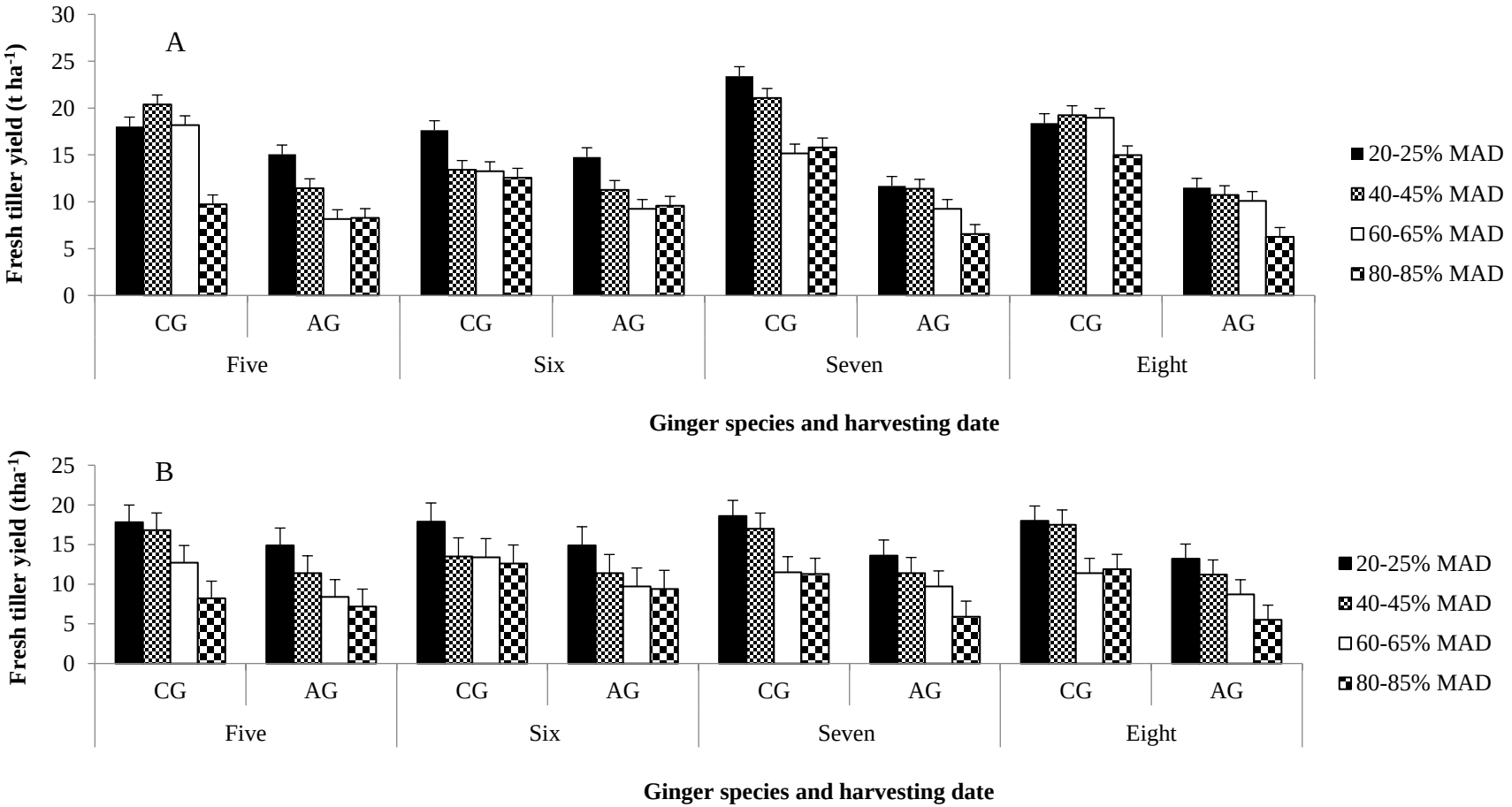


Figure 4-2: Fresh tiller yield (t.ha^{-1}) of commercial ginger (CG) and African ginger (AG) at different harvest times (five to eight months after planting) in response to four water regimes during (A) 2015/2016 and (A) 2016/2017 cropping seasons.

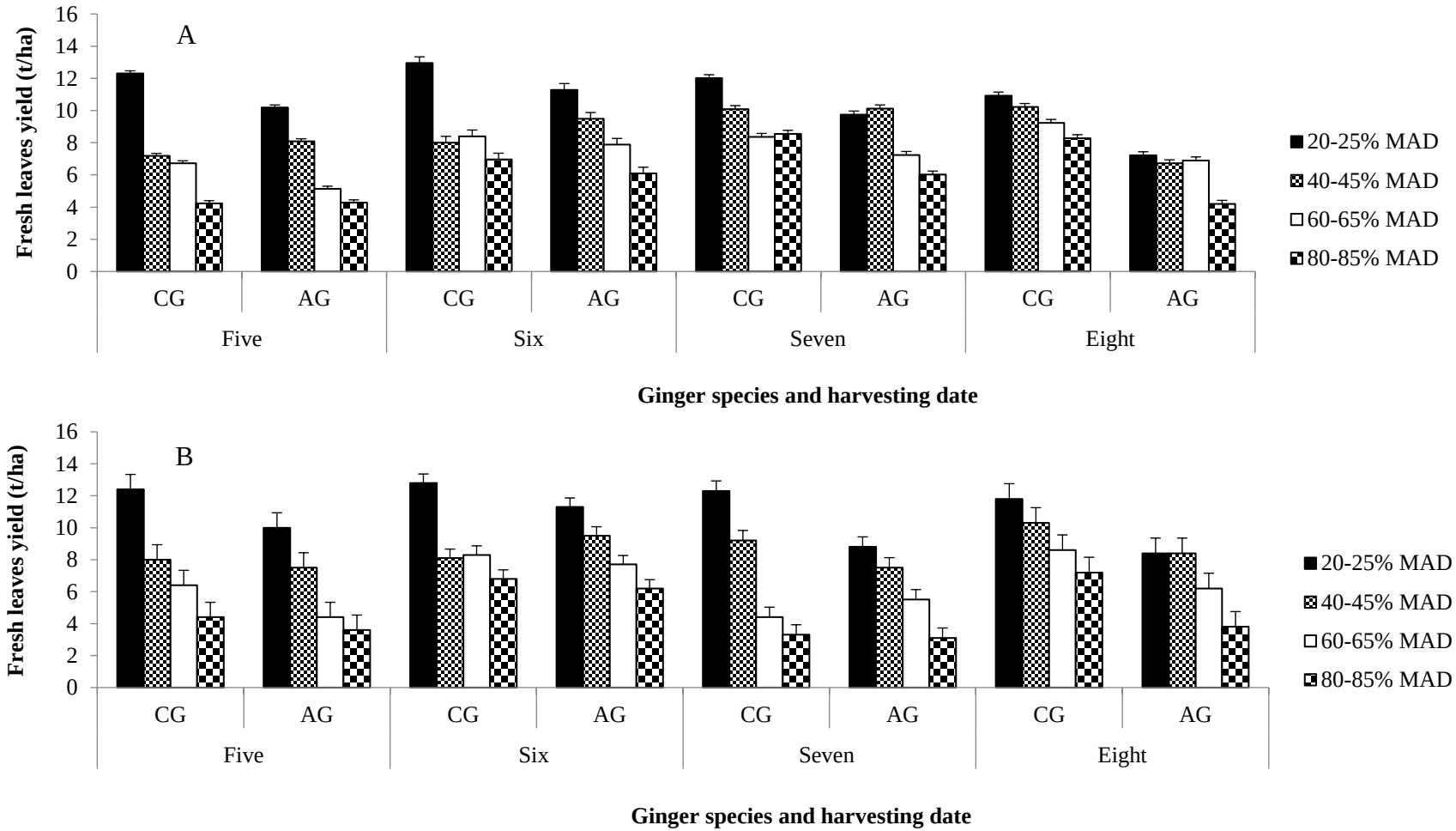


Figure 4-3: Fresh leaf yield (t.ha^{-1}) of commercial ginger (CG) and African ginger (AG) at different harvest times (five to eight months after planting) in response to four water regimes during (A) 2015/2016 and (B) 2016/2017 cropping seasons.

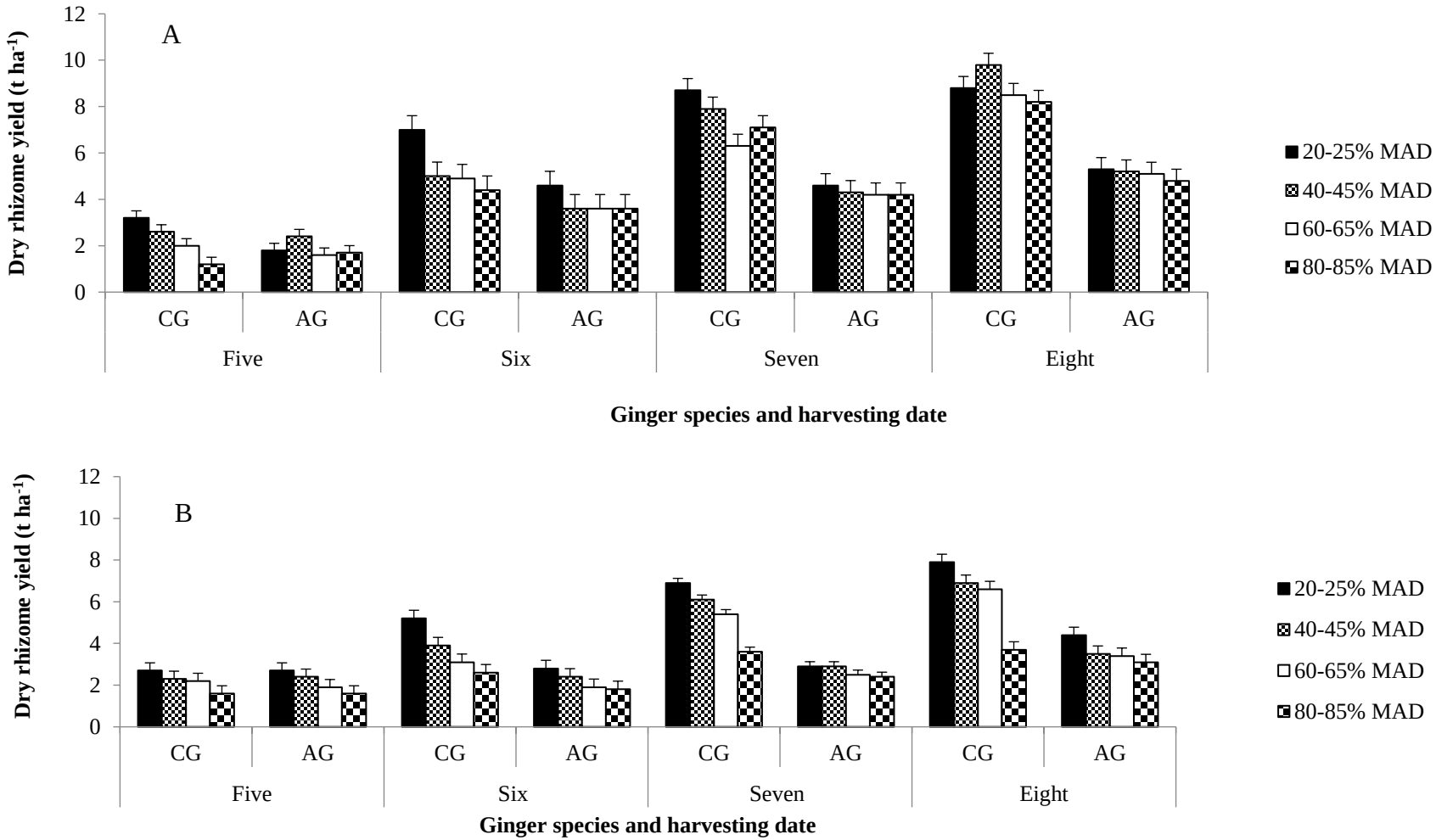


Figure 4-4: Dry rhizome yields (t.ha^{-1}) of commercial ginger (CG) and African ginger (AG) at different harvest times (five to eight months after planting) in response to four water regimes during (A) 2015/2016 and (B) 2016/2017 cropping seasons.

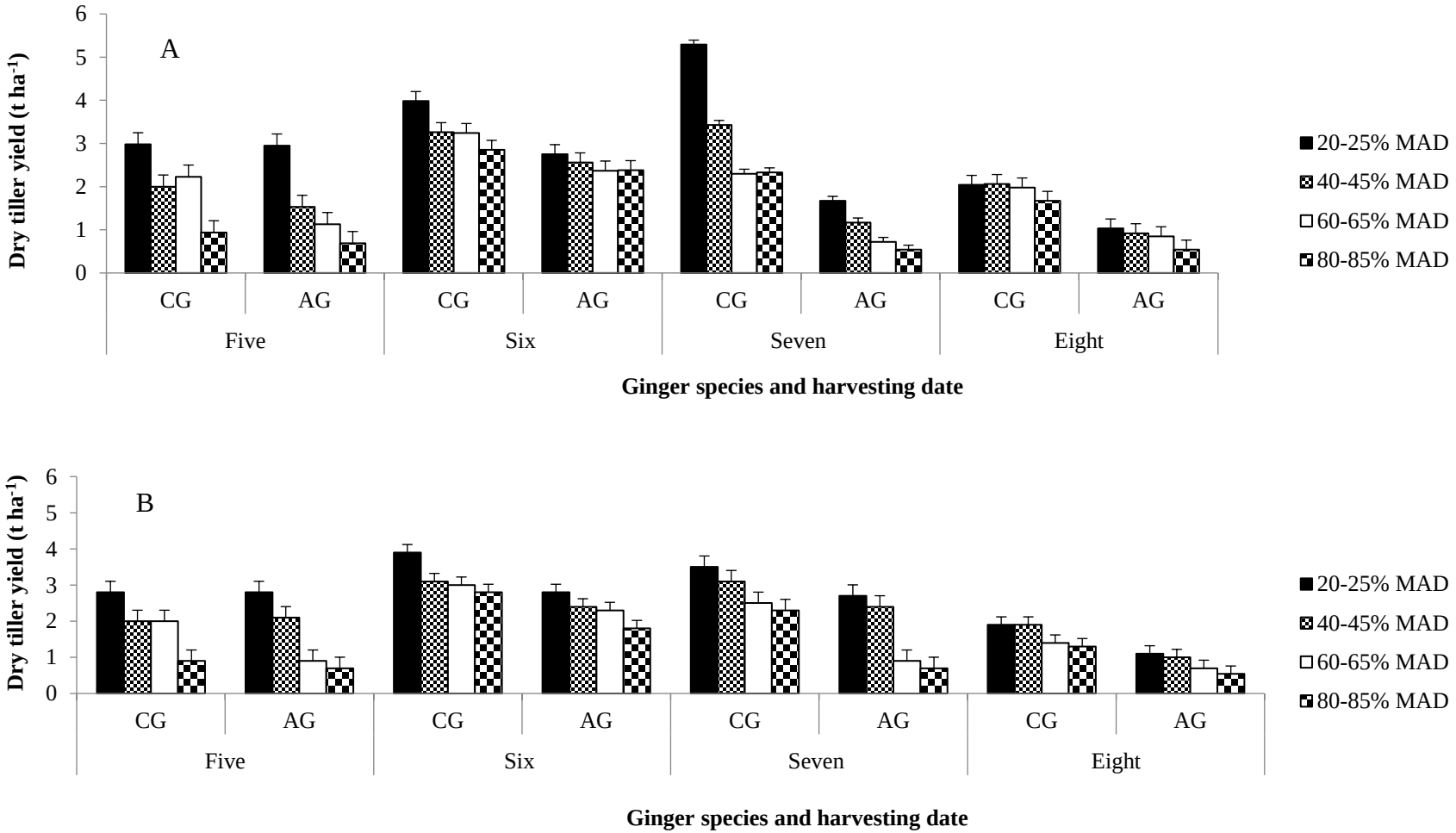


Figure 4-5: Dry tiller yields (t.ha^{-1}) of commercial ginger (CG) and African ginger (AG) at different harvest times (five to eight months after planting) in response to four water regimes during (A) 2015/2016 and (B) 2016/2017 cropping seasons.

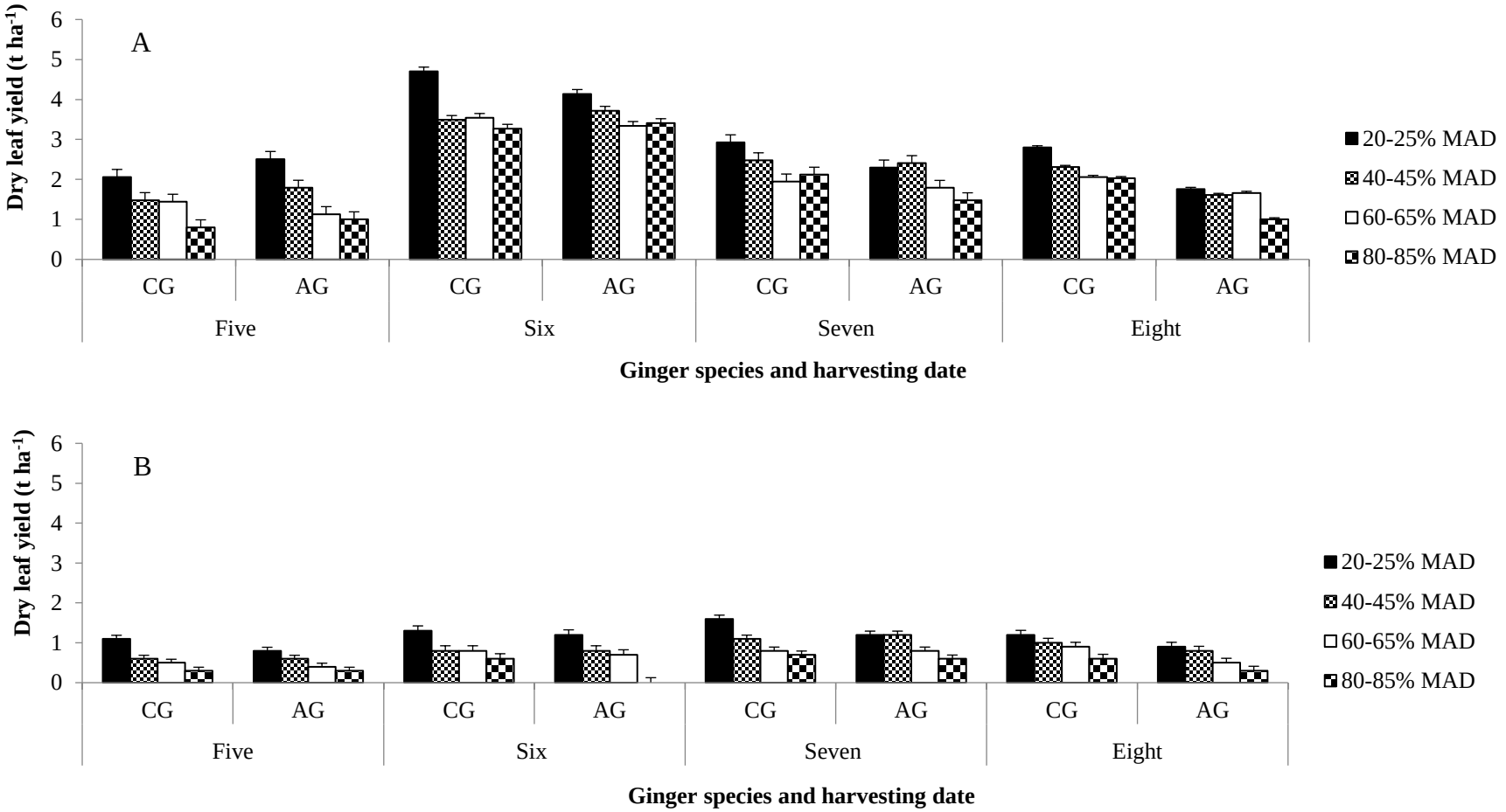


Figure 4-6: Dry leaf yields (t.ha^{-1}) of commercial ginger (CG) and African ginger (AG) at different harvest times (five to eight months after planting) in response to four water regimes for (A) 2015/2016 and (B) 2016/2017 cropping season.

4.3.4 Total flavonoid content of ginger rhizomes, leaves and tillers

In this study, the effects of four water regimes and two species of ginger were evaluated on total phenolic content, flavonoid content and antioxidant activity. Significant differences were recorded for all water regimes, species of ginger and different parts assessed at varying harvest stages of all plant species (Tables 4.3, 4.4 and 4.5). Table 4.3 indicates that the severely stressed treatment (80-85% MAD) recorded a significant increase in total flavonoid content for the rhizomes of commercial ginger (CG) and African ginger (AG) from the first harvest (month five) to the last harvest (month eight) of the 2015/2016 season. A decline in total flavonoid content was more prevalent for the leaves of CG and AG during the first harvest as compared to the last harvest (Table 4.4). The results further show that rhizomes had the highest flavonoid content compared to tillers and leaves. Flavonoid content of rhizomes also increased with the later harvest, compared to earlier harvests in both ginger species. Flavonoid content at five months after planting was lowest, as was the yield. For commercial ginger harvesting at seven or eight months after planting gave good results for flavonoids as well as dry rhizome yield. Thus, to obtain higher flavonoid content without decreasing the yield significantly, one could opt to apply moderate water stress. Water stressing African ginger did have a substantial positive impact on flavonoid content, while for commercial ginger, it did not. Hence, water stressing African ginger could help to improve flavonoid content without reducing dry rhizome yield. Leaf flavonoid content illustrated a similar trend as rhizomes, but was higher at five months than in the rhizomes, while for the other months it was lower than in rhizomes. A similar trend as for the dry tiller flavonoid content was observed for the leaves. However, it was even lower than that of the leaves at the end of the season.

