



Article

Nutrient Concentration of African Horned Cucumber (*Cucumis metuliferus* L) Fruit under Different Soil Types, Environments, and Varying Irrigation Water Levels

Mdungazi K Maluleke ^{1,*}, Shadung J Moja ², Melvin Nyathi ³ and David M Modise ⁴

¹ Department of Environmental Sciences, College of Agriculture and Environmental Sciences, University of South Africa, Tshwane 0002, South Africa

² Council of Geosciences, Water and Environment Business Unit: Geological Resource Division, Silverton, Tshwane 0002, South Africa; sjmoja@geoscience.org.za

³ Agricultural Research Council, Tshwane 0002, South Africa; Mnyathi@arc.agric.za

⁴ Faculty of Natural and Agricultural Sciences, School of Agricultural Sciences, North-West University, Potchefstroom 2520, South Africa; david.modise@nwu.ac.za

* Correspondence: malulm@unisa.ac.za

Abstract: The nutrient concentration of most crops depends on factors such as amount of water, growing environment, sunlight, and soil types. However, the factors influencing nutrient concentration of African horned cucumber fruit are not yet known. The objective of the study was to determine the effect of different water stress levels, soil types, and growing environments on the nutrient concentration of African horned cucumber fruit. Freeze-dried fruit samples were used in the quantification of β -carotene and total soluble sugars. The results demonstrated that plants grown under the shade net, combined with severe water stress level and loamy soil, had increased total soluble sugars (from 8 to 16 °Brix). Under the shade-net environment, the combination of moderate water stress level and loamy soil resulted in increased crude protein content (from 6.22 to 6.34% °Brix). In addition, the severe water stress treatment combined with loamy soil, under greenhouse conditions, resulted in increased β -carotene content (from 1.5 to 1.7 mg 100 g⁻¹ DW). The results showed that African horned cucumber fruits are nutrient-dense when grown under moderate water stress treatment on the loamy or sandy loam substrate in the shade-net and open-field environments.

Keywords: biochemical constituents; β -carotene; vitamins; micro-nutrients; growing environments



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1. Introduction

In Sub-Saharan Africa, indigenous crops have been a source of food for rural resource-poor households who experience nutritional food insecurity [1]. However, deficiencies in micronutrients, such as zinc, iron, and β -carotene, have been described as a major nutritional challenge faced by many rural households [2]. Several researchers claimed that the benefits of indigenous crops are that (i) they grow naturally in the wild [3]; (ii) are resistant to most pests and diseases; (iii) have better environmental stress tolerance; (iv) require low agricultural inputs, such as irrigation and fertilizers; and (v) have a shorter period to mature and are readily available for consumption [2]. However, most indigenous fruits and vegetables have not yet been commercialized, particularly in Southern Africa, because they are not produced under well-defined agronomic practices, and there is a lack of market value chain, since they do not have a high demand [2,4]. The nutritional composition of these crops has not been widely investigated, despite their usefulness to the communities. There appears to be scanty knowledge about their nutritional content, particularly when grown under different growing conditions. This knowledge could aid in influencing policymakers in the commercialization and products innovation in many countries, since the crop is adaptable in various growing environments. Ref. [2] reports that most of these crops have the potential to supplement several nutrients needed by

the human body, in both smaller and larger quantities. Ref. [1] iterated that there is a need to promote the consumption of indigenous crops, and that can be achieved by the investigation of their agronomical viability and qualities, such as nutritional content. The African horned cucumber fruit is palatable, with a similar taste of a mixture of banana and pineapple [5]. The internal part of the fruit contains a high moisture content, which can aid body hydration [6]. Ref. [7] the other benefits of consuming this fruit: (i) It is a source of vitamin C, and (ii) it contains biochemical compounds such as phenols, which help the body to eliminate toxins; thus, growing this crop is of the utmost important in terms of promoting biodiversity and stewardship of the natural heritage and ecosystem of the Sub-Sahara region. The objective of the study was to determine the effect of different water stress levels, varying soil types, and growing environments on the nutrient concentration of the African horned cucumber fruit.

