Effect of water regimes and harvest times on yield and phytochemical accumulation of two ginger species

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Study highlights

•Water stress treatment of 20-25% MAD increased rhizome, tiller, and leaf yield of *Zingiber* officinale.

•Severely water-stressed irrigation regime (i.e., 80-85% MAD) enhanced total flavonoid content, phenolic content, and antioxidant activity for ginger species.

•Selection of 20-25% MAD for crop yield and 80-85% MAD for secondary metabolites is desired for crop improvement strategies.

•Harvesting time and plant parts contribute to adopting plant substitution and quality improvement of ginger species.

Abstract

In South Africa, both Commercial ginger (*Zingiber officinale* Roscoe) and African ginger (*Siphonochilus aethiopicus* (Schweinf.) BL Burtt) are significant medicinal plants. This study aimed to determine the effect of water regimes on the two-ginger species' phenolic content, antioxidant properties and yield at various harvest times. During the 2015/2016 and 2016/2017 crop seasons, a field experiment was conducted. The experiment used a randomised complete block design (RCBD) and included two ginger species, four irrigation regimes, and three replicate blocks. Four different levels of soil water availability (20-25%, 40-45%, 60-65%, and 80-85% MAD) significantly increased total flavonoid and phenolic content and total antioxidant activity in both species, but the biomass yield was lower than the other treatments. The well-watered control (20-25% MAD) resulted in a significant increase in rhizome yield at different harvesting times but resulted in lower total

flavonoid and phenolic content and total antioxidant activity for both species. However, the moderately stressed treatment (40-45% MAD) obtained a higher WUE, flavonoid, phenolic and antioxidant content without reducing yield significantly in both species. The results indicated a similar trend in phytochemical constituents for leaves and rhizomes. The species can be harvested seven to eight months after planting under open field conditions. The accumulation of phytochemicals is dependent on water regime, harvesting time, and plant part; growers should consider 40-45% MAD and harvest seven to eight months after planting when growing ginger species to save the scarce resource (water), high phytochemical content and rhizome yield.

Keywords: Antioxidant; Flavonoid; Phenolics; Maximum available depletion level; Rhizome yield; and water use efficiency

1. Introduction

Medicinal plants are extremely valuable to human livelihoods and are widely recognised in South Africa as distinct and high in phytochemicals. Commercial ginger (Zingiber officinale Roscoe) and African ginger (Siphonochilus aethiopicus (Schweinf.) BL Burtt) are among the medicinal plants that are commonly used, and they are members of the Zingiberaceae family. Both plant species have traditionally been used to treat many human ailments, including malaria, asthma, headaches, and chest problems (Van Wyk, 2008). The phytochemistry in both ginger species is responsible for their health-promoting properties (Butt and Sultant, 2011). Anti-inflammatory and antioxidant components are abundant in ginger species (Cai et al., 2004). Ginger species account for various bioactivities such as phenolic substances, and organic acids are prominent due to antioxidant properties. The high antioxidant capacity of plants is due to phenolic substances, which contribute significantly to human health (Mao et al., 2019). Furthermore, the species contains volatile organic compounds such as sesquiterpene and monoterpenoid hydrocarbons and non-volatile pungent compounds such as gingerols, shogaols, and zingerone (Shukla and Singh, 2007). According to a volatile profiling study, terpenes were found in different plant parts, including the rhizome and root. On the other hand, the leaf lacked the monoterpenes found in the rhizome and root (Mokgehle et al., 2019). Ginger species have a distinct aroma and flavour due to non-volatile and volatile components. According to Ghasemzadeh et al. (2010), different ginger plant parts, such as rhizomes, stems, and leaves, differ in flavonoid, phenolic, and antioxidant compounds due to biotic and abiotic factors.

Because of their importance in traditional medicine systems and as economically valuable plants, studies on the potential effects of cultivation practices on the species are critical. Cultivation practices and climate change (increased risk of drought) had a noticeable impact on medicinal plant species' life cycles and distribution in various habitats. However, it is unclear how water stress and harvesting time may affect secondary metabolite production in medicinal plants and other physiological factors. Water stress is one of the factors responsible for the restriction of plant growth and yield for various crop species in agricultural production. Water stress alters physiological and metabolic processes by limiting gas exchange, closing stomata, and slowing photosynthesis (Shao et al., 2008). The ability of a plant species to survive under stressed conditions is determined by the plant species, growth stage, duration, and intensity of the water stress.

A previous study showed that water stress significantly impacts ginger species' growth, yield, and water use efficiency (WUE). *Zingiber officinale* is less sensitive to water stress than *S. aethiopicus* (Gatabazi et al., 2019). It is critical for achieving optimum production for commercially viable ginger species to investigate opportunities for improved plant responses to varying factors such as water regimes and harvesting time. There is a paucity of information on the effect of ginger species harvesting time and water regimes under the South African climatic conditions. Elsewhere in the world, previous reports showed improved growth for *Zingiber officinale* under constant, elevated moisture and water availability to the plant (Ravindran et al., 2004).

Xu (2000) attributed the enhanced growth of Zingiber officinale under high irrigation frequency to the availability of nutrients. Overall, irrigation restriction on the plant decreases the photosynthetic and stomatal conductance levels, affecting vegetative growth. However, in some cases, frequent irrigations may reduce plant roots' oxygen availability due to excessive moisture content in the root zone (Schroder and Liet, 2002). According to Mokgehle et al. (2017), manipulating factors such as fertilisers (especially nitrogen) can effectively increase the expression of secondary metabolites in S. aethiopicus under favourable climatic conditions. Several studies have been published on the role of medicinal plants and the mechanisms used by plants to change their cellular metabolism in response to environmental stresses (Kazan, 2013). Water stress has been studied in several plant species to see how it affects primary and secondary metabolites (Apel and Hirt, 2004; Mokgehle et al., 2017). Nakabayashi et al. (2014) attribute increased oxidative stress and photochemical inhibition to increased electron leakage towards O₂ during photosynthetic and respiratory processes in the plant cell. The ability of different plant species to cope under water-stress conditions may be related to their ability to scavenge reactive oxygen species (ROS) by increasing the activity of antioxidant enzymes during water scarcity. High antioxidant enzyme levels are required for plants to tolerate environmental stresses such as drought (Gill and Tuteja, 2010).