Water stress is regarded as a factor that causes oxidative stress and was reported to show an increase in the amounts of flavonoids and phenolic acids in willow leaves (Akula & Ravishankar, 2011). A study on *Arabidopsis* demonstrated an increase in flavonoids from three

to five days after drought, indicating that all flavonoids are drought stress-responsive metabolites and had potential to be used as positive markers and potentially mitigation for drought stress (Nakabayashi *et al.,* 2014). The leaves and tillers of both commercial and African ginger showed a significant increase in total flavonoid content with water stress (Tables 4.4 and 4.5). The markedly high flavonoid content differences between different harvest for rhizomes, leaves and tillers of both species could be attributed to seasonal variations. The influence of environmental conditions directly affects the chemical constituents and alters the metabolic levels of medicinal plants (Colling *et al.*, 2010). The reactions of the plants to water stress levels also vary significantly, depending on the intensity and duration of stress as well as plant species, plant part and its stage of development (Chaves *et al.*, 2003). In general, the total flavonoid content of rhizomes of both commercial and African increased from month six to nine (Table 4.3). The total flavonoid content of the leaves and tillers of both plant species increased for the severe water stress conditions of 60-65 and 80-85% MAD (Tables 4.4 and 4.5). The total flavonoid contents reported in our results are in agreement with previous work that characterized the synthesis of primary and secondary metabolites of ginger in response to CO_2 enrichment (Ghasemzadeh & Jaafar, 2011). Furthermore, Akula & Ravishanker (2011) reported also that CO_2 influenced plant growth and flavonoid content in the plant.

Table 4.3: Effect of irrigation regimes and harvest date (months after planting) on total flavonoid content (mg.g^{-1} QE) of rhizomes of African ginger (AG) and commercial ginger (CG). (A) and (B).

(A) Cropping season 2015/2016

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.213^{c}	1.063^{e}	1.016^{g}	1.886^{h}
	40-45	0.123^{d}	2.086^{c}	2.423^{d}	2.886^{d}
	60-65	0.313^{b}	2.273^{b}	2.613^{c}	2.290^{e}
	80-85	0.383^{a}	2.486^{a}	3.286^{b}	3.533^{c}
AG	20-25	0.126^{d}	0.663^{g}	0.713^{h}	1.926^{g}
	40-45	0.073^{e}	0.883^{f}	1.946^{f}	2.223^{f}
	60-65	0.313^{b}	1.063^{e}	2.133^{e}	4.166^{b}
	80-85	0.373^{a}	1.143^{d}	5.533^{a}	6.086^{a}
CV		2.510	0.420	0.850	0.216
LSD		0.017	0.017	0.060	0.019

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

(B) Cropping season 2016/2017

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.233^{d}	1.082^{e}	1.102^{g}	1.908^{f}
	40-45	0.233^{d}	2.186^{c}	2.433^{d}	2.774^{d}
	60-65	0.326^{c}	2.805^{a}	3.353^{c}	2.327^{e}
	80-85	0.466^{b}	2.492^{b}	3.353^{b}	3.520^{c}
AG	20-25	0.140^{e}	0.692^{g}	0.844^{h}	2.144^{e}
	40-45	0.466^{bc}	0.918^{f}	2.070^{f}	2.301^{e}
	60-65	0.466^{a}	1.082^{e}	2.238^{e}	4.265^{b}
	80-85	0.466^{a}	1.245^{d}	6.141^{a}	6.404^{a}
CV		4.061	2.680	1.384	2.280
LSD		0.037	0.120	0.104	0.210

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

Table 4.4: Effect of irrigation regimes and harvest date (months after planting) on total flavonoid content (mg.g^{-1} QE) of leaves of African ginger (AG) and commercial ginger (CG). (A) and (B).

(A) Cropping season 2015/2016

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.263^{e}	0.323^{d}	0.323^{f}	0.483^{g}
	40-45	0.430^{d}	0.253^{e}	0.253^{g}	0.483^{e}
	60-65	0.530^{b}	0.413^{c}	0.353^{e}	0.523^{d}
	80-85	0.740^{a}	0.486^{b}	0.643^{b}	0.783^{b}
AG	20-25	0.213^{f}	0.313^{d}	0.343^{e}	0.323^{g}
	40-45	0.263^{e}	0.313^{d}	0.443^{c}	0.353^{f}
	60-65	0.486^{c}	0.483^{b}	0.403^{d}	0.553^{c}
	80-85	0.740^{a}	1.013^{a}	1.173^{a}	1.026^{a}
CV		0.890	1.236	1.051	1.010
LSD		0.011	0.016	0.014	0.016

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

(B) Cropping season 2016/2017

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.249^{d}	0.257^{f}	0.366^{d}	0.485^{c}
	40-45	0.419^{c}	0.252^{f}	0.267^{e}	0.481^{c}
	60-65	0.559^{b}	0.364^{e}	0.361^{d}	0.570^{bc}
	80-85	0.760^{a}	0.355^{e}	0.680^{b}	0.686^{b}
AG	20-25	0.233^{d}	0.656^{d}	0.375^{d}	0.332^{d}
	40-45	0.273^{d}	0.826^{c}	0.447^{c}	0.464^{c}
	60-65	0.416^{c}	0.952^{b}	0.419^{c}	0.549^{c}
	80-85	0.746^{a}	1.207^{a}	1.191^{a}	1.045^{a}
CV		4.098	3.791	2.493	7.621
LSD		0.054	0.066	0.036	0.126

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

Table 4.5*:* Effect of irrigation regimes and harvest date (months after planting) on total flavonoid content ($mg.g^{-1}$ QE) of stems of African ginger (AG) and commercial ginger (CG). (A) and (B).

(A) Cropping season 2015/2016

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.290^{f}	0.203^{ef}	0.090^{f}	0.243^{d}
	40-45	0.343^{e}	0.266^{d}	0.173^{d}	0.243^{d}
	60-65	0.386^{d}	0.356^{b}	0.203^{c}	0.286^{c}
	80-85	0.486^{c}	0.533^{a}	0.283^{b}	0.413^{a}
AG	20-25	0.153^{g}	0.143^{g}	0.123^{e}	0.133^{f}
	40-45	0.390^{d}	0.186^{f}	0.113^{e}	0.206^{e}
	60-65	0.513^{b}	0.213^{e}	0.213^{c}	0.303^{c}
	80-85	0.823^{a}	0.333^{c}	0.443^{a}	0.383^{b}
CV		1.208	2.190	2.284	2.311
LSD		0.014	0.017	0.013	0.018

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

(B) Cropping season 2016/2017

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.352^{d}	0.310^{d}	0.132^{e}	0.219^{c}
	40-45	0.405^{c}	0.320^{d}	0.239^{c}	0.226^{bc}
	60-65	0.310^{e}	0.354^{c}	0.217^{d}	0.291^{b}
	80-85	0.570^{b}	0.542^{a}	0.305^{b}	0.435^{a}
AG	20-25	0.165^{e}	0.161^{f}	0.134^{e}	0.223^{bc}
	40-45	0.411^{c}	0.173^{f}	0.146^{e}	0.273^{bc}
	60-65	0.585^{b}	0.286^{e}	0.303^{b}	0.464^{a}
	80-85	0.963^{a}	0.383^{b}	0.488^{a}	0.423^{a}
CV		1.947	2.585	2.423	7.753
LSD		0.026	0.023	0.017	0.071

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

4.3.5 Total phenolic content of ginger rhizomes, leaves and tillers

In the present study, African ginger (AG) and commercial ginger (CG) were subjected to four levels of water availability to evaluate the effects of water stress on total phenolic content of rhizomes, leaves and tillers at different harvesting dates (Tables 4.6, 4.7 and 4.8 respectively). Phenolic content was higher in the rhizomes compared to tillers and leaves of both ginger species. The well-watered control (20-25% MAD) decreased the total phenolic content of the rhizomes for both commercial ginger and African ginger (Table 4.7). Rhizomes showed

significantly higher total phenolic content for both CG and AG under the severely stressed treatment (80-85% MAD) at different harvesting months after planting (Tables 4.7). Such an increase could be attributed to the ability of the plant species to scavenge reactive oxygen species (ROS) by enhancing the activities of the antioxidant enzymes during water loss (Gill & Tuteja, 2010). Water stress (60-65% MAD and 80-85% MAD) also resulted in increased total phenolic content in leaves and tillers of both plant species during different harvesting months (Tables 4.8 and 4.9 respectively). Harvesting of commercial ginger at six months after planting revealed higher total phenolic content for the stressed tillers (commercial ginger: 80-85% MAD) compared to other harvest times, while for African ginger harvest at eight months after planting resulted in higher total phenolic contents in the tillers under severe water stress (AG: 80-85% MAD) as compared to earlier harvests. Harvesting of leaves at seven months after planting demonstrated higher phenolic contents for the stressed treatment (CG: 80-85% MAD) of commercial ginger, while for African ginger the highest value was revealed when harvesting at eight months after planting for both the highly stress treatments (commercial ginger: 60-65% MAD and commercial ginger: 80-85% MAD).

The lowest means of total phenolic content for tillers were recorded under well-watered (20-25% MAD) conditions for African ginger (Table 4.8). The decrease in total phenolic content under well-watered conditions in the present study agrees with earlier findings which suggest that increased irrigation can limit certain secondary metabolite components (Battaieb *et al.*, 2010). Furthermore, Lafka *et al*. (2007) described a reduction in winery waste total phenolic content due to the increased amount of water applied. These results were conflicting with reports by Jiang & Huang (2001) and Weidner *et al.* (2009), who established that environmental stress couldcause either a decline or an increase in the content of phenolic compounds in a cell. This is despite the notion that some of the phenolic compounds, such as phenolic acids or flavonoids, are widely known and present in most plant species (Cai *et al.*,

2004). It should be noted that the adaptation period of plants to water stress might affect phenol metabolism, causing variation from one stress level to another.

Table 4.6: Effect of irrigation regimes and harvest date (months after planting) on total phenolic content (mg.g^{-1} GAE) of rhizomes of African ginger (AG) and commercial ginger (CG). (A) Cropping season 2015/2016 and (B) Cropping seasons 2016/2017.

(A) Cropping season 2015/2016

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	2.783^{g}	10.773^{f}	7.633^{h}	7.123^{h}
	40-45	4.583^{f}	11.033^{e}	8.763^{g}	8.443^{g}
	60-65	8.533^{d}	11.463^{c}	12.333^{c}	11.433^{d}
	80-85	14.686^{b}	15.753^{a}	14.633^{a}	13.743^{b}
AG	20-25	4.583^{f}	8.763^{h}	10.833^{e}	9.286^{f}
	40-45	6.653^{e}	9.563^{g}	9.663^{f}	10.033^{e}
	60-65	11.723^{c}	11.143^{d}	11.983^{d}	13.153^{c}
	80-85	17.633^{a}	12.573^{b}	13.953^{b}	16.173^{a}
CV		0.345	0.040	0.263	0.187
LSD		0.088	0.013	0.085	0.060

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

(B) Cropping season 2016/2017

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	2.659^{g}	11.243^{e}	8.073^{e}	7.146^{g}
	40-45	6.920^{f}	11.863^{cd}	9.123^{d}	9.816^{f}
	60-65	8.550^{d}	11.553^{de}	13.150^{b}	11.620^{d}
	80-85	14.936^{b}	16.113^{a}	14.566^{a}	13.650^{b}
AG	20-25	4.684^{f}	8.880^{g}	11.126^{c}	9.8166^{e}
	40-45	6.920^{e}	10.106^{f}	10.456^{c}	11.856^{c}
	60-65	11.833^{c}	12.21b^{c}	11.103^{c}	13.546^{b}
	80-85	18.030^{a}	12.626^{b}	13.360^{b}	17.560^{a}
CV		0.720	0.260	2.877	0.623
LSD		0.187	0.429	0.942	0.210

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

Table 4.7: Effect of irrigation regimes and harvest date (months after planting) on total phenolic content (mg.g^{-1} GAE) of leaves of African ginger (AG) and commercial ginger (CG) species. (A) Cropping season 2015/2016 and (B) Cropping seasons 2016/2017.

(A) Cropping season 2015/2016

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	7.063^{g}	5.530^{h}	5.313^{f}	6.223^{f}
	40-45	8.733^{e}	5.583^{g}	6.643^{d}	6.533^{e}
	60-65	9.633^{c}	6.323^{e}	7.173^{c}	7.543^{c}
	80-85	9.873^{b}	10.613^{a}	12.090^{a}	9.600^{b}
AG	20-25	4.523^{h}	6.063^{f}	3.313^{g}	4.153^{h}
	40-45	8.633^{f}	6.533^{d}	5.846^{e}	5.423^{g}
	60-65	9.183^{d}	7.123^{c}	6.643^{d}	7.383^{d}
	80-85	11.886^{a}	9.290^{b}	9.553^{b}	9.663^{a}
CV		0.346	0.064	0.093	0.066
LSD		0.086	0.013	0.019	0.013

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

(B) Cropping season 2016/2017

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	0.259^{f}	0.284^{f}	0.366^{d}	0.348^{f}
	40-45	0.509^{d}	0.247^{f}	0.264^{f}	0.484^{d}
	60-65	0.581^{c}	0.255^{f}	0.361^{d}	0.637^{c}
	80-85	0.764^{a}	0.342^{e}	0.688^{b}	0.666^{b}
AG	20-25	0.233^{g}	0.656^{d}	0.359^{de}	0.357^{f}
	40-45	0.270^{f}	0.806^{c}	0.337^{e}	0.461^{e}
	60-65	0.473^{e}	0.950^{b}	0.506^{c}	0.646^{c}
	80-85	0.736^{b}	1.231^{a}	1.191^{a}	1.049^{a}
CV		1.160	3.094	3.094	1.138
LSD		0.016	0.053	0.053	0.019

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

Table 4.8: Effect of irrigation regimes and harvest date (months after planting) on total phenolic content (mg.g-^{1} GAE) of tillers of African ginger (AG) and commercial ginger (CG) species. (A) Cropping season 2015/2016 and (B) Cropping seasons 2016/2017.

(A) Cropping season 2015/2016

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	1.080^{g}	2.680^{h}	3.470^{f}	1.570^{h}
	40-45	1.433^{e}	6.833^{d}	3.583^{e}	3.423^{f}
	60-65	3.473^{b}	7.763^{b}	4.683^{c}	3.573^{e}
	80-85	4.743^{a}	13.213^{a}	5.586^{b}	4.533^{c}
AG	20-25	0.243^{h}	2.833^{g}	2.153^{h}	1.783^{g}
	40-45	1.353^{f}	3.053^{f}	3.313^{g}	4.373^{d}
	60-65	1.573^{d}	5.733^{e}	4.153^{d}	5.003^{b}
	80-85	3.053^{c}	7.123^{c}	8.713^{a}	7.543^{a}
CV		0.918	0.437	0.121	0.051
LSD		0.056	0.077	0.015	0.005

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

(B) Cropping season 2016/2017

		Months			
Ginger species	MAD	Five	Six	Seven	Eight
CG	20-25	1.146^{g}	2.625^{g}	0.105^{f}	0.222^{e}
	40-45	1.313^{f}	7.013^{c}	0.243^{c}	0.229^{e}
	60-65	3.543^{b}	8.120^{b}	0.217^{d}	0.291^{d}
	80-85	4.743^{a}	14.023^{a}	0.315^{b}	0.438^{b}
AG	20-25	0.343^{h}	3.013^{f}	0.132^{e}	0.217^{e}
	40-45	1.460^{e}	3.190^{e}	0.144^{e}	0.306^{d}
	60-65	1.623^{d}	7.013^{d}	0.306^{b}	0.474^{a}
	80-85	3.150^{c}	8.153^{b}	0.488^{a}	0.420^{c}
CV		2.068	0.533	2.292	1.942
LSD		0.129	0.100	0.016	0.018

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$

4.3.6 Total antioxidant content of ginger rhizomes, leaves and tillers

The ferric reducing power assay (FRAP) was used to determine total antioxidant content in rhizomes, leaves and tillers of both ginger species in response to water stress and harvest date (Figures 4.7, 4.8 and 4.9), respectively. For all harvest dates, the rhizomes recorded the highest FRAP values, indicating their potential as antioxidants, under severely stressed conditions (80-85% and 60-65% MAD), followed by moderate stress (40-45% MAD) (Figure 4.7). It was

shown that antioxidants were higher within rhizomes compared to leaves (Figure 4.7 and 4.8) and tillers (Figure 4.9). Photosynthetic carbon metabolism can control or generate photochemistry that can increase secondary metabolites, such as antioxidant content in plants under water stress (Chaves *et al.,* 2002). Although environmental factors can influence secondary metabolites in plants (Ghasemzadeh *et al.,* 2010), the antioxidant activity of the plant extract largely depends on both the composition of the extract and the test system (Figueirodo *et al.,* 2008). The control plants showed lower FRAP concentrations in the rhizomes of both ginger species. In the case of leaves and tillers of both ginger species, the lowest FRAP values were found under control conditions, followed by moderately stressed conditions (Figures 4.8 and 4.9).

The increasing trend of antioxidant values obtained from different plant parts of African ginger and commercial gingerhas the potential to be used in various pharmacological preparations. The tillers also showed the highest antioxidant values for harvest five months after planting and six months, indicating their potential for antioxidant capacity at different harvest dates. The standard ascorbic acid was higher than all irrigation treatments for the rhizomes, leaves and tillers in all harvesting stages (Figure 4.7, 4.8 and 4.9). Mano (2002) reported that the sequence of events in plant tissue subjected to drought stress is firstly an increase in reactive oxygen species (ROS), followed by increases in the expression of genes for antioxidant function and increases in the level of anti-oxidative systems and antioxidants. Thus, these mechanisms may also cause the stressed treatments to increase antioxidant content in ginger species. Abiotic stresses lead to the production of reactive oxygen species (ROS) in plants. Reactive oxygen species (ROS) are toxic and may cause significant damage to lipids, proteins, DNA and carbohydrates, resulting in oxidative stress in most plants. However, in this case, the antioxidants protect plants against oxidative stress damage (Gill & Tuteja, 2010). Thus, antioxidants are important components for neutralizing oxidative damage caused by free

radicals in cells, tissue and blood (Saleh *et al.*, 2010). Antioxidants are important for delaying oxidation of other molecules by inhibiting propagation of oxidizing reaction chains by free radicals, reducing oxidative damage to the human body (Namiki, 1990). Antioxidants have various photochemistry compounds that scavenge free radicals and have shown to protect cells against oxidative stress involved in many different chronic diseases (Shukla & Singh, 2007; Kikusaki & Nakatani, 1993).

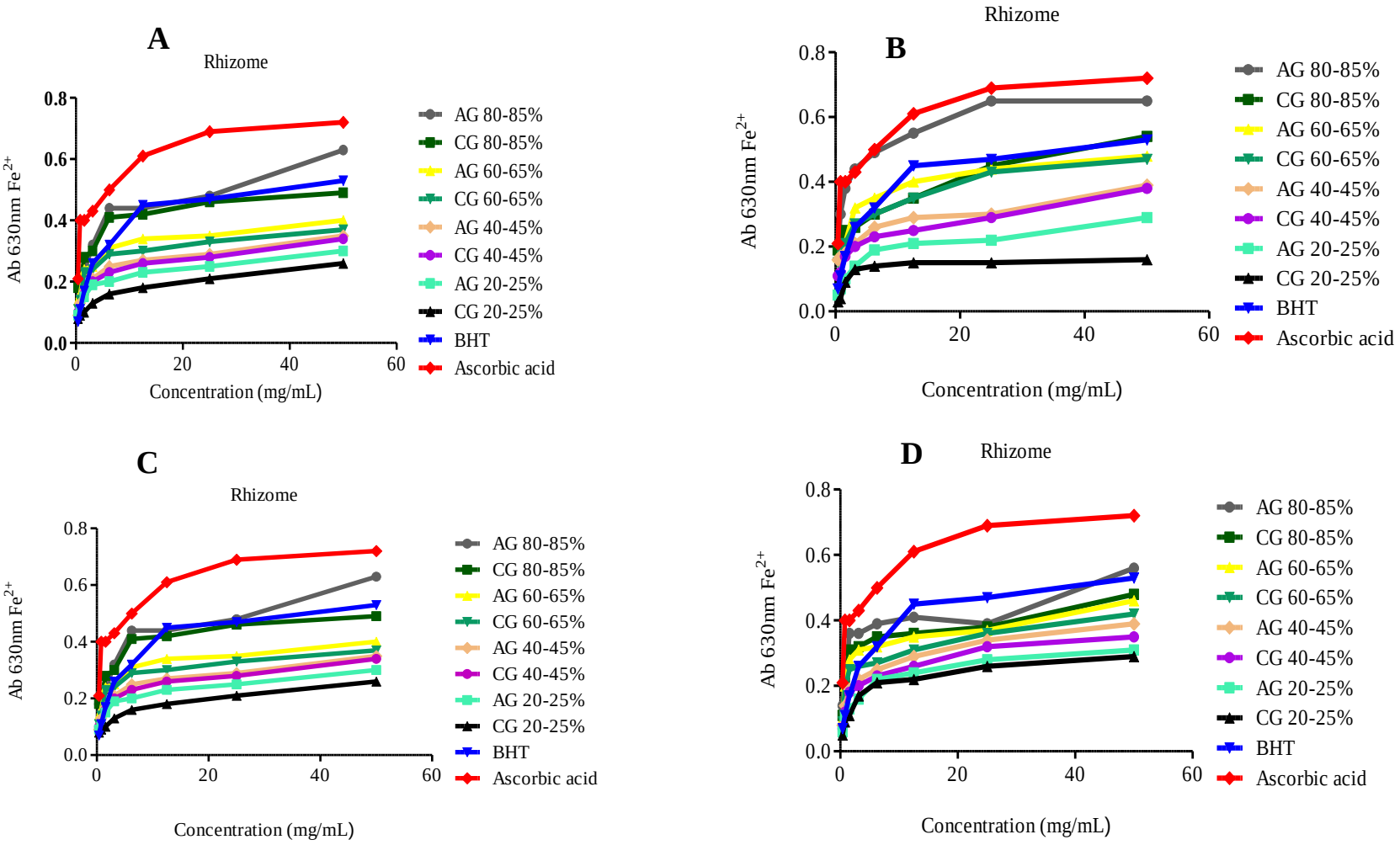


Figure 4-7: Total antioxidant content of commercial ginger (CG) and African ginger (AG) rhizomes at different harvest times, five (A), six (B), seven (C) and eight (D) months after planting in response to four water regimes during the cropping season.