2. Material and Methods

This study was conducted during the 2017/18 and 2018/19 growing seasons, under the greenhouse, shade net, and open-space environment at the Florida science campus of the University of South Africa (26°10' 30" S, 27°55' 22.8" E). Before plant cultivation, gravimetric water content (GWC) was carried out, determining the field water capacity of the soils. Briefly, dry soil was filled in a 30 cm depth planting pot, weighed, and then watered to filled capacity (3000 mL). The pots were then weighed after 72 h, when drainage was completed. The process was repeated until the soil reached permanent wilting point. Water stress levels was then determined by using the formula (e.g., 3000 mL–filled capacity $\times 75 \div 100 = 2250$ mL moderate stress, while 3000 mL $\times 35 \div 100 = 1050$ mL severe water stress). Soil samples (loamy soil and sandy loam) were analyzed for mineral and/or chemical content (Table 1), using the method followed by [8]. The above analysis was conducted at the Agricultural Research Council, Institute for Soil, Climate and Water (ARC-ISWC) in Pretoria (25° 44' 19.4" S 28° 12' 26.4" E). Sterilized growth media (loamy soil and sandy loam) were used. In addition, certified seeds of African horned cucumber were purchased from Seeds for Africa, Cape Town. A factorial experiment with two factors, i.e., soil (loamy soil and sandy loam soil) and irrigation water levels (no water stress, moderate water stress, and severe water stress), was conducted. The pot experiment was a completely randomized design with nine (9) replicates per treatment. The pots were spaced 1 m apart, and an up-rope vertical trellising was used to support the plants. On each site, pots were either filled with loamy soil or sandy loam. Each block comprised 18 plants in pots, resulting in 54 plants per site. A total of 162 plants were used for the experiment. Each site had plants used as guard plants, in order to separate the plants from the external effects outside the experimental plot. Well-established, uniform, and healthy African horned cucumber seedlings, germinated from peat substrate, that were 30 days old, were transplanted into 30 cm depth $\times$ 30 cm width. Briefly Area (depth $\times$ width) 30 cm $\times$ 30 cm = 900 cm², $A = \pi \left(\frac{d}{2} \right) \times 2 d = 286.5$ cm² planting pots, and the treatments were imposed four (4) weeks later, after establishment. Plants were well irrigated prior to imposition of the treatments. Granules fertilizers (potassium phosphate), 10 g per plant pot, were applied once every 7th day of the week during the experimental period.

The impact of soil, water, and growing environment on the nutrient composition of African horned cucumber fruit was evaluated at 12 weeks after planting during 2017/18 and 2018/2019. Prior to fruit analysis, optimization analysis of crude protein and total soluble sugars of fruit was carried out before the actual fruit analysis, whereby fruit were harvested from each irrigation water level (no-water-stress control, moderate water stress, and severe water stress), soil type, and growing environment. The goal for the fruit optimization analysis was to find the optimum value for one or more target variables among African horned cucumber fruit harvested under different treatments.

Table 1. Soil analysis for the experiment (mineral/chemical analysis).

Chemical Analysis (Micro-Minerals)						
	Fe	Mn	Cu	Zn	pH	
	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}		
SL	30.3	59.4	1.24	9.36	7.69	
L	33.2	59.8	1.27	8.96	7.74	
	P	Ca	Mg	K	Na	Total N
	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	%
SL	35.16	1900	141	243	35.5	0.105
L	34.4	1810	133	217	28.7	0.113