Growing conditions strongly influence the concentration of various secondary metabolites, which affects the metabolic pathways responsible for the accumulation of related natural products. The significance of the relationship between increased antioxidant enzyme activities and increased resistance to environmental stress is dependent on the stage of development and the duration of stress (Bajguz and Hayat, 2009). Although several studies have been published on ginger species' medicinal value, active principles, and antioxidant properties, little information is available on variable factors such as plant part, harvesting time and water regimes on yield, phytochemical constituents, and related antioxidant activity in the ginger species. As a result, this study aimed to investigate the effects of water regimes on yield and phytochemical accumulation in two ginger species harvested at different times.

2. Materials and methods

2.1. Site description

The research was conducted in a rain-shelter environment at Innovation Africa on the Hillcrest Campus of the University of Pretoria in South Africa, at an elevation of 1350 m above sea level, latitude 25°45′ S and longitude 28°16′ E. The experiment was carried out during the cropping seasons of 2015/2016 and 2016/2017.

Soil samples were collected at four depths before the experiment, namely 0-20, 20-40, 40-60, and 60-80 cm, to determine the chemical and physical properties of the study site. The samples were analysed at the Agricultural Research Council's Soil, Climate and Water (ARC-SCW, South Africa) using atomic absorption spectroscopy and ammonium acetate extraction (1 N NH40Ac). The soil of the experimental site was a sandy clay loam of the Hutton type (Soil Classification Working Group 1991). Weather data was collected from an automatic weather station (Campbell Scientific, Logan, UT, US). The monthly average air temperatures (maximum and minimum), relative humidity, solar radiation, wind speed, and monthly evapotranspiration (Fig. 1). Fertilizers were applied based on the nutrient status of the soil. A base fertiliser dressing of 170 kg ha⁻¹ phosphorus and potassium was applied, and a total of 220 kg N ha⁻¹ was used. The phosphorus was applied during land reparation, and half of the nitrogen (110 kg ha⁻¹) and 170 kg ha⁻¹ potassium were applied 45 days after planting. The remaining N was applied 90 days after planting, in accordance with the needs of *S. aethiopicus*, as described by Mokgehle et al. (2017).



Fig. 1. Monthly mean weather data measured at Innovation Africa @UP during the two growing seasons. T Max: maximum temperature, T Min: minimum temperature, ETo: daily reference evapotranspiration; RH Min: minimum relative humidity; RH Max: maximum relative humidity.

2.2. Planting material

Commercial ginger (*Z. officinale*) rhizomes were obtained from Fortuna (Pty Ltd, South Africa), while African ginger (*S. aethiopicus*) was obtained from the Agricultural Research Council–Vegetables, Industrial and Medicinal Plants campus (ARC-VIMP) in South Africa. Before planting, the ginger species were kept at a room temperature of 25 °C to ensure a high percentage of sprouting in the field (Mokgehle et al., 2019).

2.3. Irrigation treatments

The soil water content was monitored at least three times per week using a model 503DR CPN Hydroprobe neutron water metre (Campbell Pacific Nuclear, California, USA). The

neutron probe was first calibrated using gravimetric soil samples from the rain shelter. Access tubes were installed to a depth of 1.0 m in the middle of each plot between two plants in a row. Individual plots were irrigated using a pressure-compensated drip irrigation system with a water discharge rate of $1.4 \text{ L} \text{ h}^{-1}$ at a pressure range of 100-120 kPa. The irrigation treatments were determined by calculating the maximum allowable depletion (MAD) of available soil water (ASW) to a depth of 0.8 m (Zerizghy, 2012). The control treatment was watered when the soil's MAD of ASW content reached 20 and 25%. The other treatments were watered when the MAD of available soil water reached 40-45%, 60-65%, or 80-85% (Panda et al., 2003).

Eq. 1 calculated the actual depletion of available soil water (Panda et al., 2003).

Depletion of available soil water =
$$100\frac{1}{n}\sum_{i=1}^{n}\frac{FC_{i}-\theta_{i}}{FC_{i}-PWC_{i}}$$
 (1)

Where n is the number of layers of actual rooting depth used in soil water content measurement, FCi is the soil water content at field capacity for the ith layer, θ i is the measured volumetric water content, and PWPi is the soil water content at the permanent wilting point on a volume basis.

2.4. Water use

Crop water use/evapotranspiration (ET) for each plot was determined by using the soil water balance equation:

$$ET = I + P - D - Roff - \Delta S \tag{2}$$

Where *ET* denotes crop water use/crop evapotranspiration (mm), *I* represent irrigation (mm), *P* denotes precipitation (mm), *D* denotes drainage (mm), *Roff* denotes surface runoff (mm), and ΔS denotes the change in soil water storage between planting and harvest (mm). Because the plots were relatively flat, runoff and drainage were assumed to be negligible (Tesfaye et al., 2006), and irrigation was carefully managed to avoid over-irrigation and drainage.

2.5. Plant material and preparation of extract

The rhizomes, tillers, and leaves were harvested separately and dried at 50 °C for 48 h before being chopped and ground to a fine powder with a 0.45 mm sieve. The dried rhizome, tiller, and leaf powder were extracted in an ultrasonic bath (Model CPX1800-E, Merck, Cape Town, South Africa) for 1 h with 50% aqueous methanol. The extract was filtered using No. 1 Whatman filter paper. The filtrate was concentrated at 30 °C under reduced pressure using a rotary evaporator (Model RE301, Lasec SA (Pty) dried under an air stream. For analysis, the extract was dried, weighed, and stored in storage vials at 4 °C.

2.6. Determination of total phenolic content

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

2.7. Determination of total flavonoid content

Sultana et al. (2009) described a colorimetric method for estimating total flavonoid content using aluminium chloride (AlCl₃). Extracts of each ginger species' plant parts (rhizome, leaf, and tiller) were diluted with deionized water (0.3 mL), followed by 0.3 mL of 5% sodium nitrite (NaNO₂). After 5 min, at 25 °C, AlCl₃ (10%) was added and left for another 5 min before adding 0.2 mL NaOH (1.0 M). After 5 min, the reaction mixture was diluted with 1 mL of deionized water. A spectrophotometer was used to measure absorbance at 510 nm in triplicate. Three readings were taken for each sample to obtain the average results, and a standard curve was plotted using quercetin as a standard (mg of QE g⁻¹ dry mass basis) (Li et al., 2008).