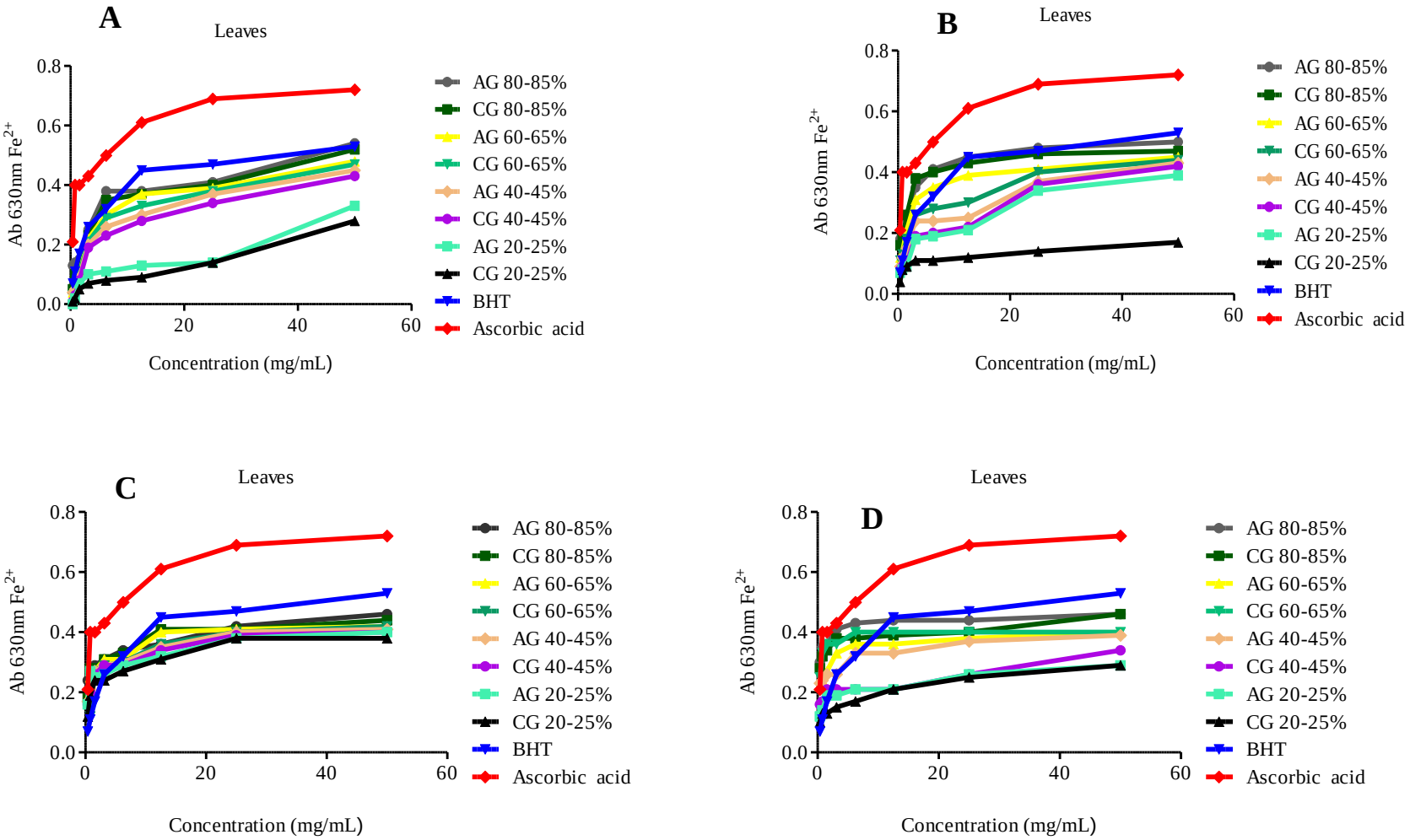


Figure 4-8: Total leaf antioxidant content of commercial ginger (CG) and African ginger (AG) at different harvest times five (A), six (B), seven (C) and eight (D) months after planting in response to four water regimes during the cropping season.

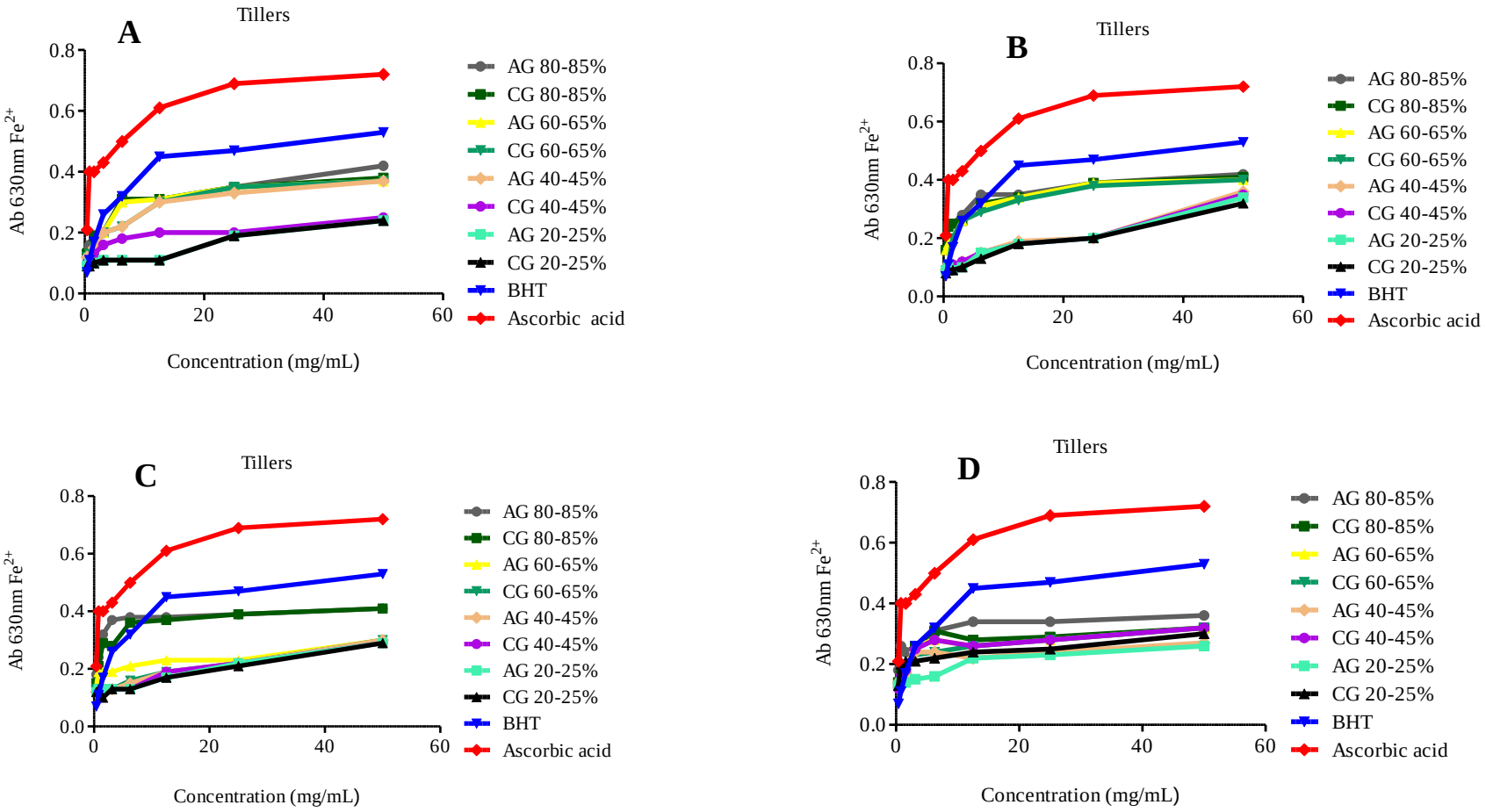


Figure 4-9: Total stem antioxidant content of commercial ginger (CG) and African ginger (AG) at different harvest times five (A), six (B), seven (C) and eight (D) months after planting in response to four water regimes during the cropping season.

4.3.7 Water use efficiency in terms of fresh and dry rhizome yields of commercial ginger (CG) and African ginger (AG).

There was a difference between water use efficiency on fresh and dry rhizomes yield among commercial and African ginger both (A) and (B) cropping seasons (Table 4.9 and 4.10). The results are clear that commercial ginger fresh rhizome water use efficiency in the first season was higher with severely water stress of CG: 60-85% MAD compared to well-watered (CG: 20-25% MAD) and less stressed treatments (CG: 40-45% MAD). The results revealed that commercial ginger increased water use efficiency compared to African ginger. However, fresh rhizome yield water use efficiency in the second seasons was higher with CG: 60-65% MAD following CG: 40-45% MAD for five months after planting compared to other treatments. Six, seven and eight months after harvesting, treatment CG: 40-45, CG: 60-65 and 80-85% MAD were higher compared to other treatments within the treatments and with African ginger. In general, it was shown that commercial ginger water use efficiency was higher compare to African ginger at all harvest intervals.

Dry rhizome water use efficiency of the first season was higher with CG:60-65% and CG:80-85% MAD; CG: 80-85% and CG:60-65% MAD; CG:80-85 and CG:60-85% MAD; CG:80-85% and CG:60-65% MAD respectively for five, six, seven and eight months after harvesting in both cropping seasons (Table 4.10). In general, dry rhizome water use efficiency was higher with commercial ginger at CG: 40-45% MAD, followed by the moderately stressed treatment (CG: 60-65% MAD). The results show that commercial ginger with 60-65% and 80-85% MAD was higher compare to other treatments. Similar results were found by Yuan *et al.* (2003), who reported a higher irrigation water use efficiency in a potato crop with less water applied.

Table 4.9: Fresh rhizome water use efficiency of commercial ginger (CG) and African ginger (AG) in response to four water stress regimes at different harvest times (from five to eight months after planting).

(A) First season 2015/2016.

	Ginger species	Five			Six			Seven			Eight		
		ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE
MAD		mm	t.ha^{-1}	t·ha^{-1} mm^{-1}	mm	t.ha^{-1}	t·ha^{-1} mm^{-1}	mm	t.ha^{-1}	t·ha^{-1} mm^{-1}	mm	t.ha^{-1}	t·ha^{-1} mm^{-1}
20-25%	CG	291.08	32.8	0.11	399.38	34.9	0.09	469.11	36.2	0.08	469.11	38.3	0.08
40-45%		135.51	24.5	0.18	260.41	26	0.10	284.47	28.2	0.10	284.47	30.4	0.11
60-65%		68.90	22.5	0.33	126.80	24.6	0.19	176.02	26	0.15	176.02	27.4	0.16
80-85%		42.53	15.8	0.37	80.70	17.8	0.22	109.02	21	0.19	109.02	22.1	0.20
20-25%	AG	280.90	23	0.08	398.90	21.3	0.05	461.55	18.9	0.04	461.55	21.5	0.05
40-45%		128.66	13.4	0.10	241.80	16.5	0.07	274.58	19.9	0.07	274.58	21.3	0.08
60-65%		71.38	10.7	0.15	138.46	16.3	0.12	183.46	20.1	0.11	183.46	21	0.11
80-85%		48.82	10.7	0.22	92.90	18	0.19	117.32	16.6	0.14	117.32	18.8	0.16

CG: Commercial ginger; AG: African Ginger

(B) Second season: 2016/2017

MAD	Ginger species	Five			Six			Seven			Eight		
		ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE
		mm	t.ha^{-1}	t.ha^{-1}mm^{-1}	mm	t.ha^{-1}	t.ha^{-1} mm^{-1}	mm	t.ha^{-1}	t·ha^{-1}mm^{1}	mm	t.ha^{-1}	t.ha^{-1}mm^{-1}
20-25%	CG	531.92	33.6	0.06	549.14	40	0.07	549.14	42	0.08	549.14	42.80	0.078
40-45%		347.57	24.9	0.07	361.79	31	0.09	361.79	41	0.11	361.79	42.10	0.116
60-65%		321.67	24.9	0.08	343.89	29.4	0.09	343.89	29.6	0.09	343.89	35.70	0.104
80-85%		329.63	16.3	0.05	328.73	28.9	0.09	328.73	28.9	0.09	328.73	34.10	0.104
20-25%	AG	525.93	13.6	0.03	542.85	15.4	0.03	542.85	19.2	0.04	542.85	22.20	0.041
40-45%		349.76	6	0.02	380.95	8.6	0.02	380.95	8.8	0.02	380.95	11.80	0.031
60-65%		347.08	5.2	0.01	362.65	6	0.02	362.65	7.3	0.02	362.65	9.7	0.027
80-85%		359.56	4.1	0.01	340.68	4.8	0.01	340.68	5.4	0.02	340.68	6.2	0.018

CG: Commercial ginger; AG: African Ginger, WUE: Water use efficiency, ET: Evapotranspiration

Table 4.10: Dry rhizome water use efficiency of commercial (CG) and African ginger (AG) in response to four water stress regimes at different harvest (from five to eight months after planting).

(A) First season 2015/2016.

	Ginger	Five			Six			Seven			Eight		
		ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE
MAD	species	mm	$t.ha^{-1}$	$t.ha^{-1}\ mm^{-1}$	mm	$t.ha^{-1}$	$t.ha^{-1}\ mm^{-1}$	mm	$t.ha^{-1}$	$t.ha^{-1}\ mm^{-1}$	mm	$t.ha^{-1}$	$r.ha^{-1}\ mm^{-1}$
20-25%	CG	291.08	3.2	0.01	399.38	7	0.02	469.11	8.7	0.02	469.11	8.8	0.02
40-45%		135.51	2.6	0.02	260.41	5	0.02	284.47	7.9	0.03	284.47	9.8	0.03
60-65%		68.90	2	0.03	126.80	4.9	0.04	176.02	6.3	0.04	176.02	8.5	0.05
80-85%		42.53	1.2	0.03	80.70	4.4	0.05	109.02	7.1	0.07	109.02	8.2	0.08
20-25%	AG	280.90	1.8	0.01	398.90	4.6	0.01	461.55	4.6	0.01	461.55	5.3	0.01
40-45%		128.66	2.4	0.02	241.80	3.6	0.01	274.58	4.3	0.02	274.58	5.2	0.02
60-65%		71.38	1.6	0.02	138.46	3.6	0.03	183.46	4.2	0.02	183.46	5.1	0.03
80-85%		48.82	1.7	0.03	92.90	3.6	0.04	117.32	4.2	0.04	117.32	4.8	0.04

CG: Commercial ginger; AG: African Ginger, WUE: Water use efficiency, ET: Evapotranspiration

(B) Second season 2016/2017

	Ginger	Five			Six			Seven			Eight		
		ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE
MAD	species	mm	$t.ha^{-1}$	$t{\cdot}ha^{-1}mm^{-1}$	mm	$t.ha^{-1}$	$t.ha^{-1}\ mm^{-1}$	mm	$t.ha^{-1}$	$t.ha^{-1}mm^{-1}$	mm	$t.ha^{-1}$	$t.ha^{-1}mm^{-1}$
20-25%	CG	531.92	2.7	0.005	549.14	5.2	0.009	549.14	6.9	0.013	549.14	7.9	0.014
40-45%		347.57	2.3	0.007	361.79	3.9	0.011	361.79	6.1	0.017	361.79	6.9	0.019
60-65%		321.67	2.2	0.007	343.89	3.1	0.009	343.89	5.4	0.016	343.89	6.6	0.019
80-85%		329.63	1.6	0.005	328.73	2.6	0.008	328.73	3.6	0.011	328.73	3.7	0.011
20-25%	AG	525.93	2.7	0.005	542.85	2.8	0.005	542.85	2.9	0.005	542.85	4.4	0.008
40-45%		349.76	2.4	0.007	380.95	2.4	0.006	380.95	2.9	0.008	380.95	3.5	0.009
60-65%		347.08	1.9	0.005	362.65	1.9	0.005	362.65	2.5	0.007	362.65	3.4	0.009
80-85%		359.56	1.6	0.004	340.68	1.8	0.005	340.68	2.4	0.007	340.68	3.1	0.009

CG: Commercial ginger; AG: African Ginger, WUE: Water use efficiency, ET: Evapotranspiration

4.4 CONCLUSIONS

In the present study, it was observed that the well-watered treatment (20-25% MAD) had higher rates for growth and yield, enhancing production. Water use was higher for the controls in both ginger species, followed by the less, moderate and severely stressed treatments. The results show that LAI was higher with African ginger in both seasons. Well-watered treatments (20-25% MAD) and less stressed of (40-45% MAD) in both species revealed the higher LAI than the moderate and severely stressed. Water-stressed irrigation regimes (i.e. 80-85% MAD) resulted in higher production of total flavonoid content, phenolic content and increased antioxidant activity. The increases were shown in all parts of the plants (rhizomes, tillers and leaves).

Comparison between ginger species revealed that commercial ginger yielded higher than African ginger. As time progressed, rhizome yield increased in both ginger species, resulting in the highest yield at the last harvest. There were variations in phenolic, flavonoid and antioxidant contents of both commercial ginger and Africa ginger over the harvest period. Flavonoids in the rhizomes increased with time in both ginger species. It was evident that at five months after planting the flavonoid content as well as the yield for both species were very low and harvesting at this stage should not be recommended for either species. For commercial ginger harvesting at seven or eight months after planting should give good results for flavonoids as well as dry rhizome yield. Also, to obtain higher flavonoid content without decreasing the yield, significantly moderate water stress may be applied.

Water stressing African ginger did have a huge positive impact on flavonoid content, and as for commercial ginger, it did not significantly reduce yield. Therefore, water stressing African ginger could help improve flavonoid content without reducing dry rhizome yield. The results demonstrated a similar trend for leaves as for rhizomes regarding flavonoid content. The

research suggests that the more plants are stressed, the higher the increase in total antioxidant content of both ginger species. Flavonoid, phenolic and antioxidant concentrations are generally enhanced in both ginger species for severe stressed treatments. This increase could either be due to water stress decline in biomass production associated with an unchanged biosynthesis rate of natural products, or to an authentic enhancement of the total secondary metabolite content such as of phenolics, flavonoids and antioxidants. The findings indicate that total phenolic, flavonoid and antioxant activities were increased with stress water regimes of both ginger species. Plants exposed to drought stress accumulate higher concentrations of secondary metabolites, including phenolics. Such increase is reported to occur in nearly all classes of natural products, such as simple or complex phenols. However, drought stress frequently enhances the concentration of secondary plant metabolites(Selmar & Kleinwachter, 2013; Valentine et al., 2003). In general, water use efficiency was higher with moderate and severely stressed treatments (CG: 60-65% and (CG: 80-85% MAD) of commercial ginger. This increasing water use efficiency was manifested in fresh rhizomes as well as dry rhizomes.

CHAPTER 5
EFFECT OF GINGER SPECIES AND WATER REGIMES ON SOIL MICROBIOLOGY

ABSTRACT

Soil microbial populations and biogeochemical cycling of nutrients, such as carbon, nitrogen, and phosphorus are essential to soil function and of great interest to assess the relative activity of soil microbial communities. The study aimed to assess soil enzymatic activity of soil in which two ginger species were grown and to evaluate carbon profiles between the water stress treatments. The experiment was conducted under a rain-shelter at the Experiment Farm on the Hillcrest campus of the University of Pretoria. The soil of the experiment site was predominately sandy clay loam of the Hutton form with a clay content of 36%. The experiment was laid out in a randomized complete block design (RCBD) with eight treatments and three replicates. Four water regimes were applied based on the maximum allowable depletion of available soil water. From the results, it was clear that African ginger was slightly more conducive to soil health than commercial ginger. Diversity indices were explicit, and a changing percentage of carbon sources was utilised, with values ranging from 0.96 to 2.41 in both ginger species. Soil samples from the commercial ginger treatments exhibited a slightly higher microbial richness than those from African ginger treatments. The soil samples from the African ginger treatments displayed slightly higher overall phosphorous (alkaline phosphatase) mineralisation activities compared to the commercial ginger treatments. The commercial ginger treatments displayed slightly higher overall carbon (ß-glucosidase) and nitrogen (urease) mineralisation activities compared to the African ginger treatments.

Keywords: *Water stress, soil microbial diversity, enzymatic activities, ginger species*

5.1 INTRODUCTION

Soil is endowed with the mixture of various elements as a result of its diverse interaction with physical, chemical and biological components under different environmental conditions. The most important region of the soil is the rhizosphere where the roots of the soil closely interact with microbes in the metabolic process for nutrient uptake, plant growth and to maintain plant health. The microbial community present in rhizosphere soil comprise of bacteria, fungi, viruses, nematodes, algae and protozoa (Raaijmakers *et al.,* 2009). Rhizosphere microbiomes can facilitate the conversion of nutrients, minerals and trace elements in the soil and suppress the occurrences of diseases (Prakash *et al.,* 2015). The continued cycling of nutrients and various activities in the soil occur through processes such as phosphate solubilisation, signal transduction and nitrogen fixation. Research on microbial diversity is crucial to understand the link between diversity, community structure and function. A range of microbiological and molecular techniques can be used to quantify the biological status of soil microbial populations, and potential activities contribute to ecosystem dynamics (Nannipieri *et al.,* 2003). The cycling of nutrients, such as carbon, nitrogen, and phosphorus is fundamental for soil function, and of great interest to assess the relative activity of soil microbial communities (Kara & Bolat, 2008). In this context, microbial community level, physiological profiles and enzymatic activity assays are often used to determine the functional diversity of soil microbial populations. The catabolic diversity of bacterial communities can also be determined based on sole-carbon substrate utilisation, using the Biolog® system.

Communities of organisms will give a characteristic reaction pattern, called a metabolic fingerprint and from the data, carbon source utilisation profiles (CSUP) are generated (Campbell *et al.,* 2003). Contrary to CSUP analysis, microbial enzymatic activity assays consist of several culture-independent methods such as determination of microbial activity without culturing microorganisms. Soil microbial enzymes fulfil essential biochemical

functions in organic matter decomposition in soil systems (Gil-Sotres *et al.,* 2005). The activity of any enzyme assayed in a soil sample is the sum of active and potentially active enzymes from all the different sources. Enzymatic activities such as microbial activity, cycling of carbon, nitrogen (ammonification, nitrification, and denitrification) and the release of inorganic phosphorus in the soil have been used to evaluate the fertility of the soil, and describe the functioning of the ecosystem.

ß-glucosidase has been reported to be a useful indicator of soil quality due to the vital role it plays in catalysing the hydrolysis and biodegradation of various β-glucosides present in plant debris decomposing in the ecosystem (Utobo & Tewari 2015). Phosphatase plays a critical role in P cycling as evidence has shown that they are correlated to P stress and plant growth. Phosphatase activity has been reported to correlate positively with soil phosphorous state, while inorganic phosphorous reduces phosphatase production (Vance *et al.,* 2003).

Similarly, urease plays a vital role in the regulation of N supply to plants, especially after urea fertilisation (Solomon *et al.,* 2010). Due to the influence of pH, temperature, organic matter content and soil moisture on microbial enzymatic activity, they are considered early indicators of ecosystem stress and can act as biological indicators of soil degradation, compared to classical and slowly changing soil properties such as organic matter. Previous reports indicated that soil microbial groups play an essential part in carbon cycling and microorganisms participate in both nitrogen and carbon cycles in the soil ecology (Hamba, 2016).