2.1. Determination of β -Carotene

The analysis of β -carotene was carried out with a Prominence-i High-Performance Liquid Chromatography–PDA model system equipped with a sample cooler LC-2030C (Shimadzu, Japan), with slight modifications (triplicate), as described by [4], since most of the compounds measured were expected to be similar to those of the current study. A mixture of approximately 0.1 g/mL of extracted sample with ice-cold hexane:acetone (1:1, *v/v*) was vortexed for two (2) minutes, before being centrifuged at 2000 rpm for two (2) minutes. The organic phase was decanted into a tube containing saturated sodium chloride solution and placed on ice. The remaining residue was similarly re-extracted until the extract was colorless. Each time, the extract separated organic phase was filtered through 0.45 μ m syringe filtered before injection into the HPLC. Chromatographic separation was achieved, using a C₁₈ Luna[®] column (150 $\times$ 4.6 mm, 5 μ) maintained at 35 °C.

An isocratic mobile phase which consisted of acetonitrile:dichloromethane:methanol (7:2:1) was used, with a flow rate of 1 mL/min, an injection volume of 20 μ L, and the detection was at 450 nm. Peak identification and quantification of the compound (β -carotene) were both achieved based on authentic β -carotene standard, which was used for plotting the calibration curves [9].

2.2. Determination of Total Soluble Sugars

The African horned cucumber fruit harvested from the greenhouse, shade net, and open field, irrigated with different water levels and soil types, were analyzed for total soluble sugars concentration (°Brix) following the method by [10]. The fruit was cut into two portions, then juice was squeezed from a fruit portion by hand to release about 0.03 mL juice onto the aperture of the hand refractometer (HI 96801 Refractometer, USA) and readings were taken immediately. About 18 fruits were measured per treatment. The aperture was washed between different juices samples, with distilled water, and dried with a soft paper towel.

2.3. Determination of Vitamin C and E

The fruit samples were freeze-dried for 72 h, using a freeze drier (HARVEST-RIGHT, Barcelona). The freeze-dried fruit slices were rigorously homogenized, using a sterilized food blender, and mixed with dried powder before nutritional analysis. The method described by [4] was followed with slight modifications (triplicate). Individual samples were weighed (1 g) into tube, followed by the addition of 5% metaphosphoric acid (10 mL). It was sonicated 15 min before centrifuged and then filtrated in the ice-cold water bath. The analysis was carried out on the model system described above, Prominence-i HLCP–PDA. A C₁₈ Luna[®] column (150/4.6 mm, 5 μ L) held at 25 μ C was used to achieve chromatographic separation. A water-based isocratic mobile phase: acetonitrile: formic acid (99:0.9:0.1) was used at a flow rate of 1 mL/min. The volume of injection was 20 μ L and 245 nm of detection was set. Depending on the calibration curve plotted by using L-ascorbic acid, sample quantification was achieved.

2.4. Determination of Total Flavonoids

The African horned cucumber fruit samples were quantified, using the aluminum chloride colorimetric method described by [4]. Catechin was used as a standard for calibration curve, and total flavonoids content was expressed in mg catechin equivalents (CEs) per dry weight.

2.5. Determination of Total Phenolic Content

Total phenolic content of the fruit samples was carried out, using [4], with a slight modification (triplicate). Garlic used as standard for plotting curve. Total phenolic content was expressed in mg garlic acid equivalents (GAEs) per g dry weight (DW).

2.6. Determination of Micro-Nutrients

Freeze-dried fruit samples were digested in a diffused microwave system (MLS 1200 Mega; Milestone S.r. L, Sorisole, Italy), and the samples were further congelated–dried, following the procedure described by [4] with minor modifications. The modifications were that samples were measured in three (3) replicates per treatment (around 15–25 mg) weighed into polytetrafluoroethylene vessels and 2 mL HNO₃ (67%, analpur) and 1 mL H₂O₂ (30%, analytical grade) added in the vessels [4f]. Every solution was diluted to 15 mL, in a deionized-water test tube, after digestion, and analyzed by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS). An ICP–MS (Agilent 7700; Agilent Technologies, Tokyo, Japan) based on quadrupole mass analyzer and octapole reaction system (ORS 3) was used to conduct the analysis. Nutrient elements, such as zinc (Zn), iron (Fe), molybdenum (Mo), copper (Cu), and manganese (Mn), were analyzed.