2.8. Determination of antioxidant activities using FRAP assay

Each sample's ferric reducing power assay (FRAP) was determined using the method described by Alothman et al. (2009). The extract's reducing antioxidant power was measured spectrophotometrically. The absorbance versus concentration of the two positive controls, butylated hydroxytoluene (BHT) and ascorbic acid, was plotted. The reaction mixture was incubated in the dark for 30 min at room temperature. The assay samples were prepared in triplicate and repeated twice. A microtiter plate reader (ELISA, Microplate Reader, California, USA) measured absorbance at 630 nm (Ndhlala et al., 2014).

2.9. Quantifying yield data

Yield parameters such as the fresh and dry mass of the rhizomes were collected. Harvesting was done at monthly intervals, starting five months after planting, with the last harvest at eight months after planting. Rhizomes 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.

2.10. Data analysis

SAS software version 9.4 was used to analyse variance (ANOVA) on the data (SA Institute, Cary, NC, USA). Duncan's multiple range test (p < 0.05) separated significantly different means. GraphPad Prism version 5.00 for Windows (GraphPad Software Inc., San Diego, CA) was used to create the graphs. The Tukey honest significant difference (HSD) test was used to separate the treatment means (p < 0.05).

3. Results

3.1. Soil water used

The results showed that *Z. officinale* used more water for well-watered treatments and moderate stress (CG: 20-25% MAD and CG: 40-45% MAD, respectively) treatments for *S. aethiopicus*. Water use was thus higher in control treatments for both species than in the other treatments. The mean water use increased from 462 mm and 469 mm for CG: 20-25% MAD and AG: 20-25% MAD in 2015/16 to 543 mm and 549 mm in 2016/17, respectively. Soil water depletion levels in plant species can differ between soil types, seasons, and irrigation systems. Soil water uptake may vary widely with management practices, location, and crop varieties.

3.2. Total flavonoid content of ginger rhizomes, leaves and tillers

The effects of four water regimes and two species of ginger on total phenolic content, flavonoid content, and antioxidant activity were investigated in this study. Significant differences were found across all water regimes, ginger species, and parts evaluated at various harvest stages (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6). The results showed that the severely stressed treatment (80-85% MAD) had a significant increase in total flavonoid content for Z. officinale and S. aethiopicus rhizomes from the first harvest (month five) to the last harvest (month eight). The change in total flavonoid content in response to irrigation regimes was more pronounced for Z. officinale, and S. aethiopicus leaves during the first harvest (month five) than the last harvest (month eight) (Table 2). Furthermore, the results show that rhizomes had the highest flavonoid content compared to tillers and leaves (Tables 1, 2 and 3). The results show that harvesting Z. officinale seven to eight months after planting yielded good results for flavonoid content. Moderate water stress could obtain a higher flavonoid content without significantly reducing yield. Water stressing S. aethiopicus and Z. officinale had a significant positive impact on the flavonoid content of the leaves. As a result, water stress treatments for the ginger species may help improve flavonoid content. The leaves showed an increasing trend in dry tiller flavonoid content observed in response to severe water stress (Tables 2 and 3). The severely stressed treatment (80-85% MAD) had the highest flavonoid content, followed by the moderately stressed treatment (60-65% MAD) for the leaves, rhizomes, and tillers of both ginger species (Tables 1, 2 and 3). With water stress, the leaves and tillers of both Z. officinale and S. aethiopicus showed a significant increase in total flavonoid content (Tables 2 and 3). The total flavonoid content of Z. officinale and S. aethiopicus rhizomes increased (Table 1).

Table 1. Effect of irrigation regimes and harvest date (months after planting) on total flavonoid content (mg.g⁻¹ QE) of African ginger (AG) and Commercial ginger (CG) rhizomes during 2015/16 (A) and 2016/17 (B) cropping seasons.

2015/2016 crop	ping season								
Ginger species	MAD	Months							
		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°				
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 foll	owed by the sa	me letter i	e letter in the same column are not significantly						
different at (p	≤ <i>0.05</i>) level ac	cording to '	Tukey's tes	st.					
2016/2017 cr	opping seasor	1							
Ginger species	MAD	Months							
		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 ^{be}	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				

Table 2. Effect of irrigation regimes and harvest date (months after planting) on total flavonoid content (mg.g⁻¹ QE) of African ginger (AG) and Commercial ginger (CG) leaves during 2015/16 (A) and 2016/17 (B) cropping seasons.

2015/2016 cropping season										
Ginger species	MAD	Months 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 ^e					
	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 foll	owed by t	the same le	tter in the	same colur	nn are not significantly					
different at (p	≤0.05) lev	vel accordir	ng to Tukey	y's test.						
2016/2017 cr	opping s	eason								
Ginger species	MAD	Months								
		Five	Six	Seven	Eight					
CG	20-25	0.249 ^ª	0.257 ^f	0.366 ^a	0.485 ^c					
	40-45	0.419 ^c	0.252 ^f	0.267 ^e	0.481 ^c					
	60-65	0.559 [₽]	0.364 ^e	0.361 ^ª	0.570 ^{bc}					
	80-85	0.760 ^a	0.355 ^e	0.680 [°]	0.686					
AG	20-25	0.233 ^d	0.656 ^d	0.375 ^d	0.332 ^d					
	40-45	0.273 ^a	0.826 ^c	0.447 ^c	0.464 ^e					
	60-65	0.416 ^c	0.952 [°]	0.419 ^c	0.549 ^e					
	80-85	0.746 ^a	1.207 ^a	1.191ª	1.045ª					
CV (%)		4.098	3.791	2.493	7.621					
LSD		0.054	0.066	0.036	0.126					

Table 3. Effect of irrigation regimes and harvest date (months after planting) on total flavonoid content (mg.g⁻¹) QE) of African ginger (AG) and Commercial ginger (CG) tillers during 2015/16 (A) and 2016/17 (B) cropping seasons.