Distinguished micro-organisms present in rhizosphere soil of some medicinal plants , through their unique and structurally divergent bioactive secondary metabolites that are most likely responsible for the high specificity of the associated microorganisms. The root-associated microbiomes are structured in distinct compartments whose compositions are affected by several factors, such as water regimes, soil type and plant genotype. Water deficit plays a

significant role in soil microbial diversity and nutrient movement in the soil. However, the microbial diversity from the rhizosphere soil in response to water regimes and its physicochemical properties has not been thoroughly investigated. Therefore, to examine the microbial community response to water regimes, we designed a multifactorial experiment to reveal interactions between soil enzymatic activity of carbon profiles and water regime treatments for African and commercial ginger.

5.1 MATERIALS AND METHODS

In this chapter, the material and methods provided in chapter three were used for further processing. The site, plant material, experiments and water use were described in chapter three sections 3.2.1 to 3.2.5.

5.1.2 Soil sampling

Eight months after planting, soils were taken from the soil of the assigned treatments by using an auger (40 cm). In order to avoid microbial contamination from different treatments, the soil auger was sterilised with 100% ethanol when moving between sampling positions. Intact soil cores were placed in sterile plastic bags, stored on ice, and transported to the laboratory. Each soil core was gently broken up along natural points of weakness and passed through a 2 mm sieve (pre-cleaned with ethanol), removing large roots and rocks. We combined replicated soil cores into one composite of 1 kg of sample soil for each plot for aggregate fractionation. A sub-sample of soil was removed immediately and dried at 105 °C for 24 h before microbial community analysis.

5.1.3 Determination of functional diversity

Whole-community substrate utilisation profiles (CSUP) were assessed on the measurements of the carbon sources. Ten grams of soil samples were diluted in sterile distilled water and inoculated into BiologEcoPlates™ (Biolog® Inc., Hayward, USA) containing 31 carbon

sources and control well, in triplicate. The plates were incubated at 28 °C. Respiration of carbon sources by microbial populations reduce the tetrazolium dye, causing a colour change which was measured twice daily over a period of 5-10 days at 590 nm to determine average well colour development (AWCD). The functional diversity of the soil microbial populations was determined using the amount and equitability of carbon substrates metabolized as indicators of richness and evenness, respectively.

5.1.4 Determination of soil microbial enzymatic activity

The enzymatic activities were assayed by estimating potential ß-glucosidase, phosphatase, and urease activities in the soil. One gram of soil for the beta-glucosidase and phosphatase was used, while 5 gram of soil was used for the urease analyses. The ß-Glucosidase and phosphatase activities were calculated following method by Dick *et al.* (1996) with slight modifications by determining the release of p-nitrophenyl after the 1 h incubation of soil with p-nitrophenyl glucoside and p-nitrophenyl phosphate, respectively using spectrophotometer at 410 nm. The urease activity was determined using the method by Kandeler & Gerber (1988), where released ammonia was spectrophotometrically measured after the 2 h incubation of soil samples with a urea solution at a wavelength of 690 nm. The urea content was calculated using a standard calibration graph designed from a series of urea concentrations

5.1.5 Statistical analyses

Data on carbon source utilisation were subjected to non-parametric statistical analyses using Statistica 13 (StatSoft Inc. Tulsa, OK, USA). One-way analysis of variance (ANOVA) and factorial ANOVA were used to determine significant differences between treatment combinations. A dendrogram was constructed using Ward's clustering algorithm, and the Euclidean distance measure, i.e. the geometric distance between variables in a multidimensional space. Homogenous grouping with Fisher Least Significant Difference (LSD) was calculated at the 5% level of significance ($p < 0.05$).

5.2 RESULTS AND DISCUSSION

5.2.1 Effect of water stress on microbial activity

The mechanism of colour development in BiologEcoPlates^{TM} is related to differences in carbon source utilization (CSU), *i.e.* food source consumption, which appears to relate to the number of viable microorganisms able to utilize the carbon sources ("food sources") within the wells of the EcoPlate. The effects of the different treatments on the active bacterial functional diversity are illustrated by using Principal Component Analysis (PCA) in Figure 5.1. The results in Figure 5.1 indicate two main clusters: Treatments AG: 20-25, AG: 40-45, AG: 80-85 and CG: 40-45% MAD in the blue circle; and treatments CG: 20-25, CG: 60-65, CG: 80-85 and AG: 60-65% MAD in the red circle. These results depict the average CSU of the soil microbial populations indicating differences in carbon source utilisation profiles between the different treatments. These variations indicate the changes in microbial functioning between the different treatments and plots. Since the 2-D ordination of the PCA might be unclear in demonstrating well-defined distinctions between groups, cluster analysis was performed as an alternative measure to enable a 2-D visualisation of the different groups illustrated in Figure 5.1. The dendogram was constructed with the aid of cluster analysis to assign treatments into groups for comparison of treatments in the same cluster and in other clusters as illustrated in Figure 5.2.

Cluster analyses (Figure 5.2) revealed two main clusters (blue and red blocks) with all the African ginger treatments clustering to the left (blue block) and treatments CG: 20-25 and CG: 60-65% aligning to the far right (red block) for commercial ginger. Results obtained show that carbon sources utilised by soil microbial communities for commercial ginger CG: 80-85% MAD closely resembles the carbon sources utilised by treatments AG: 60-65 and AG: 40-45% MAD. The carbon sources utilised in treatment CG: 40-45% MAD is more similar to carbon sources utilised in the AG treatments, than in the CG: 20-25 and CG: 60-65% MAD treatments.

The carbon source utilisation profile (CSUP) of AG: 20-25% MAD differed significantly from the CSUP of treatments CG: 20-25, CG: 60-65, CG: 80-85 and AG 60-65% MAD, while treatment CG: 20-25% MAD differed significantly from the CG: 40-45, AG: 20-25 and AG: 80-85% MAD treatments. Previous reports have shown that water stress could alter the amount and composition of root exudation which could potentially lead to the selective enrichment of certain microorganisms in the rhizosphere (Song *et al.,* 2012; Henry *et al.,* 2008). From the PCA and the dendrogram, both related to carbon source utilisation, the African ginger treatments tended to group together with the exception on AG60-65, while for commercial ginger the PCA results clustered three of the four treatments together with CG40-45 being the out layer. In the dendrogram, the commercial ginger results are even more scattered, and can be placed in three different groupings.

The plant response to water deficit could have interceded some of the compartment-specific enrichments as stress levels trigger a complex of molecular and physiological response associated with microbes that are actively reactant (Sheibani-Tezerji *et al.,* 2015). According to Zang *et al.* (2014), the plant's response under water stress conditions can enhance root growth that assists in maintaining water uptake, even as shoot growth is hindered.

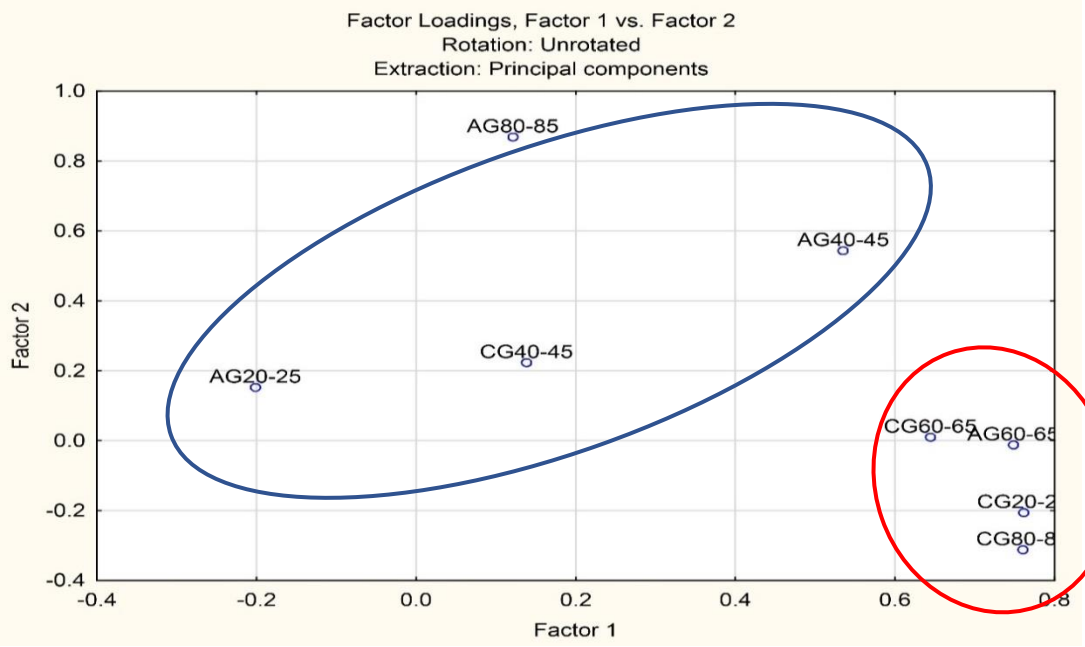


Figure 5-1: PCA ordination plot illustrating the differences in the average carbon source utilisation profiles between treatments

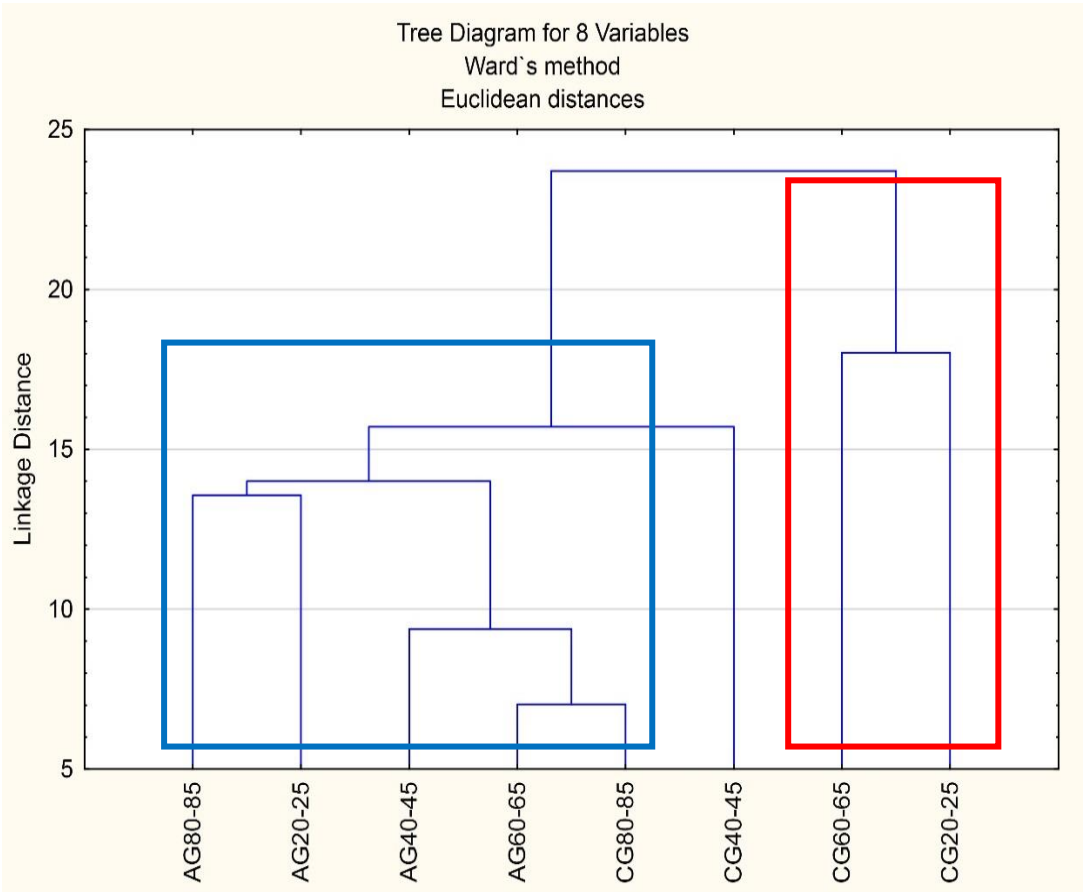


Figure 5-2: Dendrogram illustrating the carbon source utilisation profiles between sampled treatments.

5.2.2 Diversity Indices

The Shannon-Weaver substrate diversity index (*H'*) is used to quantify the functional diversity of soil microbial communities based on the number of different carbon sources utilised by soil microbial communities in BiologEcoPlates™ comparable to species richness in the soil (Lupwayi *et al.,* 2001). The Shannon-Weaver index range between 1.5 and 3.5, but rarely increased above 4.5 (Magurran, 1988). The water regime treatments varied in percentage of richness and values ranged from 0.96 to 2.40 (Table 5.1). The evenness index was used as an indication of how abundant species are within a soil microbial community. This includes the measure and how close the numbers of different microbial species are in the soil microbial community. If the abundance of different species in a community is measured, it will invariably be found that some species are rare, whereas others are more abundant. Substrate evenness assumes that a value between 0 and 1, with 1 representing a situation in which all species are equally abundant within a microbial population present in the samples. This indicates less variation in microbial populations between species, thus, less dominance, and higher diversity. The substrate evenness indices obtained in this analysis ranged between 0.33 and 0.79 (Table 5.1). The values obtained from the findings of the study were within the range of the evenness index, indicating complete evenness and a high level of microbial species diversity with no dominance of specific species. The evenness index for commercial ginger was relatively higher than that of African ginger, as was the Shannon-Weaver diversity index. This could indicate more species associated with commercial than African ginger. On the other hand, water stress also had an impact on plants receiving enough water (20-25%) and those under severe stress (80-80%) having less soil microbial species.

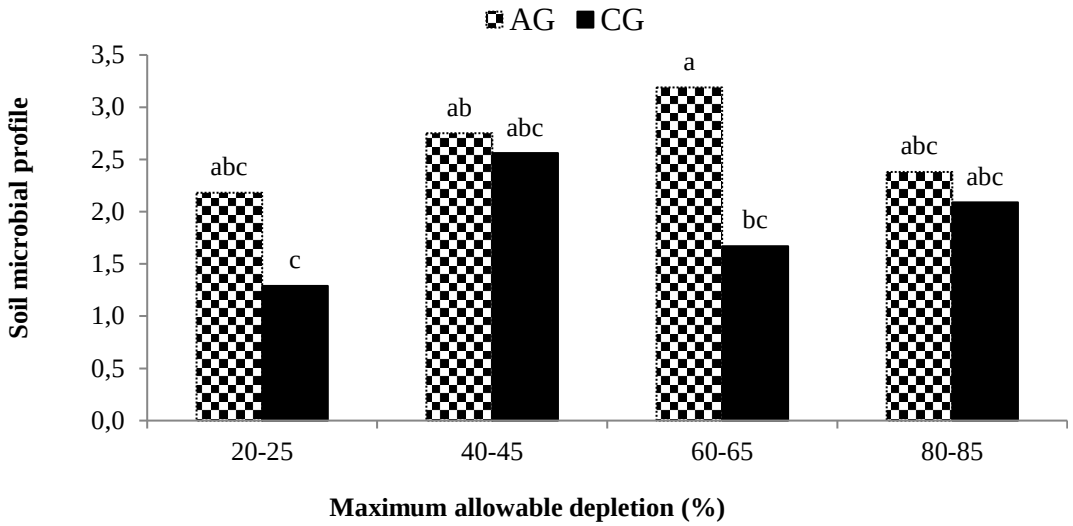
Table 5.1: Shannon-Weaver diversity index for soil microbial species richness and the Evenness index for soil microbial species abundance of soils where different ginger species were subjected to different levels of water stress.

Ginger Species	MAD (%)	Shannon-Weaver (H') (Richness)	Evenness (E) (Abundance)
CG	20-25	1.55^{abc}	0.63^{abc}
	40-45	2.06^{ab}	0.68^{ab}
	60-65	2.40^{a}	0.79^{a}
	80-85	1.71^{abc}	0.67^{ab}
AG	20-25	0.96^{c}	0.33^{c}
	40-45	1.88^{abc}	0.68^{ab}
	60-65	1.22^{bc}	0.45^{bc}
	80-85	1.53^{abc}	0.56^{abc}
CV	----	21.94	17.15
LSD	----	1.05	0.29

*Means in a column followed by the same letters do not differ significantly from each other ($p< 0.05$)

Microbial diversity was influenced differently depending on specific water regime treatments. The highest soil microbial richness and abundance was demonstrated by the CG: 60-65% MAD treatment, whereas the lowest soil microbial richness and abundance were demonstrated by the AG: 20-25%. The soil microbial richness of CG treatments with comparison to African ginger showed higher richness in all treatments, whereas, African ginger 20-25% treatment revealed lower in microbial abundance within African ginger treatments and in comparison with commercial ginger. According to literature, healthy soil is characterised by the presence of a high number (species richness), as well as the high abundance (evenness index) of the different kinds of soil bacteria present in a community. As Table 5.1, in general, the soil microbial profile of the Commercial ginger treatments demonstrated a higher richness and abundance compared to the African ginger treatments. The highest and lowest soil microbial profile in the African ginger treatments were exhibited by AG: 40-45% and AG: 20-25% MAD, respectively, whereas the highest and lowest profile in the commercial ginger treatments were displayed by CG: 60-65% and CG: 20-25% MAD respectively. The results are clear that irrigation regimes have a significant impact on soil microbial profile. The maximum availability depletion of 40-45% and 60-65% showed a higher soil microbial profile in comparison to the control of 20-

25% for commercial ginger and severe stressed treatment of 80-85%. It is important to note that these differences between 20-25%, AG;40-45% and AG: 60-65% were statistically significant only for African ginger (Figure 5.3).



Bars with the same letter do not differ significantly from each other ($p<0.05$)

Figure 5-3: Soil microbial diversity profile representing the combined microbial richness and evenness as affected by ginger species and level of water stress.

5.2.3 Soil Microbial Enzymatic Activity and Microbial Activity

The activities of four soil microbial enzymes were analysed, and results are presented in Table 5.2, which indicate the potential of soil to degrade/convert substrates from an organic form into plant-available nutrients. The higher the microbial activity (*i.e.* mineralisation rate), the faster the nutrients from organic substrates will be made available to be taken up by plant roots. Soil microbial communities associated with various treatments differed in their ability/potential to mineralise/convert carbon (ß-glucosidase), phosphorous (phosphatase), and nitrogen (urease). The results obtained on nutrient cycling did not differ significantly between the treatments for either ginger species. However, the results show significant differences between water regimes

in both ginger species. In general, water use was higher in commercial ginger than African ginger (Section 3.1.1).

As seen in Table 5.2, the African ginger treatments displayed slightly higher overall phosphorous (alkaline phosphatase) mineralisation activities compared to the commercial ginger treatments. Also, commercial ginger treatments displayed slightly higher overall carbon (ß-glucosidase) and nitrogen (urease) mineralisation activities compared to African ginger treatments. The commercial ginger treatment CG: 60-65% MAD displayed higher carbon mineralisation rates compared to treatment CG: 80-85% MAD. Thus, no significant differences were revealed. African ginger treatment AG: 60-65% MAD displayed higher phosphorous mineralisation rates compared to commercial ginger treatment.

Table 5.2: Effect of irrigation regimes on soil microbial enzymatic activities in two ginger species.

Ginger Species	Water Depletion (%)	**ß-glucosidase Activity (*p*-nitrophenol µg/g/h)**	**Alkaline Phosphatase Activity (*p*-nitrophenol µg/g/h)**	**Acid Phosphatase Activity (*p*-nitrophenol µg/g/h)**	**Urease Activity (NH_4-N µg/g/2h)**
AG	20-25	725.65[a]	666.1[a]	1499.02[a]	19.07[a]
	40-45	786.10[a]	776.3[a]	1598.84[a]	15.52[a]
	60-65	757.07[a]	850.5[a]	1496.34[a]	19.91[a]
	80-85	715.96[a]	739.1[a]	1549.57[a]	17.11[a]
CG	20-25	778.10[a]	737.0[a]	1486.89[a]	21.93[a]
	40-45	761.58[a]	744.5[a]	1536.36[a]	20.28[a]
	60-65	820.10[a]	775.5[a]	1574.0[a]	19.38[a]
	80-85	684.91[a]	722.2[a]	1512.81[a]	19.10[a]
CV		8.34	24.74	5.09	19.91
LSD		NS	NS	NS	NS

*Means in columns followed by the same letters do not differ significantly ($p>0.05$).

5.3 CONCLUSIONS

Given the results obtained, both soil microbial functional diversity and activity are sensitive to various agricultural management practices. These management practices instigated different reactions from soil microbial communities, influencing their diversity and abundance within a community, as well as their functioning. The results from measured biological indicators suggest the African ginger treatments are slightly more conducive for soil health than commercial ginger treatments. It is highly recommended that trends in SCUP and microbial activity be monitored over an extended period in order to attain a complete reflection on the impact of different rehabilitation techniques, microbial diversity as an indicator of soil fertility and health. Integration and comparison of available data with more soil quality indicator could contribute to more refined solutions. Diversity indices indicated that variations on treatments and percentage of carbon sources utilised ranged from 0.96 to 2.40 in both ginger spices. African ginger treatments exhibited slightly higher microbial productivity than commercial ginger treatments. Richness and soil microbial profile increased with commercial ginger 40-45% and 60-65%MAD. The control treatment AG: 20-25% MAD and less stressed treatment 40-45% MAD demonstrated abundance in microbial fertility. The highest soil microbial activity and abundance was showed in treatment AG: 60-65% and AG: 40-45% MAD. In general, African ginger treatments showed a slightly higher overall phosphorous (alkaline phosphatase) mineralisation activities than commercial ginger treatments. Commercial ginger treatments slightly displayed higher overall carbon (ß-glucosidase) and nitrogen (urease) mineralisation activities than African ginger treatments.

CHAPTER 6

GROWTH AND YIELD RESPONSES OF TWO GINGER SPECIES TO DIFFERENT LEVELS OF NITROGEN

ABSTRACT

Nitrogen (N) is a critical determinant of plant growth and productivity, but there is limited information on the agronomic parameters of medicinal plants. Three levels of N fertilizer (0, 1.375 and 1.625 g plant^{-1}) were used to investigate the effects of fertilization on the growth and yield parameters of two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*). The experiment was conducted in the glasshouse with six treatments (two ginger species and three levels of N fertilizer) arranged in a randomized complete block design and replicated four times. The results indicated that N application significantly affected plant height, leaf number, chlorophyll content and rhizome yield of the species for two cropping seasons. Scanning electron microscopy analyses of stomata opening revealed that a higher N level increased the number of open stomata in both ginger species. The results showed that with the application of 1.625 g N plant^{-1} both ginger growth and yield were higher compared with the lowest N fertilizer application levels. However, the growth parameters of *Z. officinale* were variably higher than those of *S. aethiopicus*. Therefore, the present study demonstrates that the application of 1.625 g N plant^{-1} to commercial ginger and African ginger can be used to improve growth and yield.