The calibration solution was prepared by appropriate dilution of the single element certified reference material with 1.000 g/L for each element (Analytika Ltd., Czech Republic) with deionized water (18.2 MΩ·cm, Direct-Q; Millipore, France). Measurement of accuracy was verified by using certified reference material of water TM-15.2 (National Water Research Institution, Ontario, Canada).

2.7. Statistical Analysis

Analysis of variance (ANOVA) was performed with a three-way ANOVA, to determine the main and interaction effects of all studied variables (crude protein, total soluble sugars, *Beta carotene*, vitamin C, vitamin E, total phenols, total flavonoids, and macro- and micro-nutrients). Homogeneity and uniformity tests were carried to determine the difference and similarities between variance. Mean separation was done by using the Fischer's unprotected least significance difference test at 5% significance level. Treatment means for each measured parameter were compared, and differences were noted. All statistical analyses were done, using GenStat (version 14, VSN, Rothamstead, UK).

3. Results

3.1. Total Soluble Sugars

Figure 1 presents the treatment interaction effect on total soluble sugars content of African horned cucumber fruit grown at different environments (greenhouse, shade net, and open field), soil types (loamy soil and sandy loam), and water stress levels (no water stress, moderate water stress, and severe water stress). The results indicated that there was no significant ($p > 0.05$) interaction between location, different water stress levels, and soil types on total soluble sugars content of African horned cucumber fruit during both growing seasons. However, fruit total soluble sugars ranged from 8.0 to 16 °Brix. In addition, the study revealed that there was a significant ($p \leq 0.05$) difference in total soluble sugars under varying water levels. Total soluble sugars among different water levels ranged from 11.4 to 14.4 °Brix. Furthermore, the results illustrated that the severe-water-stress level obtained the highest total soluble sugar content (14.4 °Brix), while the lowest content was observed from the no-water-stress (control) water level, with 11.4 °Brix.