2015/2016 cropping season												
Ginger species	MAD	Months										
		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 ^e	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 foll	owed by t	he same le	tter in the s	ame colun	nn are not significantly							
different at (p	≤ 0.05) lev	el accordir	ig to Tukey	's test.								
2016/2017 cr	opping se	ason										
Ginger species	MAD	Months										
		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 ^{be}							
	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							

3.3. Total phenolic content of ginger rhizomes, leaves and tillers

In this study, S. aethiopicus and Z. officinale (CG) were subjected to four levels of water availability to assess the effects of water stress on the total phenolic content of rhizomes, leaves, and tillers at various harvesting dates (Tables 4, 5 and 6). The phenolic content of both ginger species' rhizomes was higher than that of the tillers and leaves (Tables 4, 5 and 6). The total phenolic content of the rhizomes was lower in the well-watered control (20-25%) MAD) for both Z. officinale and S. aethiopicus (Table 4). Under the severely stressed treatment (80-85% MAD), rhizomes had significantly higher total phenolic content for CG and AG at different harvesting months after planting (Tables 4). During other harvesting months, the stressed and severely water-stressed treatments (60-65% MAD and 80-85% MAD) showed increased total phenolic content in leaves and tillers of both plant species (Tables 5 and 6). Six months after planting, the harvesting of Z. officinale tillers revealed a higher total phenolic content for the severely stressed treatment CG: 80-85% MAD than in other months (Table 6). However, the total phenolic contents of the tillers under severely stressed treatment (AG: 80-85% MAD) were higher seven months after planting compared to earlier harvests (Table 6). Harvesting the leaves of Z. officinale seven months after planting revealed higher phenolic contents for the severely stressed treatment (CG: 80-85% MAD). For the moderate (60-65% MAD) and severely stressed treatments (80-85% MAD),

Siphonochilus aethiopicus showed the highest value when harvested at six and seven months, respectively. The well-watered treatment yielded the lowest total phenolic content for the tillers (20-25% MAD) for *Z. officinale* and *Siphonochilus aethiopicus* (Table 6).

Table 4. Effect of irrigation regimes and harvest date (months after planting) on total phenolic content (mg.g ⁻¹	
GAE) of African ginger (AG) and Commercial ginger (CG) rhizomes during 2015/2016 (A) and 2016/2017 (B)
cropping seasons.	

2015/2016 crop Ginger species	ping seaso MAD	on Months			
0		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 ^e	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 foll different at (p 2016/2017 cr	lowed by t ≤0.05) lev copping s	the same let vel according eason	ter in the san g to Tukey's	ne column a test.	re not significantly
Ginger species	MAD	Rivo	Civ	Sources	Fight
66	20.25	2 6508	11 040 ⁶	0.070 ^e	7 1 46 ⁸
60	20-25	2.039- 6.020 ^f	11.243	0.073 0.102 ^d	7.140- 0.916 ^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
110	40-45	6.920 ^e	10.106 ^f	10.456 ^c	11.856 ^c
	60-65	11.833°	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 $(p \le 0.05)$ level according to Tukey's test.

Table 5. Effect of irrigation regimes and harvest date (months after planting) on total phenolic content (mg.g⁻¹ GAE) of African ginger (AG) and Commercial ginger (CG) leaves during 2015/2016 (A) and 2016/2017 (B) cropping seasons.

2015/2016 cropping season											
Ginger species	MAD	Months									
		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 foll different at (p-	owed by t <0.05) lev	the same let rel accordine	ter in the sa g to Tukey's	me column test.	are not significantly						
2016/2017 cr	opping se	easons	5 - , -								
Ginger species	MAD	Months									
		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						

Table 6. Effect of irrigation regimes and harvest date (months after planting) on total phenolic content (mg.g⁻¹ GAE) of African ginger (AG) and Commercial ginger (CG) tillers during 2015/2016 (A) and 2016/2017 (B) cropping seasons.

2015/2016 cropping season												
Ginger species	MAD	Months										
		Five Si	x	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 fol	lowed by t	the same lett	er in the sam	ie column a	are not significantly							
different at (p	≤0.05) lev	vel according	to Tukey's t	est.								
2016/2017 c	ropping s	eason										
Ginger species	MAD	Months										
		Five Si	x	Seven	Eight							
CG	20-25	1.146 ^g	2.625 ^g	0.105 ^r	0.222 ^e							
	40-45	1.313 ^r	7.013 ^c	0.243 ^c	0.229 ^e							
	60-65	3.543⁵	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 ⁿ	3.013 ^r	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 [₽]	0.488 ^a	0.420 ^c							
CV (%)		2.068	0.533	2.292	1.942							
LSD		0.129	0.100	0.016	0.018							

3.4. Total antioxidant content of ginger rhizomes, leaves and tillers

The ferric reducing power assay (FRAP) determined the total antioxidant content in ginger species' rhizomes, leaves, and tillers in response to water stress and harvest date (Figs. 2, Fig. 3, Fig. 4). Under severely stressed conditions, the rhizomes had the highest FRAP values for all harvest dates, indicating their potential as antioxidants (80-85% and 60-65% MAD), followed by 40-45% MAD (Fig. 2). Antioxidants were more abundant in rhizomes than leaves or tillers (Figs. 2, 3 and 4). The lowest FRAP values were observed in the rhizomes, leaves and tillers of *Z. officinale* and *S. aethiopicus* under well-watered conditions (20-25% MAD), followed by moderate and severely stressed conditions (Figs. 2, 3, and 4).

The tillers had the highest antioxidant values five and six months after planting, indicating their antioxidant capacity at various harvest dates. The standard ascorbic acid had higher FRAP values than the four irrigation regime treatments for the rhizomes, leaves, and tillers (Figs. 2, 3 and 4). The antioxidant activity of the rhizomes for the severely stressed (80-85% MAD) treatment for *Z. officinale* and *S. aethiopicus* was highest from five and six months after harvesting to seven and eight months after harvesting (Fig. 2A-D). The butylated hydroxytoluene (BHT) standard and treatments AG: 60-65%, CG: 60-65%, AG: 40-45% and CG: 40-45 MAD clustered together in the leaves during month five after harvesting



Fig. 2. Total rhizome 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.



Fig. 3. 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.



Fig. 4. Total tiller 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.

(Fig. 3A). The well-watered treatment (20-25% MAD) for *Z. officinale* and *S. aethiopicus* had the lowest antioxidant activity. At all harvests, the tillers' results showed that ascorbic acid and BHT had the highest antioxidant activity compared to other treatments (Fig. 4).

3.5. Effect of water stress on rhizome yield of two ginger species

There were significant interactions between irrigation regimes and ginger species for fresh and dry rhizome Table 7A and B) yields. The results showed a gradual increase in the fresh rhizome yield of the well-watered control (20-25% MAD) from five to eight months after planting for both plant species during the 2015/2016 and 2016/2017 cropping seasons (Table 7A and B). The highest dry rhizome yield was reported seven to eight months after planting *Z. officinale* during both cropping seasons (Table 7A and B). The fresh (38.3 versus 21.5 t ha⁻¹) and dry (8.8 versus 5.3 t ha⁻¹) rhizome yields eight months after planting were higher for *Z. officinale* as compared to *S. aethiopicus* for the well-watered control (20-25% MAD). The stressed treatments (60-65% MAD and 80-85% MAD) recorded the lowest fresh and dry matter yields across all months for *S. aethiopicus* species, although differences were not always significant.