Keywords: *Nitrogen fertilizer rate, growth, nitrogen, nitrogen use efficiency and yield*.

6.1 INTRODUCTION

Owing to their ability to produce bioactive compounds used in a wide variety of medicines and food products, medicinal plant derivatives are considered important sources of active ingredients to be used in drug development and synthesis. The demand for medicinal plants has increased enormously due to the use of biologically active compounds in food, the pharmaceutical and health-care industries (Singh *et al.,* 2014). The growing importance and utilization of medicinal plants can be valued from the economic outlook with global trade in herbs estimated at over US$14 billion per annum and projected to increase to US$5 trillion in 2050 (Bhowmik *et al.*, 2009).

African ginger *(Siphonochilus aethiopicus)* and commercial ginger (*Zingiber officinale*) are members of the Zingiberaceae family used in various food ingredients and medicines. Both plant species produce underground rhizomes varying in shape (Figure 6.1). The species have been reported to possess antidiabetic properties, antioxidant properties and volatile oils (Ghasemzadeh *et al.,* 2010). These species have immense potential for economic development and poverty reduction through income generation for smallholder farmers. The production and cultivation of medicinal plants arenot fully utilized to its potential because of a lack of improved agronomic practices. Among agronomic practices employed in medicinal plant production, nitrogen (N) is a critical measure in improving growth and yield. Nitrogen regulates plant metabolic processes and is critical to synthesize amino acids, which are the building elements of protein, nucleotides, chlorophyll, and numerous other metabolites and cellular components (Nunes-Nesi *et al.,* 2010). The application of N fertilizers at the correct recommended rate and timing has the potential to improve growth and yield (Chaturvedi, 2006).

In contrast, high N application has been found to decrease secondary metabolites in medicinal plants (Ibrahim *et al.,* 2011a). Optimum N fertilization has a favourable effect on root growth

and distribution in the soil (Wang *et al.*, 2014). The application of N and other elements (phosphorus [P] and potassium [K]) significantly increases vegetative growth of moringa plants (Isaiah, 2013). Also, N fertilization significantly increases plant height, fresh weight and dry weight of *Ficus deltoidea* (Sheikh & Ishak, 2016).

Although studies of cultivation techniques in medicinal plants have been undertaken previously, the agronomic and physiological responses to different N levels are often still unknown. Due to increasing demand for ginger species for medicinal purposes, it is, therefore, necessary to investigate ways to improve its growth and quality. Therefore, the study aimed to investigate the effect of different N levels on the growth and yield of two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) under controlled conditions.

6.2 MATERIALS AND METHODS

6.2.1 Experiment site and treatments

A glasshouse experiment was conducted at the Experimental Farm of the University of Pretoria, South Africa. Two ginger species, African ginger (*Siphonochilus aethiopicus*) and commercial ginger (*Zingiber officinale*) were grown for two cropping seasons (2015/2016 and 2016/2017). Six treatments were arranged in a randomised complete block design replicated four times. Artificial growth media (perlite and coir medium) were used and supplemented with N fertiliser at different rates (Table 6.1). Ginger rhizomes (*S. aethiopicus* and *Z. officinale)* weighing 20–30 g were planted in polypropylene bags filled with 30 L perlite. Three months after planting, once the rhizomes were enlarged, 20 L coir medium was added on top of the perlite to increase the total medium volume to 50 L . Phosphorus and potassium were applied as a basal application at a rate of 1.312 g. plant^{-1}. Three months after planting, N treatments were applied at the rate of 0, 1.375 or 1.625 g plant^{-1}. Nitrogen was applied in the form of limestone ammonium nitrate (LAN; 28%), P as 14% superphosphate and K as 50% potassium

chloride. Plants were irrigated three times a week using drip spaghetti irrigation for 30 min at each application.

Table 6.1: Glasshouse experiment design for ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) in response to nitrogen levels and nitrogen combinations.

Ginger species	Treatment (g N. plant^{-1})	Treatment equivalent per hectare (kg N. ha^{-1})
CG	0	0
	1.375	220
	1.625	260
AG	0	0
	1.375	220
	1.625	260

CG: Commercial ginger; AG: African ginger

6.2.2 Temperature (°C) recorded in the glasshouse during the experimental period

Temperature data were obtained from an automatic portable data HOBO logger (MicroDaQ.Com.LTD, USA). The data logger was installed in the glasshouse at the experimental site. The monthly mean over the two cropping season starting in February and ending in August are presented in Figure 6.1.

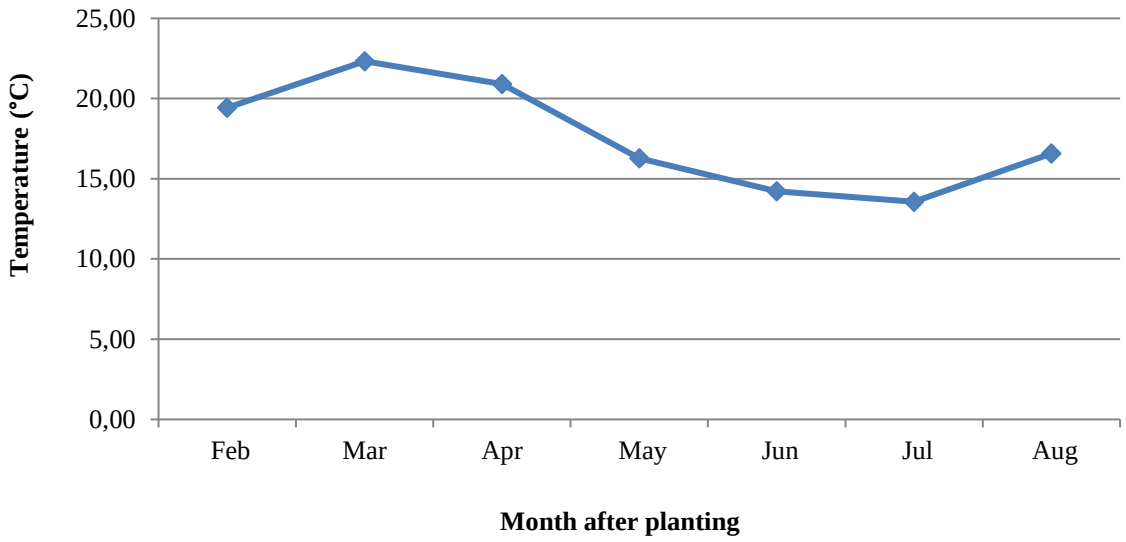


Figure 6-1: Mean monthly temperatures (°C) during the experimental period.

6.2.3 Growth parameters

Growth parameters, i.e. plant height, the number of stems and leaves per plant were measured and sampled every month from each treatment. Plant height (cm) was measured from the base of a plant to the apex using a measuring tape. A number of stems and leaves were monitored by manual counting. Data on plant growth were measured at monthly intervals for both seasons.

6.2.4 Plant chlorophyll content

Plant chlorophyll content was measured once a month. Three leaves were marked for data representation, and chlorophyll measurements were taken at different stages of plant development. Chlorophyll was measured using SPAD meter (Minolta Co Ltd, Japan) to estimate plant N status.

6.2.5 Open stomata parameter

Fresh leaves were collected from each polyprop bag to evaluate the stomatal variations between the treatments. A leaf area of 10 mm x 10 mm was cut from each leaf and fixed immediately into 3% (mass/volume) of glutaraldehyde aqueous solution of 0.05 M phosphate buffer at pH 7.0. The samples were then thoroughly rinsed with distilled water, and the procedure was repeated three times. The samples were then post-fixed into osmium tetraoxide (1% m/v) for 2 hours and dehydrated in a series of ethanol concentrations and ranging from 30, 50, 70, 90 and 100 % (m/v) for 15 minutes and repeated three times (Eiasu *et al.,* 2012). The samples were dried in a critical point drying apparatus (Bio-RAD e300, Watford, England). After drying, the dried samples were mounted on copper stubs and then coated with gold in a vacuum coating unit (Polaron E5200C, Watford, England). In order to observe the total and open stomata number, the coated samples were analysed under a JSM 840 scanning electron microscope (JEOL, TOKYO, JAPAN) at 350 magnifications.

6.2.6 Yield

Each pot was labelled, and the plant material sampled for yield. The yield was measured at harvest ten months after planting. Rhizomes were removed from the polyprop bags, and fresh rhizomes were weighed. The rhizomes were dried in an oven at 50 °C for three days, and the dry weights recorded.

6.2.7 Nitrogen use efficiency (NUE)

Nitrogen use efficiency (NUE) is the N accumulation capacity from the initial plant parts to the harvested plant parts (Roberts, 2008). Both fresh and dry rhizomes were used to calculate NUE using the formula:

$$AE = (Y-Yo)/F \qquad (6.1)$$

Where AE is agronomic efficiency, Y is crop yield with applied N g. plant^{-1}; Yo is a control treatment without N application, and F is the amount of N fertiliser applied (g. plant^{-1}).

6.2.8 Data analysis

Data analysis was subjected to analysis of variance (ANOVA) using Statistical Analysis Software 9.4 (SAS), Institute, Cary, NC, USE. The significant different means were separated using Tukey's honest significant difference test ($p \leq 0.05$).

6.3 RESULTS AND DISCUSSION

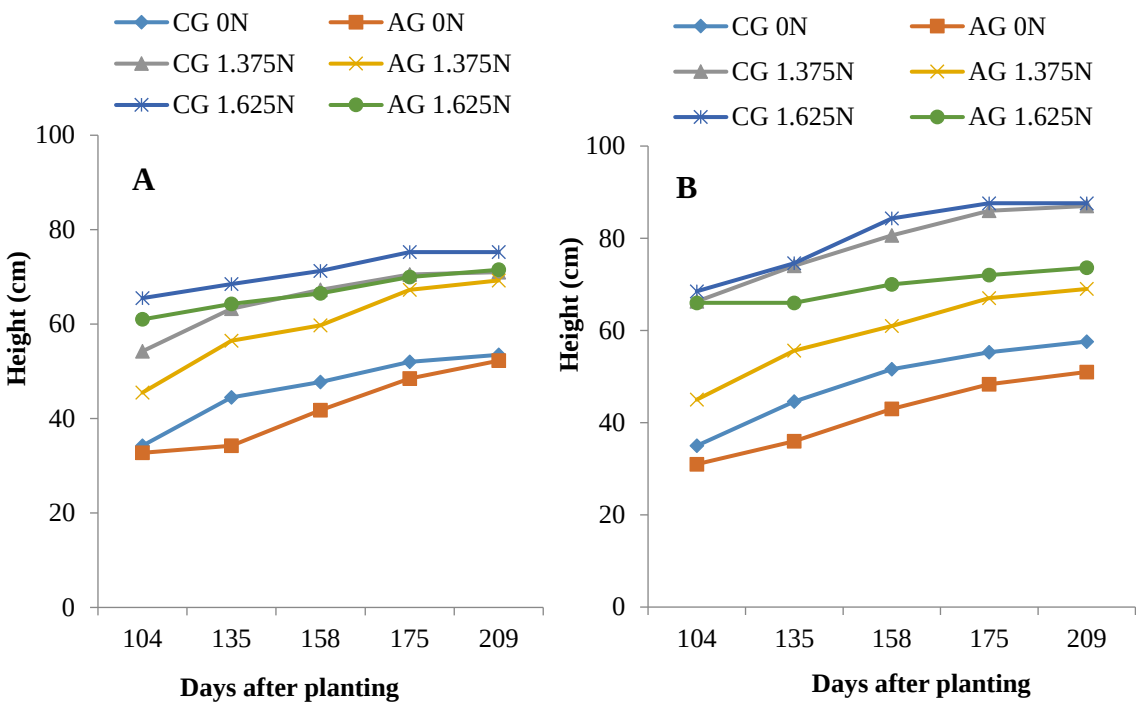
6.3.1 Growth parameters

6.3.1.1 Plant height

The effect of N fertiliser levels on the height of the two ginger species during 2015/2016 and 2016/2017 seasons are presented in Figure 6.2A and B. The results showed significant differences ($p<0.05$) between the two species and treatments. Commercial ginger recorded the highest plant height with the application of 1.625 g. N plant^{-1} for both cropping seasons. African ginger also exhibited increased plant height with the application rate of 1.625 g. N

plant^{-1} in both seasons. However, the heights were significantly lower compared to commercial ginger in all treatments. Consistent with the study hypothesis, the control (0N) exhibited reduced plant height for both species and cropping seasons (Figure 6.2A and 6.2B). Decreased plant biomass in response to 0 N was previously reported for *Pelargonium sidoides* (Mofokeng *et al.,* 2015).

Across the treatments in this study, N addition showed no significant effect on plant height for the two years, indicating that season would not be the primary limiting factor for N application to the two species. The increased plant height with an increase in N application may be attributed to the plant frequent use of nitrogen by plants for cell elongation during active cell division. Also, nitrogen increases meristematic cells and thus increases plant height (Kavanova *et al.,* 2008). Attoe & Osodeke (2009) attributed the increase of height biomass of *Zingiber officinale* to increased N application. The increase in plant height at 104 to 209 days after planting with N application at different rates can be associated with the accumulation of nitrate and proteins as main forms of nitrogen mostly used in vegetative plant tissues



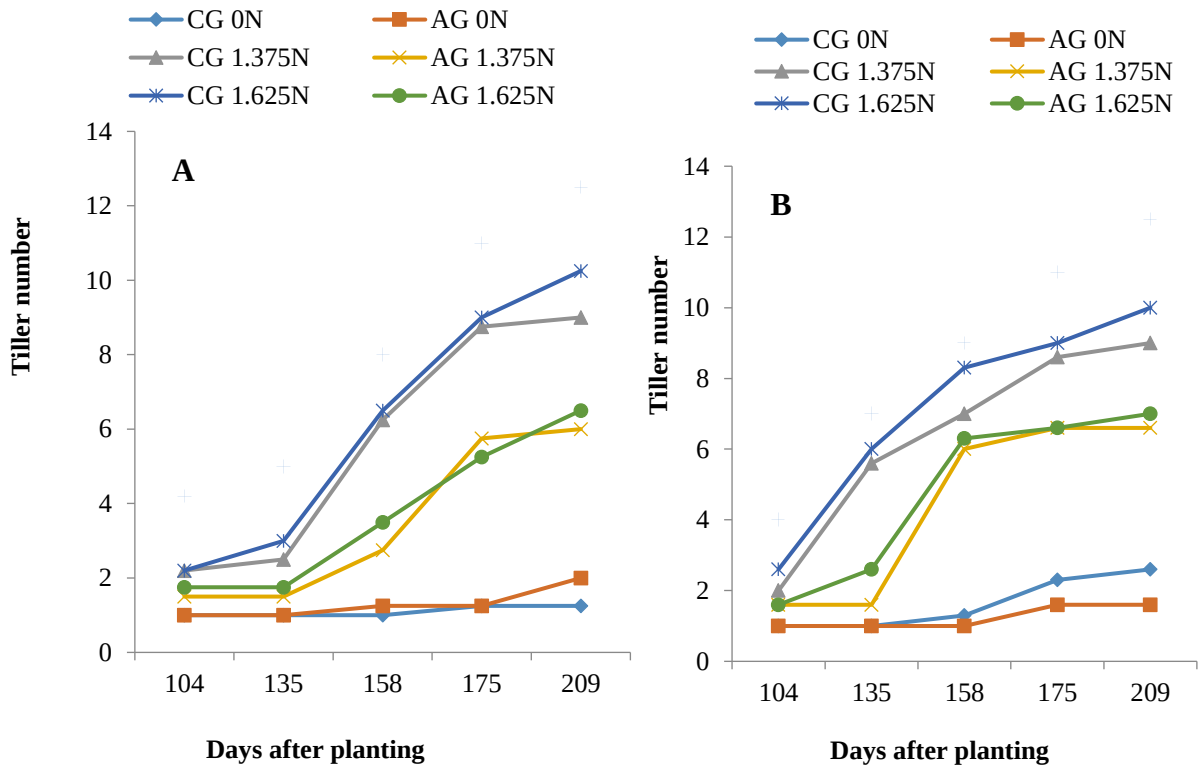
CG: Commercial ginger; AG: African ginger

Figure 6-2: Plant height in response to different nitrogen (N) application rates on two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during cropping season (A) 2015/2016 and (B) 2016/2017.

6.3.1.2 Number of tillers per plant

One-way ANOVA showed that the number of tillers per treatment for the two species was significantly affected by nitrogen level during the two cropping seasons (Figure 6.3A and 6.3B). Commercial ginger exhibited the highest tiller number with the application of 1.375 and 1.625 g. N plant^{-1} for both cropping seasons. In comparison with the no-N treatment, the number of tillers per plant was low from 104 to 209 days after planting for African ginger during both cropping seasons. Likewise, in both cropping seasons, 0 N application reduced the numbers of tillers per plant for commercial ginger compared to treated plants (Figure 6.3A and B). The results show that N application improved the number of tillers per plant from 135 to 209 days after plant for both ginger species and cropping seasons. The differences in the number of tillers between the two species gradually increased with increasing N rates (Figure

6.3A and 6.3B). The application of the highest levels of N also increased the number of tillers for various plant species (Crook & Ennos, 1995). The promoting effect of N on the number of tillers can be explained based on N supply increasing the number of meristematic cells and their growth leading to the formation of shoots (tillers) in addition to leaf expansion and number (Lawlor, 2002). Asafa & Akanbi (2017) attributed the increase in growth parameters of Ginger (*Zingiber officinale* L.) to the application of N fertilizer. (James, 2008). Furthermore, different conclusions on yield-related parameters (number of leaves and propagule weight), were reported in which it did not increase or even reduce following the application of N fertilizer (Asafa & Akanbi 2017).



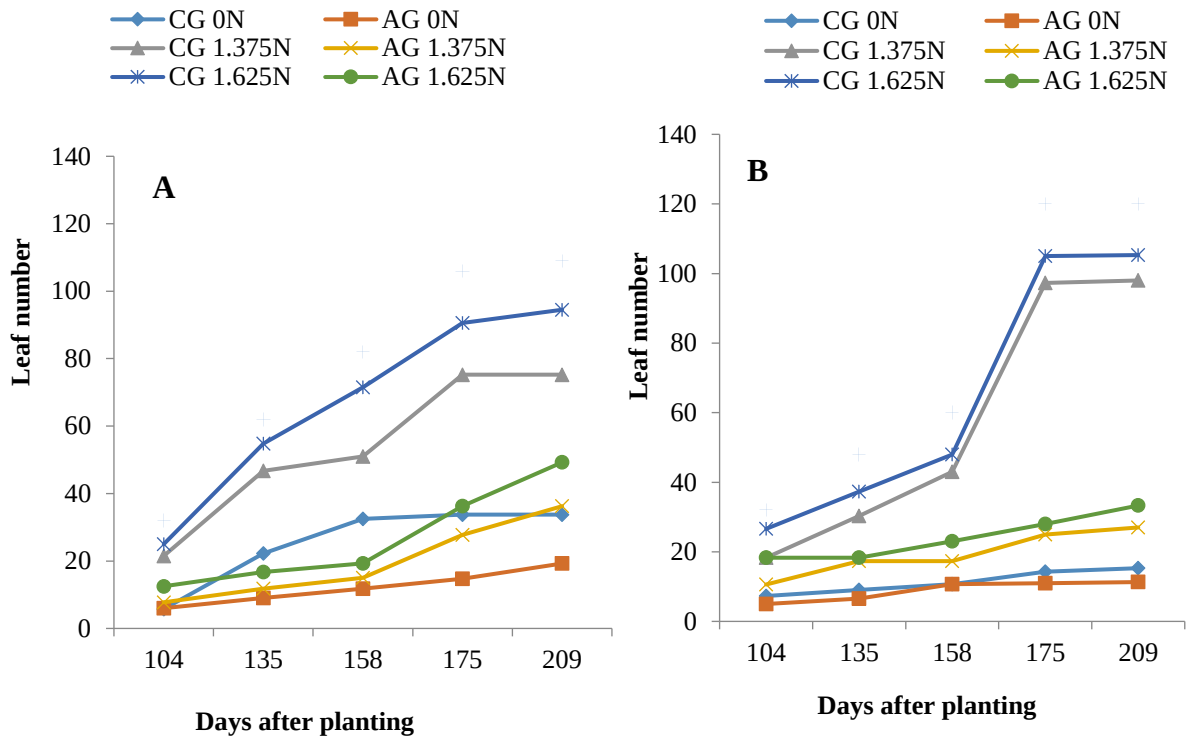
CG: Commercial ginger; AG: African ginger.

Figure 6-3: Number of tillers in response to different nitrogen (N) application rates for two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during cropping season (A) 2015/2016 and (B) 2016/2017.

6.3.2.2 Leaf number

The number of leaves per plant as influenced by nitrogen fertilization rates ($p<0.05$). It was observed that the application of 1.625 and 1.375 g. N plant^{-1} increased the production of leaf number in commercial ginger for the two cropping seasons (Figure 6.4A and 6.4B). The number of leaves was also enhanced for African ginger with the application of 1.625 g N. plant^{-1}, although the numbers of leaves were lower (Figure 6.4A and 6.4B). It should be considered that the absorption and assimilation of N by the two ginger species might vary according to genotypic factors. The contribution of leaf N remobilization to various plants was reported to be cultivar dependent, varying from 50 to 90% (Masclaux-Daubresse *et al.*, 2008). The results showed an increasing trend in the number of leaves per plant with days after planting. The data showed an enormous increase from 158-209 days after planting with the application of 1.375 and 1.625 g. N plant^{-1} for commercial ginger during the 2016/2017 cropping season (Figure 6.4B).

Dohleman *et al*. (2012) observed higher above-ground plant biomass in response to greater N concentrations. In contrast, the additions of N did not promote mass accumulation and leaf longevity in eucalyptus plants as compared to phosphorus fertilization (Laclau *et al*., 2009). Murillo-Amador *et al*. (2006) attributed the decrease in growth and leaf biomass to competitive inhibition between ions of the fertilizer applied. Jaćimović *et al*. (2010) found no effect of increasing N doses on the shoot and dry leaf mass of sweet basil.