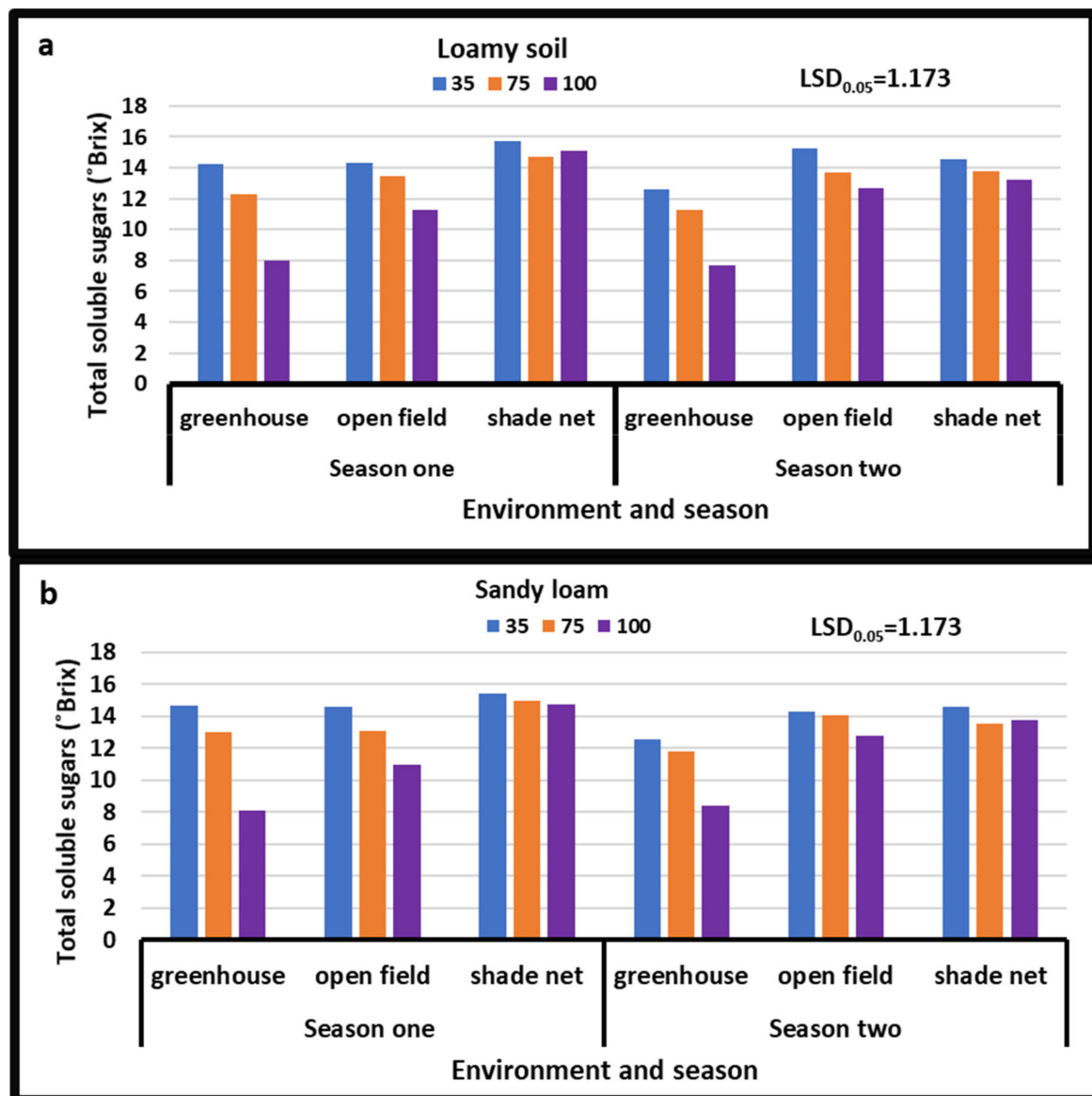


Figure 1. Treatment effect on the total soluble sugars content of African horned cucumber fruit grown in different environments; (a) effect of different water stress levels and loamy soil, in different environments, during different seasons (2017/18, season one; and 2018/19, season two); (b) effect of different water stress levels and sandy loam, in different environments, during different seasons (2017/18, season one; and 2018/2019, season two); 35 means severe water stress, 75 means moderate water stress, and 100 means no water stress (control). $LSD_{0.05}$ is the least significant difference of means.

3.2. Crude Proteins

For crude protein content, the results of the study showed that there was no significant ($p > 0.05$) difference in crude protein content between interaction of growing environment, water stress levels, and soil types (Figure 2). However, the results delineated that fruit crude protein ranged from 6.22 to 6.29%. Moreover, the results of the study demonstrated two extremes: The treatment of no water stress and severe water stress combined with both soil types (loamy soil and sandy loam) at growing conditions (greenhouse and shade net) during both seasons decreased crude protein content from 6.29 to 6.22% (Figure 2a,b), whereas the treatment of severe water stress combined with loamy soil at shade-net conditions increased crude protein content from 6.22 to 6.29% (Figure 2a). In addition, results showed evinced that there was a significant ($p \leq 0.05$) difference for crude protein content under different growing environment. Crude protein under varying growing environments

ranged from 6.24 to 6.28%. Moreover, results showed that shade-net growing environment obtained the highest crude protein, at 6.28%, while the greenhouse environment expressed the lowest content, at 6.24%.