3.6. Water use efficiency of fresh and dry rhizome of two ginger species

There was a difference between water use efficiency on fresh and dry rhizomes yield among *Z. officinale* and *S. aethiopicus* for the 2015/2016 and 2016/2017 cropping season (Table 8A and B). The results for 2015/2016 show that the fresh rhizome of the *Z. officinale* gave the highest water use efficiency for the stressed 60-85% MAD and the severely stressed 80-85% MAD regimes, as compared to the well-watered CG: 20-25% MAD and less stressed CG: 40-45% MAD (Table 8A). In contrast, in 2016/2017, the results in Table 8B show the highest water use efficiency for both ginger species and the well-watered treatments (20-25% MAD and 40-45% MAD) five months after harvesting.

The water use efficiency for fresh rhizome yield in the second cropping season showed higher efficiency for the stressed treatment CG: 60-65% MAD five months after harvesting. During six, seven and eight months after harvesting, treatment of CG: 40-45, CG: 60-65 and 80-85% MAD exhibited higher water use efficiency compared to the well-watered CG: 20-25% MAD (Table 8B). The results indicated that *S. aethiopicus* had the lowest water use efficiency, specifically at the well-watered treatment (AG: 20-25% MAD), which recorded the lowest values across different months after planting in the 2015/2016 cropping season (Table 8B). The results showed that *Z. officinale* had higher water use efficiency than *S. aethiopicus* during different harvesting periods.

The water use efficiency for dry rhizome yield in the first cropping season was higher for the treatment 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 all the months of harvesting (Table 8A). Although the highest water use efficiency was reported for *Z. officinale* for treatments CG: 40-45% MAD and the severely stressed CG: 60-65% MAD, the variants were not significantly different to *S. aethiopicus* values (Table 8A and B).

(A) First season 2015/2016																			
	Species		Fiv	ve		Si	ix				Seven					Eight			
MAD	Ginger	ET	Yie	eld	WUE	E	Т	Yield	WUE		ET	Yie	ld	WUE		ET		Yield	WUE
		mm	kg		kg∙ha ⁻¹	m	m	kg.	kg·ha ⁻¹	L	mm	kg.		kg∙ha ⁻¹		mm		kg.ha ⁻¹	kg∙ha ^{−1} mm ^{−1}
			ha	_1	\rm{mm}^{-1}			ha ⁻¹	\rm{mm}^{-1}			ha ⁻	-1	\rm{mm}^{-1}					
20-25%	CG	291.0	08 32	800	112.7	39	99.38	34900	87.39		469.11	362	200	77.17		469.1	1	38300	81,64
40-45%		135.5	51 24	500	180.80	20	60.41	26000	99.84		284.47	282	200	99.13		284.4	17	30400	106,87
60-65%		68.90) 22	500	326.56	12	26.80	24600	194.00		176.02	260	000	147.71		176.0)2	27400	155,66
80-85%		42.53	3 15	800	371.50	8	0.70	17800	220.57		109.02	210	000	192.63		109.0)2	22400	205,47
20-25%	AG	280.9	90 23	000	81.88	39	98.90	21300	53.37		461.55	189	900	40.95		461.5	55	21500	46,58
40-45%		128.6	56 13	400	104.15	24	41.80	16500	68.24		274.58	199	900	72.47		274.5	58	21300	77,57
60-65%		71.38	3 10	700	149.90	13	38.46	16300	117.61		183.46	201	00	109.56		183.4	16	21000	114,47
80-85%		48.82	2 10	700	219.17	93	2.90	18000	193.76		117.32	166	500	141.49		117.3	32	18800	160,25
CG: Commerc	cial ginger;	AG: Afric	can ging	er															
(B) Season	: 2016/20	17																	
S	pecies		Five			Six				Seve	n				Eigh	t			
MAD G	inger E	Т	Yield	WU	E	ET	Yiel	d WI	JE	ET	Yi	eld	WU	E	ET		Yield	1	WUE
	n	nm	t.ha ⁻¹	kg∙h	a^{-1}	mm	t.ha	^{−1} kg-	ha ⁻¹	mm	t.l	1a ⁻¹	kg-ł	1a ⁻¹	mm		t.ha	-1	kg∙ha ⁻¹ mm ⁻¹
				mm	-1			mn	n ⁻¹				mm	1					
20-25% C	G 5	31.92	33600	63.1	7	549.14	400	00 72,	84	549.	14 42	2000	76.4	18	549.	14	4280	00	77,94
40-45%	3	47.57	24900	71.6	54	361.79	310	00 85.	69	361.	79 41	000	113	.33	361.	79	4210	00	116,37
60-65%	3	21.67	24900	77.4	41	343.89	294	00 85.	49	343.	89 29	9600	86.0	07	343.	89	3570	00	103,81
80-85%	3	29.63	16300	49.4	45	328.73	289	00 87.	91	328.	73 28	3900	87.9	91	328.	73	3410	00	103,73
20-25% A	.G 5	25.93	13600	25.8	36	542.85	154	00 28.	37	542.	85 19	9200	35.3	37	542.	85	2220	00	40,90
40-45%	3	49.76	6000	17.1	6	380.95	860	0 22.	58	380.	95 88	300	23.1	10	380.	95	1180	00	30,98
60-65%	3	47.08	5200	14.9	98	362.65	600	0 16.	55	362.	65 73	300	20.1	[3	362.	65	9700)	26,75
80-85%	3	59.56	4100	11.4	10	340.68	480	0 14.	09	340.	68 54	100	15.8	35	340.	68	6200)	18,20

Table 7. Water use efficiency of fresh rhizomes of ginger species in response to four water stress regimes at different times (5 – 8 months) after planting.