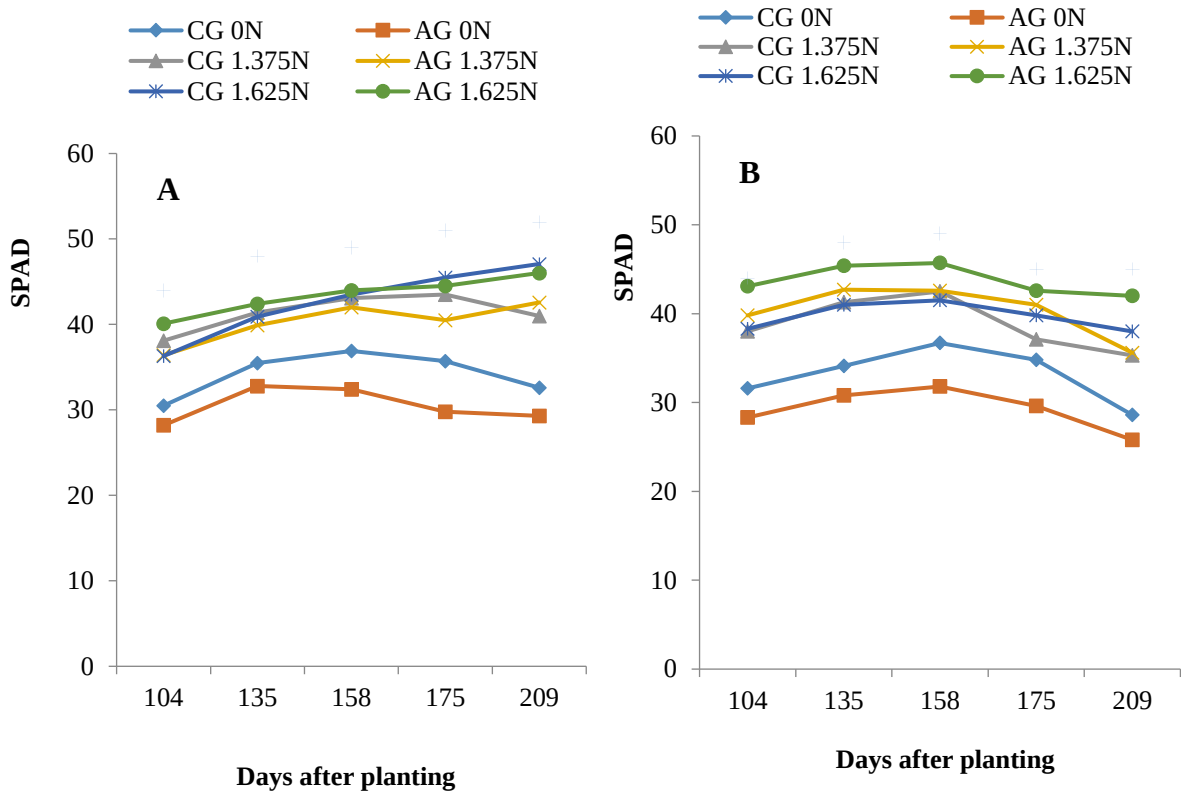
CG: Commercial ginger; AG: African ginger

Figure 6-4: Leaf number in response to different nitrogen (N) application rates for two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during cropping season (A) 2015/2016 and (B) 2016/2017.

6.3.2.3 Chlorophyll content

The chlorophyll content differed significantly ($p<0.05$) among the two species and N application rates. Maximum chlorophyll content was observed with the application of 1.625 g. N plant^{-1} for African ginger during the 2016/2017 cropping season (Figure 6.5B). For both African ginger and commercial ginger, SPAD meter leaf readings at different days after planting showed lower values for non-N treated plants during both cropping seasons. Differences between ginger species for SPAD readings were notable (Figure 6.5A and 6.5B). The results show that it should be possible to select for species differences in levels of chlorophyll content per unit leaf area at different planting dates using a SPAD 502. This is in agreement with the observation that SPAD chlorophyll measurements have intermediate productivity and are of use in selection (Gutiérrez-Rodriguez *et al.*, 2000). Previous studies

reported on the significant correlation at different harvesting times between SPAD readings and N fertilizer rate (Debaeke *et al.,* 2006). According to Cartelat *et al*. (2005), chlorophyll readings can be useful in detecting N deficiencies in growing crops. However, the SPAD meter cannot be used to make accurate predictions of how much N to apply to a crop during the growing season. A positive correlation was also observed between chlorophyll meter readings and leaf N in dry land and irrigated pumpkins (Swiader & Moore, 2002). Argenta *et al.* (2004) indicated that a portable chlorophyll meter (SPAD 502) is an instantaneous tool to assess plant N status. Swiader & Moore (2002) also reported on the potential usefulness of the SPAD 502 chlorophyll meter as an N management tool in estimating plant N status. However, the SPAD values in response to N requirements could vary with plant species and growing conditions.



CG: Commercial ginger; AG: African ginger

Figure 6-5: Chlorophyll content in response to different nitrogen (N) application rates on for two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during cropping season (A) 2015/2016 and (B) 2016/2017.

6.3.2.4 Open stomatal pores

The stomatal patterns of African ginger and commercial ginger leaves in response to fertilizer N rates were evaluated by SEM (Figures 6.6A-F, Figures 6.7A-F) in both cropping seasons. African ginger recorded a higher number of open stomatal pores compared to that of commercial ginger in both seasons. The results indicated higher open stomatal pores under higher nitrogen conditions compared to the plants supplied with less N (Figures 6.6A-F, Figures 6.7A-F) for both cropping seasons. In agreement with our observations, high nitrogen fertilization was also described to increase open stomatal pores in potato crop by Yan *et al*. (2012). Average percentage open stomata ranged from 37% to 60% for commercial ginger, while for African ginger it ranged from 50% to 68.2% for the first season. It was shown that African ginger had a higher percentage of open stomata compared to commercial ginger with 0 and 1.625 g. N plant^{-1}.

Data of the open stomata from the species (i.e. African ginger and commercial ginger) differed significantly indicating the effect of N fertilizer rates prominent in the 2016/2017 season. The mechanism and role of stomata in protecting plants from damage have been described by Raven (2014). Furthermore, Ghasemzadeh *et al*. (2010) indicated that stomatal opening and regulation plays a significant role in the control of photosynthesis rate. In African ginger leaves, high N fertilization promoted a two-fold increase on stomata pores. The increased number of open stomata pores in African ginger indicated its adaptive capability to varying environmental conditions. Lawson & Blatt (2014) attributes the increased stomatal opening to an increase in photosynthetic rate and activities.

Table 6.2: Total stomata and open stomata percentage of commercial ginger (CG) and African ginger (AG) in response to N fertiliser rates.

Species	Treatment	2015/2016			2016/2017		
		Total stomata	Open stomata	Open stomata (%)	Total stomata	Open stomata	Open stomata (%)
CG	0N	25	12	48	47	15	31.9
	1.375N	43	16	37.2	46	28	60.8
	1.625N	46	29	63.	47	34	72.3
AG	0N	46	23	50	47	27	57.4
	1.375N	41	28	68.2	47	28	59.5
	1.625N	45	30	66.6	46	41	89.1

CG: Commercial ginger; AG: African ginger

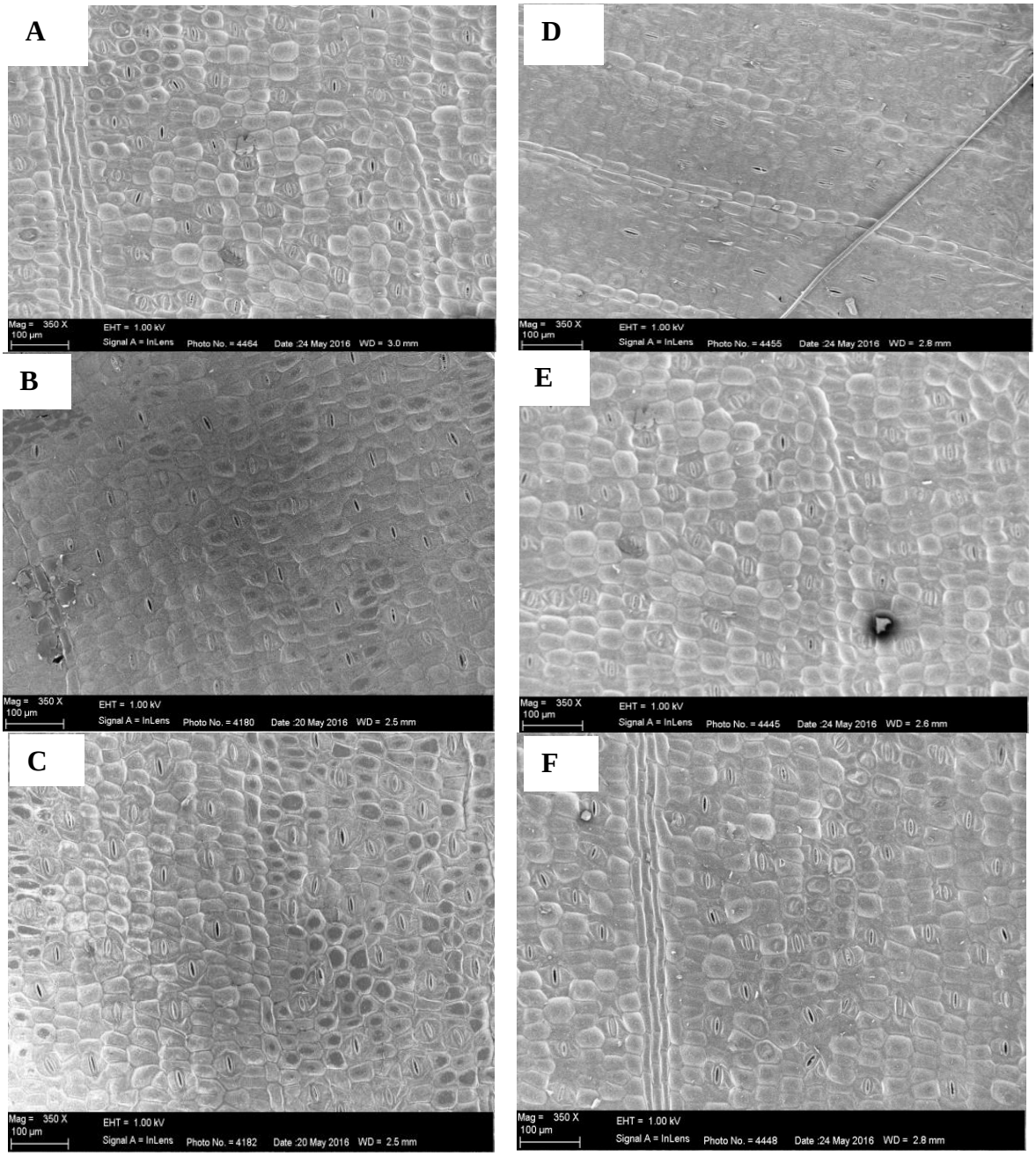


Figure 6-6: A-F: Scanning microscope images of the adaxial side of *S. aethiopicus* and *Z. officinale* leaves as affected by different nitrogen application rates in the 2015/2016 season (A: AG-0N g plant^{-1}; B: AG-1.375 g plant^{-1}; C: AG-1.625 N g plant^{-1}, D: CG-0N; E: CG-1.375 and F: CG-1.625 N g plant^{-1}).

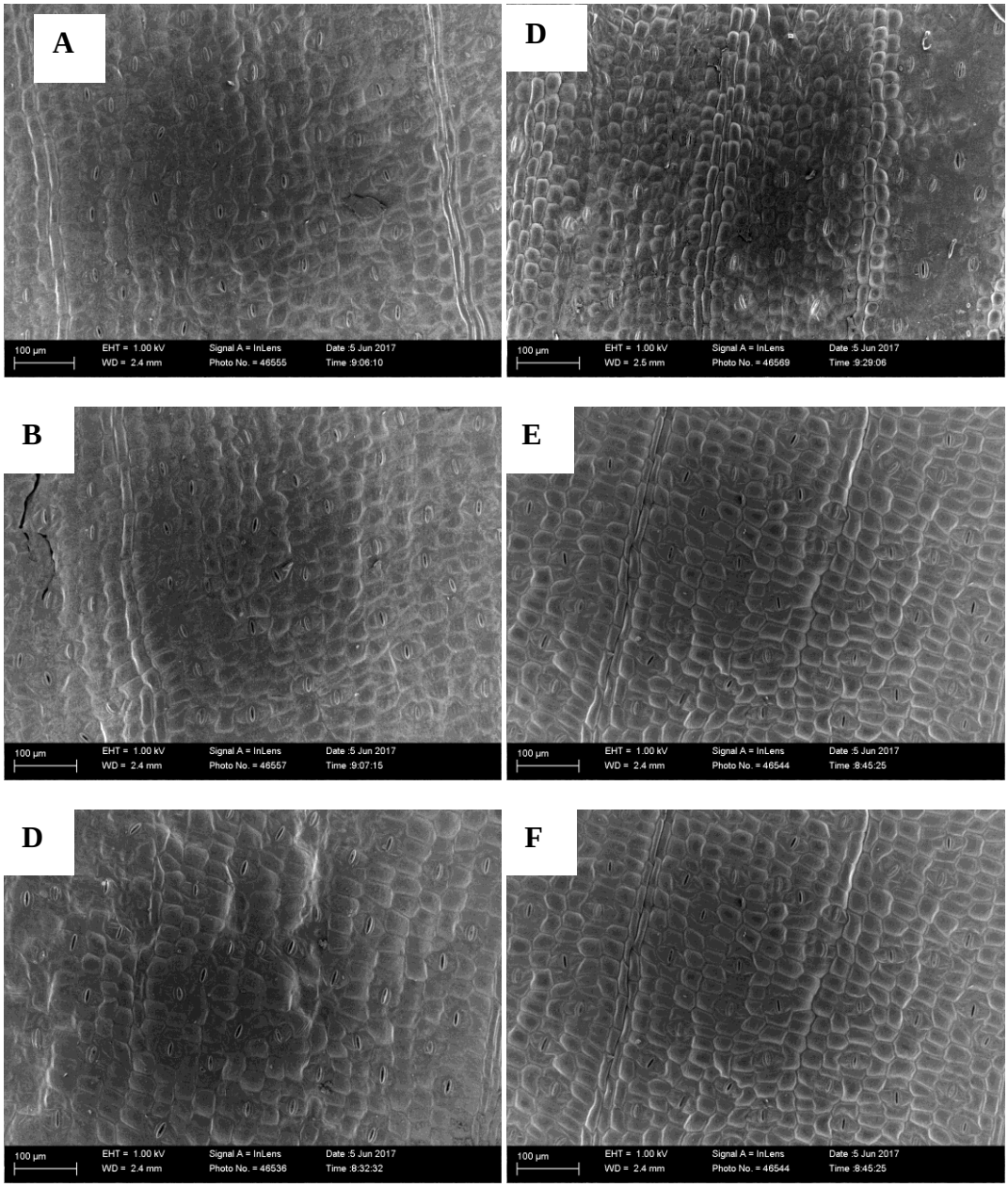


Figure 6-7: A-F: Total stomata and stomatal open of *S. aethiopicus* and *Z. officinale* leaves in response to different nitrogen (N) application rates in the 2016/2017 season. Images represent the following; A: AG-0N g. plant^{-1}; B: AG-1.375 g. plant^{-1}; C: AG-1.625 N g. plant^{-1}, D: CG-0N; E: CG-1.375 and F: CG-1.625 N g. plant^{-1}.

6.3.3 Yield parameters

6.3.3.1 Fresh and dry rhizome mass

The results on fresh and dry rhizome mass revealed significant differences for African ginger and commercial ginger in response to varying N fertilizer application rates (Figure 6.8A-D). Commercial ginger recorded increased rhizome fresh mass for both cropping seasons in response to all fertilizer N application rates as compared to African ginger. The increase in fresh rhizome mass was observed when commercial ginger received 1.625 N g. plant^{-1} for the two growing seasons. Application of 1.375 N g. plant^{-1} proved optimum and elevated the fresh rhizome mass in the 2015/2016 growing season (Figure 6.8A-B). Akinyemi *et al.* (2014) reported on the improved growth and yield in response to fertiliser application of ginger. Correspondingly, an enhancement in rhizome dry weight, was recorded for commercial ginger treatments for both cropping seasons (Figure 6.8C-D). The results coincided with the findings of Rathke *et al*. (2006), who reported that an increase in N application could enhance root yield in *Brassica napus* L. The effect of N on plant growth may improve biochemistry and physiology of plants, and these could impact on yield (Lawlor, 2002). Nitrogen acted as a key component and proved to have a significant effect on various attributes studied at different growth stages (Xu *et al*., 2012). This showed that the increased rates of N fertilizers contributed much to the growth of the two species.

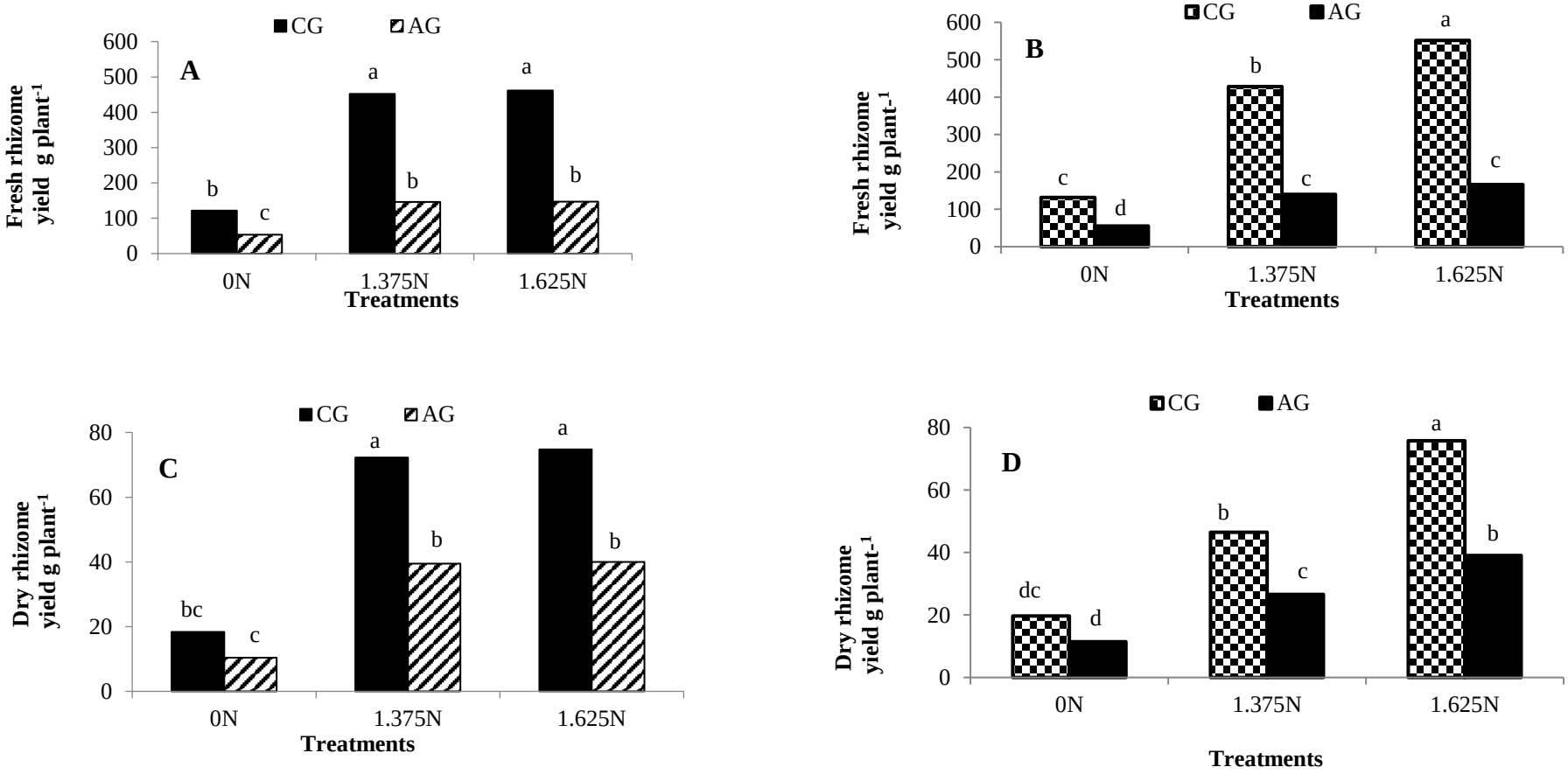


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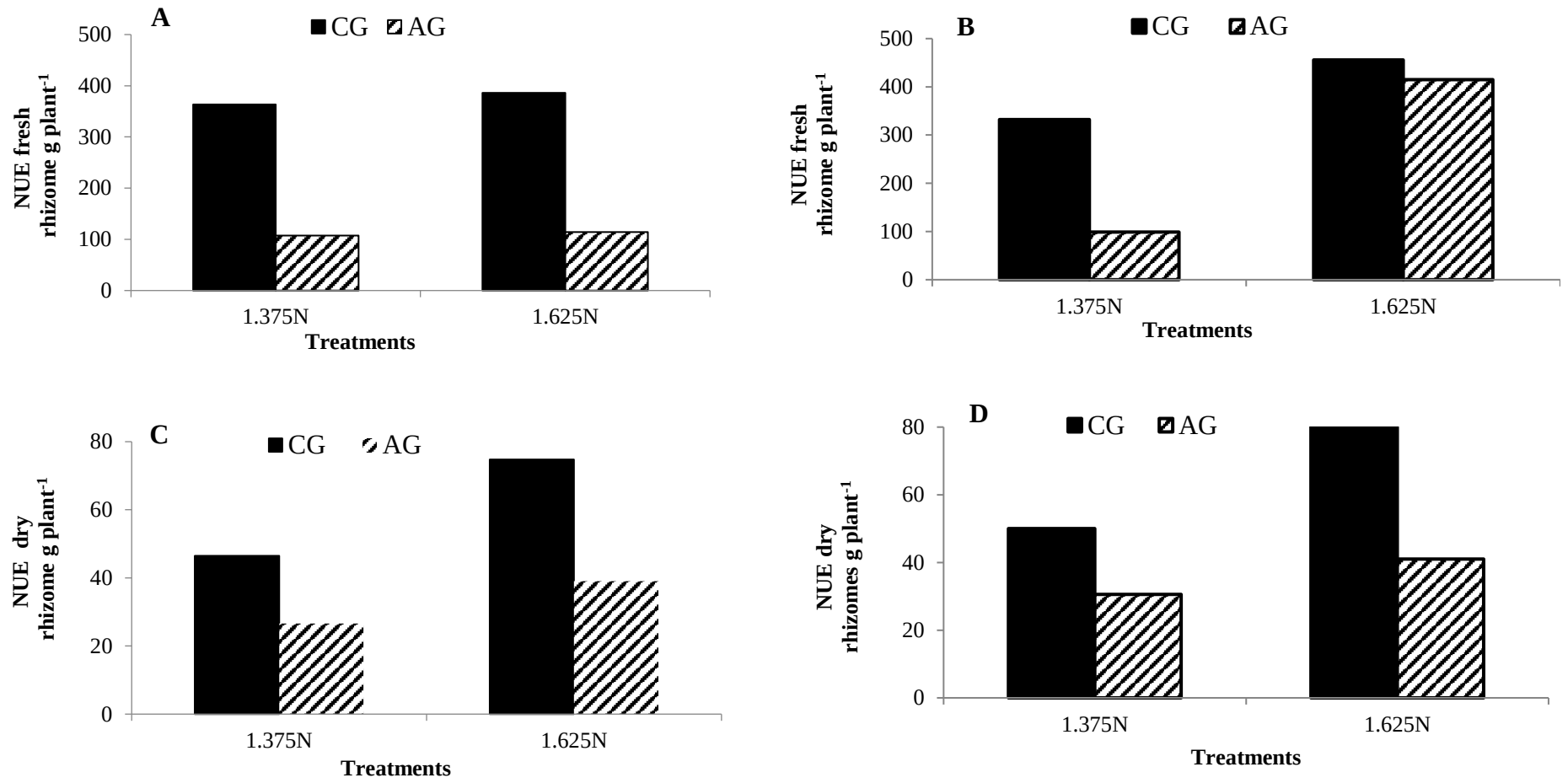
Figure 6-8: A-D: Fresh and dry rhizome yield of two ginger species in response of different nitrogen (N) application rates during cropping season (A and C) 2015/2016 and (B and D) 2016/2017, respectively.

6.3.3.2 Nitrogen use efficiency (NUE) of rhizome fresh and dry mass

Nitrogen use efficiency (NUE) was influenced by N application rates for rhizome fresh and dry mass (Figure 6.9A-D). Nitrogen use efficiency in a crop plays a significant role by increasing crop yield due to the regulation of crop metabolic processes (Xu *et al.,* 2012). The highest N use efficiency ranged from 386.3 to 455.5 g plant-^{1} for fresh rhizomes during both the 2015/2016 and 2016/2017 cropping seasons. Similar increases in NUE were observed for dry rhizome for commercial ginger for all fertilizer N treatments during both cropping seasons (Figure 6.9C and 6.9D). The findings by Dordas (2015) demonstrated new information on the relationship between NUE, N application, chlorophyll meter readings and N leaf concentration of oregano. A previous report by Fageria & Baligar (2005) supported the findings of this study, indicating that the application of N improved yield and NUE. The NUE at 1.375 and 1.625 N g plant^{-1} for African ginger during 2015/2016 cropping season may be related to saturation of N absorption capacity by plants at higher N rate (Fageria *et al.,* 2003). Also, the findings on nutritional variability demonstrated that plants grown at the lowest nutrient concentrations would inevitably have the highest utilization measure because of dilution effects (Fageria *et al.,* 2013). According to Baligar *et al*. (2001), reduced NUE N application could be a result in loss of mechanisms such as volatilisation, denitrification and leaching, or temporary unavailability.



Bars in individual figures with the same letter do not differ significantly from each other ($p<0.05$)

Figure 6-9: Nitrogen Use Efficiency (NUE) of fresh and dry yield rhizomes of two ginger species in response to different nitrogen (N) application rates during cropping season (A and C) 2015/2016 and (B and D) 2016/2017 respectively.

6.4 CONCLUSIONS

The present study demonstrates that growth parameters (height, tiller and leaf number) of *Z. officinale* and *S. aethiopicus* were significantly affected by N fertilization rates. *Z. officinale* exhibited higher measures of plant height, the number of tillers, chlorophyll content and leaf number. Scanning electron microscopy (SEM) analyses on open stomata revealed that higher nitrogen level increased open stomata in both ginger species and both cropping seasons. African ginger showed higher open stomata percentage compared to commercial ginger, indicating the African ginger was more resistant than commercial ginger. The researchers also showed that fresh and dry mass of commercial ginger were higher in both seasons compare to African ginger. In general, the results showed that commercial ginger exhibited higher fresh and dry mass plus N use efficiency than African ginger in both seasons. The results of the present study suggest that optimal levels of N can be used to maximize the production of ginger species. The results of this study can be useful to elucidate the use of N fertiliser levels on yield of both commercial and African ginger.

CHAPTER 7

PHYTOCHEMICAL PROFILING OF TWO GINGER SPECIES IN RESPONSE TO NITROGEN FERTILISER LEVELS

ABSTRACT

Medicinal plants are becoming more important due to their medicinal value and high demand for human use. South Africa is endowed with a diversity of medicinal plant varieties, and approximately 27 million South Africans depend on them as traditional medicines. Secondary metabolites such as flavonoids and phenolic acids are important sources rich in enzyme inhibitors and antioxidants. There is limited information on the phytochemical profiling of medicinal plants in response to nitrogen fertilizer applications. This study aims to determine the effect of nitrogen on quality (total flavonoid, phenolic and antioxidant content) and determine antimicrobial activity of commercial (CG) and African ginger (AG). A glasshouse experiment with two ginger species grown in an artificially grown medium (perlite and coir medium conducted at the Experimental farm of the University of Pretoria for two seasons (2015/2016 and 2016/2017). The six treatment combinations (2 species x 3 N levels) were arranged in a randomized complete block design (RCBD) and replicated four times. The results obtained revealed that total phenolic, flavonoid and antioxidant content increased with increasing nitrogen levels in both ginger species. The study also showed that nitrogen fertiliser increased the inhibition of bacterial and fungi such as *E.coli* and *C. albicans* when treated with commercial and African ginger extracts. The findings of the study suggest that nitrogen fertiliser levels of up to 1.625 g. plant^{-1} should be used for higher phytochemical activity.

Keywords: *Antibacterial, antifungal, antioxidant, nitrogen use efficiency, flavonoid and phenolic content.*

7.1 INTRODUCTION

Cultivation of medicinal plants is becoming more important due to the high demand for human use (Amujoyegbe *et al.*, 2012). South Africa is well endowed with a diversity of medicinal plants used daily for various purposes such as in traditional medicine, for water, for shelter and as fuel (van Wyk & Gericke, 2000). There are approximately 27 million South Africans, depending on traditional medicines (Dauskardt, 1990). Although food crops dominate the global market in terms of nutrition, medicinal plants such as ginger and garlic are still utilized for aroma, flavour and as agents of antioxidant potential for health improvement (Barta *et al.*, 2006).

Secondary metabolites can reduce the risk of chronic illness (Siro *et al*., 2008; Vina *et al*., 2006). Flavonoids as biochemistry compounds are regarded as a rich source in terms of enzyme inhibitors, pigments and antioxidants, providing defence mechanism (Harborne & Williams, 2000; Caretto *et al*., 2015). Both flavonoids and phenolics are found in various foods and are also important in the human health as lipid peroxidation inhibitors, free radical-mediated inhibitors and reducing for reactive oxygen species (ROS) (Heim *et al*., 2002; Rauha *et al.*, 2000). Previous studies have indicated that the intake of antioxidants reduce the risk of many diseases such as diabetes, cardiovascular diseases and can reduce the risk of cancer (Mclarty, 1997; Hertog *et al.*, 1995; Kuo, 1997). Flavonoids and antioxidant are mainly found in the chloroplast and play a significant role by controlling cell growth and in regulating whole plant development (Agati *et al.*, 2012). Flavonoids act mostly as antioxidants, antiviral, antiallergenic and anti-inflammatory agents, which generally reduce free radical formation and scavenge free radicals (Pietta, 2000).

Zingiber officinale (commercial ginger) is a tropical plant high in medicinal value, while it is mostly used as a spice (Kambaska & Santilata, 2009). Its rhizomes are used in tea, cakes, as a food spice, and eaten raw or cooked as a vegetable (Ghasemzadeh *et al.*, 2010). *Zingiber*

officinale has many culinary and therapeutic uses with anti-inflammatory, antimicrobial and antioxidant potential. Also, the plant possesses health-enhancing properties due to the high content of shogaols, gingerols, paradols and volatile constituent such as sesquiterpenes and monoterpenes (Masood & Taussef, 2011). Furthermore, it possesses numerous pharmacological activities, including cardiovascular, anti-inflammatory, anti-cancer and glucose lowering properties (Shukla & Singh, 2007).

Siphonochilus aethiopicus (African ginger) belongs to the Zingiberaceae family and is distributed in different regions of Africa, especially Ethiopia, southwards from Senegal and to the northern and eastern parts of South Africa (Smith, 1997; van Wyk *et al.*, 1997). The rhizomes are commonly used in traditional medicine to treat malaria, coughs, cold chewed to clear nasal passages and used by women to relieve pain during menstruation (Hutchings *et al.*, 1996).

Nitrogen plays an important role in plant production and regulates metabolic processes, such as photosynthesis (Xu *et al.,* 2012). Nitrogen regulates plant organism's health, functioning and structural integrity (Tewari *et al.*, 2004). It also plays a significant role in bulb formation, skin colour development and plant elongation (Mozumder *et al.*, 2007). Nitrogen is also an important element that promotes and enhances secondary metabolites production in plants (Aires *et al.*, 2006). Stewart (2001) reported that plant nutrient availability could be an important factor in determining secondary metabolism and antioxidant production. Previous reports on the effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (*Achillea collina* Becker ex Rchb.) showed that nitrogen starvation decrease plant growth, leaf protein content, amino acids and primary metabolism, while the phenolic and antioxidant content were increased (Giorgi *et al.*, 2009).

Nitrogen fertiliser is important element and plays a significant role in changing antioxidant system and in fatty acid composition of chloroplast membranes from coffee *Arabica L*. Nitrogen application may reduce the negative effect of reactive oxygen species and contribute to a better photosynthetic apparatus which will contribute in reducing the production of reactive molecules (Ramalho *et al.,* 1998). Alizadeh *et al.* (2010) reported that total phenolic and antioxidant activity and essential oil composition were increased with 1g N-plant^{-1} on *Satureja hortensis* L. (Lamiaceae). However, little is known about the effect of nitrogen on flavonoid, phenolic and antioxidant contents, as well as antimicrobial activity of commercial and African ginger species. This study aims to determine the effect of nitrogen levels on flavonoids, phenolics, antioxidant and antimicrobial activity of commercial (CG) and African ginger (AG).

7.2 MATERIALS AND METHODS

In this chapter, the material produced in chapter six was used for further processing. The experiment site and treatments and temperature recorded in the glasshouse during the experiment period were described in chapter six sections 6.2.1 to 6.2.2.

7.2.1 Plant collection

Rhizomes of commercial and African ginger were harvested ten months after planting in both seasons 2015/2016 and 2016/2017. Fresh rhizome yields were first measured; thereafter two gram of rhizomes from each treatment was dried in an oven at 50 °C for three days. Rhizomes were grounded into a fine powder using FRITSH pulverisette 19.

7.2.2 Determination of total phenolic content

The total phenolic content of the crude extract was determined using Folin–Ciocalteu reagents with analytical grade gallic acid as the standard (mg GAE/g dry weight basis) (Ghasemzadeh *et al.,* 2010). An amount of 1 mL of the extract was mixed with 1.0 mL Folin–Ciocalteu phenol reagent. After 5 min, 7.5% sodium carbonate (2.0 mL) was added to the mixture. After being

kept in total darkness for 2 hours to complete the reaction, the absorbance was measured at 750 nm using a spectrophotometer. Total phenolic content amounts were calculated using a gallic acid calibration curve. The results were expressed as mg gallic acid (GAE) per dry plant matter (Chaovanalikit & Wrolstad, 2004).

7.2.3 Determination of total flavonoid content

The aluminium chloride ($AlCl_3$) colorimetric method was used to estimate total flavonoid content as described by Sultana *et al*. (2008). Extracts of each rhizome of *Zingiber officinale* and *Siphonochilus aethiopicus* material (0.1 mL) were diluted with deionized water (0.3 mL) followed by 0.3 mL of 5% sodium nitrite ($NaNO_2$). After 5 minutes, $AlCl_3$ (10%) was added at 25 ^oC and at after another 5 minutes, 0.2 mL of NaOH (1.0 M) was added. The reaction mixture was diluted with 1 mL of deionized water and allowed to stand for 5 minutes. Absorbance was measured at 510 nm in triplicates using a spectrophotometer. For each sample, three readings were taken to get the averaged results, and a standard curve was plotted using quercetin as a standard (mg of QE g^{-1} dry weight basis).

7.2.4 Determination of total antioxidant activities using FRAP assay.

The ferric reducing power assay (FRAP) of each sample was determined according to the method described by Benzi & Stain (1996). The reducing antioxidant power of the extract was measured with a spectrophotometer. The two positive controls Butylated hydroxytoluene (BHT) and ascorbic acid were expressed by graphically plotting absorbance against concentration. The mixture reaction was performed and incubated in the dark at room temperature for 30 min. Samples for assay were prepared in triplicate and repeated twice. The absorbance was read at 630 nm using a microtiter plate reader (ELISA, Microplate Reader, California, USA).

7.2.5 Determination of antimicrobial activity.

7.2.5.1 Preparation of plant extracts

Plant samples of both ginger species were extracted using 70% ethanol in a sonication bath containing ice for 30 min with 20 ml/g (w/v) of extract. The crude extracts were filtered under vacuum through Whatman No 1 filter paper. The extracts were concentrated in vacuum at 35 °C using a rotary evaporator (Insert Model No., Company name). Further, the percentage yield of extracts from each extracting solvent was calculated as the ratio of the mass of the dried to the mass of the ground plant sample. The concentrated extract was subsequently dried at room temperature under a stream of cold air and stored at 4 °C until they were used for the various assays.

7.2.5.2 Antibacterial activity

The microbes strain used Gram negative bacteria *Escherichia coli*. The bacteria growth was done as described by Eloff (1998) and as detailed by Ncube *et al.* (2011). The cultures were incubated overnight at 37^{o}C in an orbital shaker and gram-negative (*Escherichia coli* ATCC 11775) bacteria strains were diluted with sterile Mueller-Hinton broth to give a final of approximately 10^{8} Colony Forming Units (CFU ml^{-1}). Dried crude organic plant extracts were suspended in 70% ethanol to a concentration of 50 mg ml^{-1}, while others were dissolved in distilled water to the same concentration. Then 100 µl of each extract was placed in a 96-well micro-titre plate for bacteria strain analysis. A similar two-fold serial dilution of neomycin (Sigma-Aldrich, German) 0.1 mg ml^{-1} was used as a positive control and 100µl of bacterial culture was added to each well. Water and 70% ethanol was included as negative and solvent control, respectively. The plates were covered with Parafilm and incubated at 37 °C for 24 hours. Bacterial growth was indicated by adding 50 µl of 0.2 mg ml^{-1} p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, Germany) and incubated further for 2 h at 37 ^{o}C. Since the colourless Tetrazolium salt is biologically reduced to a red product due to the presence of active

organisms, the MIC value were recorded as the concentrations in the last wells in which no colour change was observed after adding the INT indicator. Bacteria growth in the wells was indicated by a reddish-pink colour.

7.2.5.3 Antifungal activity

The MIC antifungal activity of the extracts against *Candida albicans* (ATCC 10231) was determined using the micro-dilution bioassay as described by Eloff (1998) and modified for fungi (Masoko *et al,* 2007), as detailed by Ncube *et al.* (2011). An overnight yeast culture was prepared in Yeast Malt (YM) Broth. Four hundred microliters of the overnight culture were added to 4 ml of sterile saline and absorbance was read at 530 nm. The absorbance was adjusted with sterile saline to match that of a 0.5 M McFarland standard solution. From this standardised *Candida* stock, a 1:1000 dilution with sterile YM broth was prepared, giving a final inoculum of approximately 10^6 CFU ml^{-1}. Dried organic extracts were suspended in 70% ethanol to a concentration of 50 mg $ml^{-1,}$ and water extracts were dissolved in water to the same concentration. One hundred microlitres of each extract were serially diluted two-fold with a dilution of Amphotericin B (Sigma-Aldrich, Germany) (2.5 mg ml^{-1}) and used as the positive control, while water and 70% ethanol was used as negative control and solvent control respectively. Dilute fungal cultural (100 µl) was added to each well. The plates were covered with Parafilm and incubated at 37 ^oC for 24 hours, after which 50 µl (0.2 mg ml^{-1} INT) was added and incubated for a further 24 hours at 37 ^oC. Wells that remained clear had inhibition of fungal growth. MIC values were recorded as the lowest concentration that inhibited fungal growth after 48 hours. To determine the fungicidal activity, 50 µl of sterile YM broth was added to all the clear wells and further incubated at 37 °C for 24 hours, after which the minimum fungicidal concentration was recorded as the last clear wells. The assay was replicated twice.

7.2.5.4 Statistical analysis

Data analysis was subject to analysis of variance (ANOVA). The significant different means F-test were done and separated using the Tukey honest significant difference test ($p \leq 0.05$).

7.3 RESULTS AND DISCUSSION

7.3.1 Total phenolic content of rhizomes of commercial ginger (CG) and African ginger (AG) as affected by different N fertiliser levels.

African ginger (AG) and commercial ginger (CG) species were subjected to three different nitrogen levels. Total phenolic content of rhizomes for both ginger species is illustrated in Table 7.1. The highest N application level of 1.625 g N plant^{-1} increased total phenolic content to 13.42±1.60 mg GAE g^{-1} for commercial ginger and 9.14±1.16 mg GAEg^{-1} for African ginger. The lowest total phenolic content was demonstrated for the control treatment, indicating as low as 4.88±1.52 mg GAEg^{-1} and 4.17±1.91 mg GAEg^{-1} for commercial and African ginger, respectively. Similar results were reported for the effects of nitrogen availability on the expression of constitutive and induced chemical defences in tomato (Stout *et al.,* 1998). It has shown that mineral fertilizers increase the concentration of antioxidants and vitamin B_1 in plants (Mozafa, 2008). Also Zhang *et al*. (2016) reported the same results that fertiliser increased antioxidant concentration. A study on elevated carbon dioxide levels resulted in an increase in flavonoids and phenolic compounds which resulted in increased antioxidant activities in *Zingiber officinale* (Ghasemzadeh *et al.,* 2010, Ghasemzadeh *et al., 2012*). Tisserat & Vaughn (2001) reported that carbon-nutrient balance increases under an elevated atmospheric CO_2 environment indicating that a greater amount of carbohydrates can be allocated to the plant's secondary metabolism, resulting in the production of higher amounts of carbon-based secondary metabolites. Higher photosynthetic activity may be the result of increased plant nutrient content, which will increase in secondary metabolites (Hemming & Lindroth, 1999). Azaizeh *et al.* (2005) reported that photosynthesis decreased with poor soil

fertility resulting in decrease secondary metabolites. Nitrogen is one of the fertiliser elements that regulate plant phenolic content (Aires *et al.,* 2006).

Table 7.1: Rhizome phenolic content of two ginger species as affected by nitrogen application levels.

Rhizome/ Phenolic content (mg GAEg^{-1})			
		2015/2016	2016/2017
Ginger Species	Treatment (g. plant-1)	Mean	Mean
CG	0N	4.880^{e}	4.962^{e}
	1.375N	7.302^{d}	6.557^{d}
	1.625N	13.415^{a}	12.400^{a}
AG	0N	4.170^{f}	2.422^{f}
	1.375N	7.862^{c}	6.860^{c}
	1.625N	9.142^{b}	8.140^{b}
CV		0.07	0.130
LSD		0.013	0.020

CG: Commercial ginger; CG: African ginger. Values in a column followed by the same letter do not differ significantly from each other ($p<0.05$)

7.3.2 Total flavonoid content for rhizomes of commercial ginger (CG) and African ginger (AG) as affected by different N fertiliser levels.

Total flavonoid content of commercial and African ginger rhizomes, as influenced by N fertiliser levels are indicated in Table 7.2. The results revealed that N has a positive impact on the total flavonoid content of both ginger species. The total flavonoid content of rhizomes was higher with increased N fertiliser application with 5.23±0.10 QE g^{-1} and 4.75±0.13 mg QE g^{-1} at the highest rate of 260 kg ha^{-1} for commercial and African ginger, respectively. The lowest flavonoid content in 2015/2016 was 2.03±0.00 mg QE g^{-1} for both ginger species. Application of fertilisers may affect secondary metabolites and nutrient composition in the plant. The inorganic fertilisers significantly change and influence secondary plant metabolites, and this could correlate with plant growth and biomass development (Mitchell *et al.,* 2007). The effects of fertiliser on yield, essential oil composition, total phenolic content and antioxidant activity have also been reported in *Satureja hortensis* (Alizadeh *et al.,* 2010).

Ibrahim *et al*. (2011) reported that the highest nitrogen fertiliser rate of 270 kg N ha^{-1} increased total flavonoids in *Labisia pumila* Blume (*Kacip Fatimah*) plants compared to the N control treatment. Availability plant nutritional can significantly increase antioxidant content in plants (Stewart, 2001); Munene *et al*., 2017). It has been showed that nitrogen fertiliser plays an important role by increasing yield and quality, such as secondary flavonoids content (Aires *et al*., 2006). Flavonoid content has been shown to improve the antioxidant potential in plants (Ghasemzadeh *et al.,* 2010). Cesco *et al.* (2012) reported that nutrient availability affects chemical composition, including the biotic and abiotic activity of flavonoids.