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

(A) 2015/2	(A) 2015/2016 cropping season													
	Species	-	Five		Six			Seven			Eight			
MAD	Ginger	ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE	ET	Yield	WUE	
		00.00	kg_ha=1	kg.ha ⁻¹ mm ⁻¹	JT100	kg.ha-	kg.ba ⁻¹ mm ⁻¹	ITRIDO.	kg.ha ⁻¹	kg.ha=1 mm=1	ana	kg.ha ⁻¹	kg.ha ⁻¹ mm ⁻¹	
20-25%	OG	291.08	3002	10.31	399.38	7000	17.53	469.11	8700	18.56	469.11	8800	18,76	
40-45%		135.51	2500	19.19	260.41	5000	19.20	284.47	7900	27.77	284.47	9800	34,45	
60-65%		68.90	2000	29.03	126.80	4900	38.64	176.02	6300	35.79	176.02	8500	48,29	
80-85%		42.53	1200	28.22	80.70	4400	54.52	109.02	7100	65.13	109.02	8200	75,21	
20-25%	AG	280.90	1800	6.41	398.90	4600	11.53	461.55	4600	9.97	461.55	5300	11,48	
40-45%		128.66	2400	18.65	241.80	3600	14.89	274.58	4300	15.66	274.58	5200	18,94	
60-65%		71.38	1600	22.42	138.46	3600	26.00	183.46	4200	22.89	183.46	5100	27,80	
80-85%		48.82	1700	34.82	92.90	3600	38.75	117.32	4200	35.80	117.32	4800	40,91	
CG: Comm	ercial ginger	; AG: African ginge	r, WUE: Wate	r use efficiency, ETo	c Evapotranspiratio	C16								
(B) 2010	5/2018 crop	ping season												
	Species		Five		Six			Seven			Eight			
MAD	Ginger	ET	Yield	WUE	ET	Yield	WUE	EL	Yield	WUE	ET	Yield	WUE	
		100.000	kg.ha=1	kg-ha ⁻¹ mm ⁻¹	mim	kg.ha ⁻¹	kg.ha ⁻¹ mm ⁻¹	maina.	kg.ba-	kg.ha=1mm=1	mm	kg.ha~i	kg.ha=1mm=1	
20-25%	OG	531.92	2700	5.08	549.14	5200	9.47	549.14	6900	12,57	549.14	7900	14,39	
40-45%		347.57	2300	6.62	361.79	3900	10.78	361.79	6100	16,86	361.79	6900	19,07	
60-65%		321.67	2200	6.84	343.89	3100	9.02	343.89	5400	15,70	343.89	6600	19,19	
80-85%		329.63	1600	4.85	328.73	2600	7.91	328.73	3600	10,95	328.73	3700	11,26	
20-25%	AG	525.93	2700	5.13	542.85	2800	5.18	542.85	2900	5,34	542.85	4400	8,11	
40-45%		349.76	2400	6.86	380.95	2400	6.30	380.95	2900	7,61	380.95	3500	9,19	
60-65%		347.08	1900	5.47	362.65	1900	5.24	362.65	2500	6,89	362.65	3400	9,36	
80-85%		359.56	1600	4.45	340.68	1800	5.28	340.68	2400	7,04	340.68	3100	9,10	

Table 8. Water use efficiency of dry rhizomes of ginger species in response to four water stress regimes at different times (5 – 8 months) after planting.

CG: Commercial ginger; AG: African ginger, WUE: Water use efficiency, ETo: Evapotranspiration.

4. Discussion

Zingiber officinale used more water for well-watered and stress treatments (CG: 20-25% MAD and CG: 40-45%, respectively) than *S. aethiopicus*. The moderate and severely stressed treatments (60-65% MAD and 80-85% MAD) reported slightly higher water use for *S. aethiopicus* than *Z. officinale* at various months after harvesting (Gatabazi et al., 2019). According to Zhang et al. (2004), as the irrigation interval lengthens (higher MAD percentage), the topsoil layer dries out more and the proportion of water taken up from deeper soil layers increases. The two ginger species' recorded yield components and phytochemical profiles were significantly affected by the different total available water depletion levels. The water use increased from 462 mm and 469 mm for CG: 20-25% MAD and AG: 20-25% MAD, respectively, to 543 mm and 549 mm. During the 2016/2017 cropping season, the maximum water use for well-watered *Z. officinale* and *S. aethiopicus* was 549 mm and 543 mm, respectively. According to Jabro et al. (2017), soil water depletion levels in plant species can vary depending on soil texture and pore size distribution.

The current study's well-watered control (20-25% MAD) decreased the total flavonoid and phenolic content of ginger species' rhizomes, leaves, and tillers. The severely stressed treatment (80-85% MAD) had higher total flavonoids and phenolics but a lower yield. Water stress is thought to be a factor that causes oxidative stress and has been linked to an increase in flavonoids in willow leaves (Akula and Ravishankar, 2011). According to the current study, an increase in flavonoid content was observed three to five days after the onset of drought, indicating that all flavonoids are drought stress-responsive metabolites with the potential to be used as positive markers and potentially mitigate drought stress (Nakabayashi et al., 2014). Environmental factors directly affect the chemical constituents of medicinal plants and alter their metabolic levels (Colling et al., 2010). The reactions of plants to water stress levels vary significantly, depending on the intensity and duration of the stress and the plant species, plant part, and stage of development (Chaves et al., 2003). The increase in flavonoids and phenolics under extreme conditions could be attributed to the plant species' ability to scavenge reactive oxygen species (ROS) by increasing the activities of antioxidant enzymes during water loss (Gill and Tuteja, 2010).

The decrease in total phenolic content under well-watered conditions observed in the current study agrees with previous findings that suggest that increased irrigation can limit specific components to improve secondary metabolites (Battaieb et al., 2010). Furthermore, Lafka et al. (2007) reported decreased total phenolic content due to increased water application. These findings contradicted those of Jiang and Huang (2001) and Weidner et al. (2009), who found that environmental stress either decreases or increases the content of phenolic compounds in cells. Although some phenolic compounds, such as phenolic acids or flavonoids, are well-known and found in most plant species (Cai et al., 2004). It should be noted that plant adaptation to water stress may affect phenol metabolism, causing variation from one stress level to the next.

The current study's antioxidant levels, like flavonoids and phenolics, indicate that the antioxidant defence system is not compromised under long-term water deficit conditions. The severely stressed treatments (80-85% MAD) had higher FRAP values than the well-watered treatments. The findings suggest that a lack of water may increase antioxidant levels depending on the crop species. In contrast, severe or long-term water deficiency has reduced antioxidant activity (Pan et al., 2008). The antioxidant content of ginger species includes various photochemistry compounds that scavenge free radicals and protect cells from

oxidative stress, which is linked to multiple chronic diseases (Shukla and Singh, 2007; Sedghi et al., 2012). According to Mano (2002), the sequence of events in drought-stressed plant tissue is first 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. The increasing antioxidant levels obtained from various plant parts of *S. aethiopicus* and *Z. officinale*, particularly under water stress conditions be used in various pharmacological preparations. An excellent strategy for testing plant extract's antioxidant activity in different crop species is based on the extract's composition and the test system (Figueiredo et al., 2008). Such characteristics will also contribute to the benefits of antioxidants, which protect plants from oxidative stress damage (Gill and Tuteja, 2010). Such mechanisms should be investigated further to determine the impact of other environmental conditions on secondary metabolites in plants (Ghasemzadeh et al., 2010).