Table 7.2: Rhizome flavonoid content of two ginger species as affected by different N levels

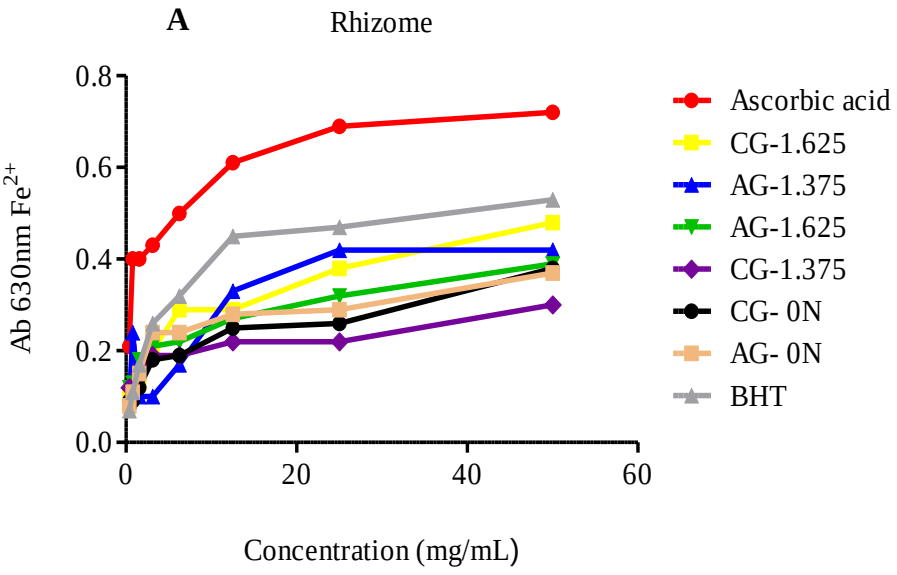
Flavonoids (mg QEg^{-1})			
Ginger Species	Treatment (g.$plant^{-1}$)	2015/2016 Mean	2016/2017 Mean
CG	0N	2.039^{e}	2.060e
	1.375N	3.403^{c}	3.460^{c}
	1.625N	5.234^{a}	5.315^{a}
AG	0N	2.039^{e}	2.037^{e}
	1.375N	3.039^{d}	3.045^{d}
	1.625N	4.755^{b}	4.740^{b}
CV	------	3.658	0.232
LSD	------	0.287	0.0108

CG: Commercial Ginger, AG: African ginger. Values in a column followed by the same letter are not significantly different ($p<0.05$)

7.3.3 Total antioxidant content of the rhizomes of commercial ginger (CG) and African ginger (AG) as affected by different N fertiliser levels.

The result of ferric reducing power in rhizomes extracts of African ginger (AG) and commercial ginger (CG) in response to nitrogen levels with positive controls (BHT and Ascorbic acid) are presented in Figure 7.1. The extracts showed a difference in antioxidant activity of African and commercial ginger species. The total antioxidant content in plant species was higher when applying the highest N rate of 1.625 g $plant^{-1}$ in the second season for both ginger species. The antioxidant content was lower for control treatments in both species.

African ginger showed highest FRAP values for the treatment with 1.625 g N plant^{-1} compared to the application of 1.375 and control treatment for the first season. In the second season, however, showed that African ginger exhibited the highest antioxidant activity with the application 1.375 g N plant^{-1}. The increase in antioxidant activity may also be due to higher flavonoid and phenolic content in both gingers species. It is well established that the accumulation of secondary metabolites such as antioxidants strongly depends on growing conditions, such as nitrogen fertilisation. The increase in antioxidants is due to the higher alkaloids and cyanogenic glocoside affected by nitrogen content in plants (Tavarini *et al*., 2015). Increased N levels greater than 200 mg/L increased antioxidants, phenolics and flavonoids in *Lavandula angustifolia* Mill (Chrysargyris *et al*., 2016). Reports on tomatoes showed that mineral nutrition plays an important role in antioxidant levels (Mitchell *et al.*, 2007). Alizadeh *et al.* (2010) reported similar results on *Satureja hortensis*. Similar results were found on the effect of water and nitrogen fertiliser on herb and essential oil yield of oregano (Said-al Ahl *et al.*, 2009). Nguyen & Niemeyer (2008) also reported that nitrogen fertiliser levels improved total antioxidant content in basil (*Ocimum basilicum* L).



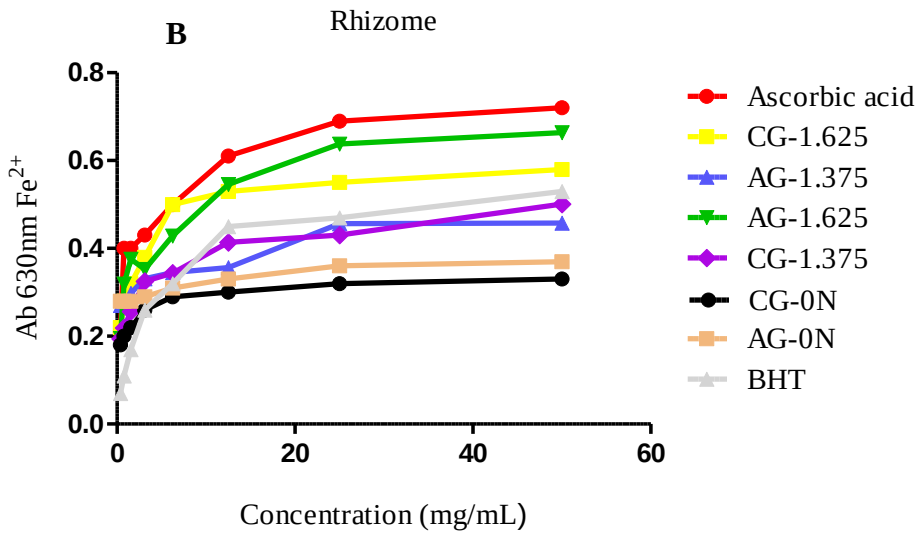


Figure 7-1: Ferric reducing power of the rhizome extracts from African ginger (AG) and commercial ginger (CG) in response to nitrogen levels for the (A) 2015/2016 and (B) 2016/2017 seasons. (Positive controls are BHT and Ascorbic acid).

7.3.4 Antibacterial activity

The results of the antibacterial activity of both ginger species during 2015/2016 and 2016/2017 are presented in Table 7.3. Extracts of both ginger species inhibited microbial growth, thus showing good antibacterial activity. The antibacterial activity ranged from 0.39 to 3.12 and 0.78-3.128 mg ml^{-1} in the 2015/2016 and 2016/2017 seasons, respectively. The highest antibacterial activity observed was 0.39-0.78 and 0.78 MIC mg ml^{-1} with 1.625 N mg/plant for African and commercial ginger, respectively. The results are clear that the highest N fertiliser levels increased antibacterial activity, compared to the control treatments. This increasing activity was shown for both ginger species in both seasons. The results of the present study show that ginger species (commercial and African ginger) possess antimicrobial activities, due to high antioxidant activity in ginger species. The results show that ginger species have an anti-fungal effect on *E.coli* growth. Also, the results from these two ginger species may suggestion some degree of effectiveness in treating *E.coli* bacterial infection. In general, both seasons showed similar antibacterial activity.

Table 7.3: Effect of N levels on the minimum inhibitory concentration of antibacterial activity

E.coli MIC (mg ml^{-1})			
Ginger Species	Treatment (g. $plant^{-1}$)	2015/2016	2016/2017
CG	0N	3.12	3.125
	1.375N	0.78	1.56
	1.625N	0.78	0.78
AG	0N	1.56	3.125
	1.375N	0.39	0.78
	1.625N	0.39	0.78

CG: Commercial ginger; AG: African ginger

7.3.5 Antifungal activity

The results minimum inhibitory concentrations to prevent fungal growth for both ginger species are presented in Table 7.4. The results showed a good antifungal activity by both ginger species during both seasons. The antifungal activity observed ranged between 0.19 to 3.12 mg ml^{-1} in the first season and 0.04 to 3.12 for the second season in both species. For commercial and African ginger, the results showed the highest inhibition of fungal growth at the highest N fertiliser level of 1.625 g. N $plant^{-1}$ compared to the lowest N fertiliser application. However, the data shows that African ginger did not show any difference in the minimum inhibitory concentration of antifungal between 1.375 and 1.625 N g $plant^{-1}$. In comparison, African ginger shows a trend of increased of fungal depression with increasing N level from 1.375 to 1.625 g N $plant^{-1}$. Control treatments showed similar MIC in the first and second season for commercial ginger. The MIC for control treatments showed the lowest antifungal inhibition, compared to the other N fertiliser application rates. However, both ginger species demonstrated a higher anti-fungal effect on *C. albicans* growth.

Table 7.4: Effect of N levels on the minimum inhibitory concentration of antifungal activity

C. albicans MIC (mg ml^{-1})			
Ginger Species	Treatment (g. $plant^{-1}$)	2015/2016	2016/2017
CG	0N	3.12	1.56
	1.375N	1.56	0.39
	1.625N	0.39	0.04
AG	0N	0.39	3.125
	1.375N	0.19	0.19
	1.625N	0.19	0.19

CG: Commercial ginger; AG: African ginger

7.4 CONCLUSIONS

This study demonstrated that different nitrogen fertiliser levels can have a significant impact on phenolic, flavonoid and antioxidant contents in commercial and African ginger species. Total phenolic content increased with increasing nitrogen levels in both ginger species. The highest phenolic content was 13.40±1.60 mg GAE g^{-1} for commercial and 9.14±1.16 mg GAE g^{-1} for African ginger. In general, total phenolic content was higher for commercial ginger, compared to African ginger in both cropping seasons. The results showed that rhizomes had higher total flavonoids content at the highest fertilizer rate of 1.625 g N $plant^{-1}$ in both ginger species. Accumulation of secondary metabolites such as flavonoids, phenolics and antioxidants in plants is strongly influenced by nitrogen fertilisation. The results also clearly indicated that increasing nitrogen fertiliser levels increased the total antioxidant content of commercial ginger and African ginger. The results, therefore, show that both ginger species can be an excellent source of antioxidants that can be used for medicinal purposes. In general, both *E.coli* MIC and *C. albicans* MIC (mg ml^{-1}) were higher at higher nitrogen application levels. The results show that both ginger species can be used as antimicrobials for treating bacterial and fungal pathogens. In conclusion, nitrogen levels of 1.625 N g $plant^{-1}$ improved plant quality aspects such as antioxidant, flavonoid and phenolic content in commercial and African ginger species. Further research is needed to determine the effects of varying N levels.

CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL CONCLUSIONS

The majority of South Africans still rely on medicinal plants to treat various ailments, as they are considered safe, easily accessible and affordable, especially within rural communities. This is in contrast to the high cost of modern drugs, inaccessibility of modern health services and cultural acceptability of modern medicine. Most medicinal plants, even today, are collected from the wild, and the continued over-harvesting and exploitation of many traditional medicinal plants have become a threat to the country's species diversity. As a result, many medicinal plant species are endangered and even extinct in the wild. Irrigation water demand can be regarded as a derived demand for food, and it requires good management of existing water supplies. Fertiliser application, either through the basal or foliar application, is important for increasing plant yield and quality. Optimization of cultivation practices such as irrigation, integrated nutrient management and optimized plant densities can comprehensively contribute to meet the ever-increasing demand of ginger cultivation and conserve its biodiversity. Both ginger species were selected based on their medicinal value. In South Africa, medicinal plant species such as African ginger (*Siphonochilus aethiopicus*) play a significant role in income generation through the sale of plant material for health benefits. *Siphonochilus aethiopicus* is listed in the African Herbal Pharmacopoeia, among the 51 most important medicinal plants in sub-Saharan Africa. In order to understand the variability between ginger species and their responses to different irrigation regimes and nitrogen management, field trials were conducted with the following aims: to assess ginger species quality (total flavonoid, phenolic and antioxidant contents) under different soil water regimes; to evaluate the effects of water regimes on soil microbial diversity for two ginger species; to investigate growth and yield of

two ginger species in response to nitrogen level; and to examine quality (total flavonoid, phenolic and antioxidant contents) of two ginger species in response to nitrogen level.

The study has provided the information needed on the tolerance of ginger species to different levels of water supply. The amount of water used was higher under well-watered treatments, compared to stress treatments for both ginger species. Growth parameters such as height, leaf number and stem number varied between species and irrigation treatments. Irrigation treatment effects on plant growth depended on the plant species. Leaf area index and FI_{PAR} values were higher in African ginger than commercial ginger in both seasons. This was even though African ginger had fewer leaves, which thus reflect the bigger size of individual leaves of African ginger as compared to commercial ginger. Scanning electron microscopy images showed that both ginger species had more stomatal pores and open stomata under well-watered than stressed conditions. African ginger had a higher percentage open stomata in all stressed treatments, compared to commercial ginger and this shows that African ginger is probably more tolerant to water stress than commercial ginger. The study demonstrates that fresh and dry yields were higher for commercial ginger compared to African ginger. Even at severely water stressed conditions the fresh and dry rhizome yields for commercial ginger were higher than those of African ginger. Water use efficiency in terms of fresh commercial ginger was higher at moderate water stress (CG-60-65% MAD). However, for dry matter yield, water use efficiency was highest for either CG-60-65% or CG-40-45% MAD. The results of our study suggest that water stress had a highly significant effect on the growth, yield and WUE of ginger species. The well-watered treatment (20-25% MAD) had higher values for growth and yield, enhancing the production in both species. These could be due to a higher percentage of open stomata in the well-watered treatments that was always significantly higher than that of the stressed treatments. Severe water stress (i.e. 80-85% MAD) resulted in higher production of total

flavonoid content, phenolic content and increased antioxidant activity. These increases were shown in all parts of the plants (rhizomes, tillers and leaves). There were variations in phenolic, flavonoid and antioxidant contents of both commercial ginger and Africa ginger over the harvest period. Flavonoids in the rhizomes increased with time in both ginger species. It was evident that at five months after planting the flavonoid content as well as yield for both species were still very low and harvesting at this stage should not be recommended for either species. For commercial ginger, harvesting between seven to eight months after planting resulted in high flavonoid content and dry rhizome yield. The study suggests that the optimum time for harvesting the rhizomes of ginger species is eight months after planting, to ensure a high level of secondary metabolites. This study has indicated the importance of the age of the plant for the accumulation of secondary metabolites and pharmaceutical quality in the two ginger species. The accumulation of secondary metabolites is due to the elevated diffusion resistance caused by stomatal closure resulting in the concentration of CO_2 being much lower within the stressed plant leaves. As a result, much less NADPH + H^+ can be consumed within the Calvin cycle for the fixation and reduction of CO_2. Accordingly, a much greater share of the energy has to be dissipated. Although the corresponding processes (non-photochemical quenching, photorespiration and the xanthophyll cycle) are enhanced by feedback mechanisms, numerous electrons are transferred to molecular oxygen. The superoxide radicals generated, subsequently produce a wide range of further Reactive Oxygen Species (ROS). Due to the stress-related induction of superoxide dismutase (SOD) and ascorbate peroxidase (APX), superoxide radicals are detoxified and thus production of large amounts of ROS is prevented. The study findings further indicated that higher flavonoid content is attainable without decreasing the yield significantly under moderate water stress. The research suggest that the more plants are stressed, the higher the increase in total antioxidant content for both ginger species. Water stress regimes did have an impact on soil microbial diversity and the soil microbial profile.

Diversity indices clearly indicated variations between treatments and percentage of carbon sources utilised ranged from 0.96 to 2.40 in both ginger spices. African ginger treatments improved microbial productivity. Richness and soil microbial profile increased with commercial ginger at 40-45% and 60-65% MAD.

The nitrogen study demonstrated that all measured growth parameters (height, tiller and leaf number) of *Z. officinale* and *S. aethiopicus* were significantly affected by N fertilization rates. Commercial ginger exhibited higher measures of plant height, number of tillers, chlorophyll content and leaf number than African ginger. Higher nitrogen levels increased open stomata percentage in both ginger species. Regarding species, open stomata percentage was higher for African ginger, compared to commercial ginger. Fresh and dry yields of commercial ginger were higher, compared to African ginger, for the same N treatment. The results were clear that commercial ginger exhibited higher fresh and dry yield N use efficiency than African ginger.

Highest N fertiliser levels increased antibacterial activity for both ginger species in both seasons. The findings of the study indicated that ginger species possess antimicrobial activities and showed anti-fungal effects against C. albicans growth. Also, the results from the two ginger species indicated some degree of effectiveness in treating *E. coli* bacterial infection. The study thus showed that ginger species can be used as antimicrobials for treating bacterial and fungal pathogens. The findings of this study indicated that increased nitrogen levels lead to accumulation of secondary metabolites such as flavonoids, phenolics and antioxidants in plants. This increase could be due to the higher alkaloid and cyanogenic glocoside contents as a result of increased nitrogen in the plant.

8.2 GENERAL RECOMMENDATIONS

- It is recommended to maintain the percentage depletion of plant available water between 20-25% for both ginger species, as it has a positive effect on growth and yield.

To increase the production of total flavonoids, phenolics and antioxidant activity in both ginger species, water stress at 80-85% MAD in the period preceding harvesting is recommended.

- Since water stress reduces growth and biomass accumulation, but increases secondary metabolite production, it is recommended to determine the growth stage at which water stress should commence.
- To determine the amount of flavonoids, phenolics and antioxidants produced under water stress conditions in African and commercial ginger species, further assays are recommended.
- It is highly recommended that whole-community substrate utilisation profiles (CSUP) and microbial activity be monitored over an extended period of time in order to attain a more complete reflection on the impact of different rehabilitation techniques, as microbial diversity is an indicator of soil fertility and health.
- The research recommends that optimal levels of N be used to improve the production and quality of both ginger species. Since yield and quality were still increasing at the highest N level used, further trials with higher N levels should be undertaken to determine the optimum level.
- Furthermore, modelling can be used to explore optimum water and N levels for best yield and quality.

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APPENDECES

Summary of ANOVA tables (Total water use rain shelter)

Table 1: Effect of irrigation on the water use (mm) during the first growing seasons

(Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	3.42	8.88	259.44	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
	TRT	7	423658.41	60522.63	766.58	<.0001
	REP	2	310.03	155.01	1.96	0.1772
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.97	4.17	16.71	400.85	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
	TRT	7	174270.72	24895.81	89.06	<.0001
	REP	2	16.514	8.25	0.03	0.9710

TRT= Treatment, REP= Repetition, DF= Degree of freedom

II. Summary of ANOVA table (Rain shelter fresh rhizome yield)

Table 2: Effect of irrigation regimes on fresh rhizome yield (kg.ha^{-1}) 240 days after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	4.23	844.86	19943.88	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
	TRT	7	3239711069	462815867	648.38	<.0001
	REP	2	283682	141841	0.20	0.8221
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	6.05	1598.84	26384.21	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	4910695730	701527961	274.43	<.0001
		2	12769341	6384671	2.50	0.1181

TRT= Treatment, REP= Repetition, DF= Degree of freedom

III. Summary of ANOVA table (Rain shelter dry rhizome yield)

Table 3: Effect of irrigation on dry rhizome yield ($kg.ha^{-1}$) 240 days after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	3.04	129.26	4244.83	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	129383601.3	18483371.6	1106.17	<.0001
		2	123904.3	61952.2	3.71	0.0510
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	1.30	72.64	5586.91	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	120671745.2	17238820.7	3266.16	<.0001
		2	31470.6	15735.3	2.98	0.0834

TRT= Treatment, REP= Repetition, DF= Degree of freedom

IV. Summary of ANOVA table (Rain shelter WUE fresh and dry rhizome yield)

Table 4: Effect of irrigation on WUE fresh rhizome yield ($kg \cdot ha^{-1}mm^{-1}$) 240 days after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	6.45	5.38	83.35	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	46974.09	6710.58	231.72	<.0001
		2	56.17	28.08	0.97	0.4032
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.98	8.55	5.73	67.00	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
	7	35076.73	5010.96	152.48	<.0001	
	2	65.64	32.82	1.00	0.3931	

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 5: Effect of irrigation on WUE dry rhizome yield ($kg \cdot ha^{-1}mm^{-1}$) 240 days after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	5.72	1.00	17.55	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	1659.14	237.02	234.62	<.0001
		2	12.03	6.01	5.96	0.0134
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	4.88	0.68	14.06	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	723.11	103.30	219.34	<.0001
		2	0.94	0.47	1.01	0.3905

TRT= Treatment, REP= Repetition, DF= Degree of freedom

V. Summary of ANOVA table (Rain shelter LAI destructive harvest period)

Table 6: Effect of irrigation regimes on LAI destructive five months after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.80	18.85	0.56	2.99	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	17.58	2.51	7.90	0.0006
		2	0.97	0.48	1.53	0.2514
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.90	14.88	0.36	2.44	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	16.719	2.38	18.07	<.0001
		2	0.08	0.04	0.32	0.7323

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 7: Effect of irrigation regimes on LAI destructive six months after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.84	16.28	0.40	2.46	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	12.34	1.76	10.96	<.0001
		2	0.20	0.10	0.64	0.5424
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.91	12.42	0.32	2.63	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	16.82	2.40	22.46	<.0001
		2	0.21	0.10	1.00	0.3932

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 8: Effect of irrigation regimes on LAI destructive seven months after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.79	15.07	0.349	2.31	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	6.44	0.92	7.54	0.0007
		2	0.07	0.03	0.29	0.7523
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.88	18.04	0.42	2.35	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	18.43	2.63	14.58	<.0001
		2	0.22	0.11	0.63	0.5450

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 9: Effect of irrigation regimes on LAI destructive eight months after planting first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.69	24.96	0.45	1.80	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	6.05	0.86	4.27	0.0101
		2	0.50	0.25	1.25	0.3175
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.96	4.35	0.08322	1.90	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	2.76949	0.39564	57.12	<.0001
		2	0.00002	0.00001	0.00	0.9980

TRT= Treatment, REP= Repetition, DF= Degree of freedom

VI. Summary of ANOVA table (Rain shelter microbial Diversity)

Table 10: Effect of irrigation on Shannon-Weaver richness and Evenness index illustrating soil microbial species abundance (Tukey's Studentized Range)

Shannon-Weaver Richness	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.72	21.94	0.36	1.66	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	4.46	0.63	4.76	0.006
		2	0.49	0.24	1.85	0.194
Evennes (Soil microbial abundance)	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.75	17.15	0.10	0.60	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	0.45	0.064	6.01	0.002
		2	0.01	0.007	0.66	0.532

TRT= Treatment, REP= Repetition, DF= Degree of freedom

VII. Summary of ANOVA table (Glasshouse quality)

Table 11: Effect of irrigation on total phenolic content for rhizomes of commercial ginger (CG) and African ginger (AG) as affected by different N fertiliser levels first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	0.0725	0.00565	7.79	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	221.1404	44.22809	1384532	<.0001
		2	0.0002	0.00008	2.57	0.0934
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	0.1304	0.0089	6.89	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	222.8314	44.5662	551336	<.0001
		2	0.0008	0.0002	3.35	0.0474

TRT= Treatment, REP= Repetition, DF= Degree of freedom

Table 12: Effect of irrigation on total flavonoid content for rhizomes of commercial ginger (CG) and African ginger (AG) as affected by different N fertiliser levels first season (Tukey's Studentized Range)

First season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	3.65	0.12	3.41	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	36.18	7.23	462.89	<.0001
		2	0.12	0.04	2.75	0.0793
Second season	**Dependent Variable**	**R-Square**	**Coeff Var**	**Root MSE**	**Mean**	
		0.99	0.2326	0.0080	3.44	
	Source	**DF**	**Type III**	**Mean Square**	**F Values**	**Pr>F**
		7	36.9336	7.3867	115118	<.0001
		2	0.0003	0.0001	1.62	0.2260

TRT= Treatment, REP= Repetition, DF= Degree of freedom