The reduction in fresh and dry yield under stressed, and severe water stress treatments (60-65% MAD and 80-85% MAD) can be attributed to crop canopy and biomass reduction which impact negatively on yield (Yuan et al., 2003). Mofokeng et al. (2015) reported significantly higher plant height and fresh root yield for the well watered control than for the water stressed treatments. The decrease in fresh and dry rhizome yield for 60-65% MAD and the severely stressed 80-85% MAD treatment can probably be attributed to accelerated senescence and shedding of leaves under water stress (Munné-Bosch and Alegre, 2004). Furthermore, water stress has been reported to significantly reduce plant growth and affect various physiological and biochemical processes in plants (Leithy et al., 2006; Bettaieb et al., 2010; Ekren et al., 2012; Hassan et al., 2012; 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; Anjum et al., 2011). Vazin (2013) results showed that stressed treatments reduced the yield components of cumin (Cuminum cyminum L.). Previous results on Ocimum basilicum were consistent with the findings of this study (Khalid 2006). This supports the findings of Cifre et al. (2005), who reported the increase in yield that was directly associated with an increase in the amount of water irrigated. Similar results were reported by Yuan et al. (2003), who showed that the less water is applied, the higher the irrigation water use efficiency. The increase in irrigation rate resulted in significantly higher growth and productivity of the plants such as Ocimum basilicum L, employing a positive effect on all the determinant parameters of growth development (Ekren et al., 2012).

5. Conclusions

This study evaluated four water regimes and two ginger species at various harvesting times. The severely water-stressed irrigation regime (i.e., 80-85% MAD) resulted in higher total flavonoid content, phenolic content, and antioxidant activity. Increases were seen in all parts of the plants (rhizomes, leaves and tillers). Water stressing *S. aethiopicus* significantly impacted flavonoid content, but it did not on rhizome yield. However, increasing rhizome, tiller, and leaf yield by irrigating *Z. officinale* at 20-25% MAD was recorded. Comparing ginger species revealed that *Z. officinale* yielded higher than *S. aethiopicus*. As time progressed, rhizome yield increased in ginger species, resulting in the highest yield at the last harvest. Our findings indicate that water stress (e.g. 80-85% MAD) treatment increased phytochemical accumulation in *S. aethiopicus. Zingiber officinale* harvesting at seven or

eight months after planting should give good results for flavonoids as well as dry rhizome yield. Also, significantly moderate water stress (40-45% MAD) may be applied to obtain higher phytochemical content without compromising the biomass yield. This trend manifested in both seasons in fresh rhizomes and dry rhizomes.

These findings will aid in saving water by adopting moderate stress (40-45% MAD) during cultivation practices without compromising the quality of produce the pharmaceutical companies require. The species can be harvested seven to eight months after planting under open field conditions, which improves water use efficiency, crop yield, and secondary metabolites quality.

Declaration of Competing Interest

The authors declare no conflict of interests that could influence the work reported in this paper.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Akula, R., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6, 1720–1731.

Alothman, M., Bhat, R., Karim, A.A., 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem. 115, 785–788.

Anjum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., Lei, W., 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6, 2026–2032.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Bahreininejad, B., Razmjoo, J., Mirza, M., 2013. Influence of water stress on morpho-physiological and phytochemical traits in Thymus daenensis. Int. J. Plant Prod. 7, 151–166.

Battaieb, I.N., Zakhama, W.A., Wannes, M.E, Marzouk, B., 2010. Water deficit effect on *Salvia officinalis* fatty acids and essential oils composition. Sci. Hortic. 120 (2), 271–275.

Bajguz, A., Hayat, S., 2009. Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol. Biochem. 47, 1–8.

Bettaieb, I., Knioua, S., Hamrouni, I., Limam, F., Marzouk, B., 2010. Water-deficit impact on fatty

acid and essential oil composition and antioxidant activities of cumin (*Cuminum cyminum* L.) aerial parts. J. Agric. Food Chem. 59, 328–334.

Butt, M.S., Sultan, M.T., 2011. Ginger and its health claims: molecular aspects. Crit. Rev. Food Sci. Nutr. 51, 383–393.

Cai, Y., Luo, Q., Sun, M., Corke, H., 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74, 2157–2184.

Cifre, J., Bota, J., Escalona, J.M., Medrano, H., Flexas, J., 2005. Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera L.*): an open gate to improve water-use efficiency. Agric. Ecosyst. Environ. 106, 159–170.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to rought - from genes to the whole plant. Funct. Plant Biol. 30 (3), 239–264.

Colling, J., Stander, M.A., Makunga, N.P., 2010. Nitrogen supply and abiotic stress influence canavanine synthesis and the productivity of in vitro regenerated *Sutherlandia frutescens* microshoots. J. Plant Physiol. 167, 1521–1524.

Ekren, S., Sönmez, Ç., Özçakal, E., Kurtta, Y.S.K., Bayram, E., Gürgülü, H., 2012. The effect of different irrigation water levels on yield and quality characteristics of purple basil (*Ocimum basilicum* L.). Agric. Water Manag. 109, 155–161.

Figueiredo, A., Barroso, J., Pedro, L., Scheffer, J., 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr. J. 23, 213–226.

Gatabazi, A., Marais, D., Steyn, J.M., Araya, H.T., Mofokeng, M.M., Mokgehle, S.N., 2019. Evaluating growth, yield, and water use efficiency of African and Commercial ginger species in South Africa. Water. 11, 548.

Ghasemzadeh, A., Jaafar, H.Z.E., Karimi, E., Rahmat, A., 2010. Antioxidant activities, total phenolics and flavonoid content in two varieties of Malaysia young Ginger (*Zingiber Officinale*). Molecules. 15, 4324–4333.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.

Hassan, H.S., Sule, M.I., Musa, A.M., Abubakar, M.S., Hassan, A.S., 2012. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. Afr. J. Tradit. Complement. Altern. Med. 9, 250–256.

Jabro, J.D., Stevens, W.B., Iversen, W.M., 2017. Field performance of three real-time moisture sensors in sandy loam and clay loam soils. Arch. Agron. Soil Sci. 64, 930–938.

Jiang, Y., Huang, B., 2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J. Exp. Bot. 52 (355), 341–349.

Khalid, K.A., 2006. Influence of water stress on growth, essential oil, and chemical composition of herbs (*Ocimum* spp.). Int. Agrophys. 20, 289–296.

Kazan, K., 2013. Auxin and the integration of environmental signals into plant root development. Ann. Bot. 12, 1655–1665. Lafka, T.I., Sinanoglou, V., Lazos, E.S., 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem. 104, 1206–1214.

Li, H.B., Wong, C.C., Cheng, K.W., Chen, F., 2008. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. Food Sci Tech. 41, 385–390.

Leithy, S, El-Meseiry, TA, Abdallah, EF., 2006. Effect of/biofertilizer, cell stabilizer and irrigation regime on rosemary herbage oil yield and quality. J. Appl. Sci. Res. 2 (10), 773–779.

Mao, Q.Q., Xu, X.Y., Cao, S.Y., Gan, R.Y., Corke, H., Beta, T., Li, H.B., 2019. Bioactive compounds and bioactivities of Ginger (*Zingiber officinale* Roscoe). Foods (Basel, Switzerland) 8 (6), 185.

Mano, J.I., 2002. Early events in environmental stresses in plants. Induction mechanisms of oxidative stress. Oxid. Stress Plants. 217–245.

Mofokeng, M.M., Steyn, J.M., Du Plooy, C.P., Prinsloo, G., Araya, H.T., 2015. Growth of Pelargonium *Pelargonium sidoides* DC. In response to water and nitrogen levels. S. Afr. J. Bot. 100, 183–189.

Mokgehle, S.N., Tesfay, S.T., Makgato, M.J., Araya, H.T., 2019. Phytochemical profiling and soluble sugars of African ginger (*Siphonochilus aethiopicus*) from different growing regions in South Africa. S. Afr. J. Plant Soil. 1–7.

Mokgehle, S.N., Tesfay, S.T., Araya, H.T., Du Plooy, C.P., 2017. Antioxidant activity and soluble sugars of African ginger (*Siphonochilus aethiopicus*) in response to irrigation regimen and nitrogen levels. Acta Agr Scand B-S P. 67.

Munné-Bosch, S., Alegre, L., 2004. Die and let life: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol. 31, 203–216.

Nakabayashi, R., Yonekura-Sakakibara, K., Urano, K., Suzuki, M., Yamada, Y., Nishizawa, T., Matsuda, F., Kojima, M., Sakakibara, H., Shinozaki, K., Michael, A.J., Tohge, T., Yamazaki, M., Saito, K., 2014. Enhancement of oxidative and drought tolerance in Arabidopsis by over accumulation of antioxidant flavonoids. Plant J. 77 (3), 367–379.

Ndhlala, A.R., Mulaudzi, R., Ncube, B., Abdelgadir, H.A., Du Plooy, C.P., Van Staden, J., 2014. Antioxidant, antimicrobial and phytochemical variations in thirteen *Moringa oleifera* Lam. Cultiv. Mol. 19, 10480–10494.

Panda, R.K., Behera, S.K., Kashyap, P.S., 2003. Effective management of irrigation water for wheat under stressed conditions. Agric. Water Manag. 63, 37–56.

Pan, Y., Wang, K., Huang, S.Q., Wang, H.S., Mu, X.M., He, C.H., Ji, X.W., Zhang, J., Huang, F.J., 2008. Antioxidant activity of microwave-assisted extract of longan (Dimocarpus Longan Lour.) peel. Food Chem. 106 (3), 1264–1270.

Ravindran, P.N., Nirmal Babu, K., Shiva, K.N., 2004. Botany and crop improvement of ginger. In: Ravindran, P.N, Babu, Nirmal (Eds.), Ginger: The Genus Zingiber. CRC Press, pp. 15–86.

Shao, H.B., Chu, L.Y., Jaleel, C.A., Zhao, C.X., 2008. Water-deficit stress-induced anatomical changes in higher plants. Crit. Rev. Biol. 331, 215–225.

Sultana, B., Anwar, F., Ashraf, M., 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14 (6), 2167–2180.

Schroder, F.G., Liet, J.H., 2002. Irrigation Control in Hydroponics. Hydroponic Production of Vegetables and Ornamentals. Athens. Publication Embryo, pp. 263–298.

Sedghi, M., Seyed, S.R, Pirzad, A.R., Amanpour-Balaneji, B., 2012. Phytohormonal regulation of antioxidant systems in petals of drought-stressed pot marigold (*Calendula officinalis* L.). J. Agric. Sci. Technol. 14, 869–878.

Soil Classification Working Group. Soil classification, 1991. A Taxonomic System for South Africa. Department of Agricultural, Pretoria, South Africa.

Shukla, Y., Singh, M., 2007. Cancer preventive properties of ginger: a brief review. Food Chem. Toxicol. 45, 683–690.

Tesfaye, K., Walker, S., Tsubo, M., 2006. Radiation interception and radiation use efficiency of three grain legumes under water deficit conditions in a semi-arid environment. Eur. J. Agron. 25, 60–70.

Van Wyk, B.E., 2008. A broad review of commercially important southern African medicinal plants. J. Ethnopharmacol. 119, 342–355.

Vazin, F., 2013. Water stress effects on cumin (*Cuminum cyminum* L.) yield and oil essential components. Sci. Hortic. 151, 135–141.

Weidner, S., Kordala, E., Brosowska-Arendt, W., Karamać, M., Kosińska, A., Amarowicz, R., 2009. Phenolic compounds and properties of antioxidants in grapevine roots (*Vitis vinifera L.*) under low-temperature stress followed by recovery. Acta Soc. Bot. Pol. 78, 279–286.

Xu, K., 2000. Effects of mulching with straw on the photosynthetic characteristics of ginger. Chin. Veg. 2, 18–20.

Yuan, B.Z., Nishiyama, S., Kang, Y., 2003. Effects of different irrigation regimes on the growth and yield of drip-irrigated potato. Agric. Water Manag. 63, 153–167.

Zhang, Y., Kendy, E., Qiang, Y., Changming, L., Yanjun, S., Hongyong, S., 2004. Effect of soil water deficit on evapotranspiration, crop yield, and water use efficiency in the North China Plain. Agric. Water Manag. 64, 107–122.

Zerizghy, M.G., 2012. Integrating rainfall-runoff and evaporation models for estimating soil water storage during fallow under in-field rainwater harvesting. PhD Thesis, University of the Free State, Bloemfontein, South Africa.