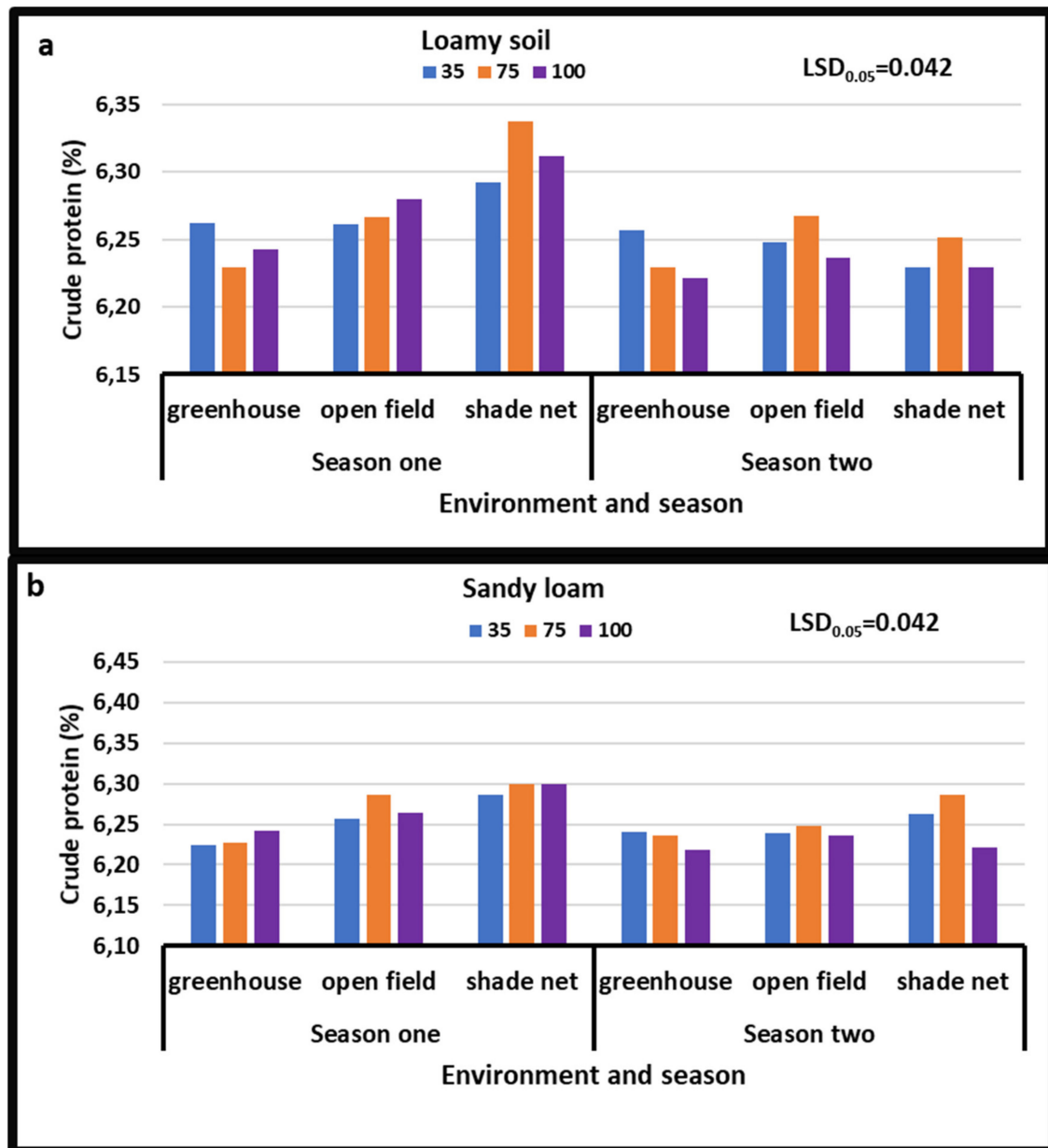


Figure 2. Treatment interaction effect on crude protein content of African horned cucumber fruit; (a) interaction effect of different water stress levels and loamy soil, in different environments, during season one (2017/2018); (b) interaction effect of different water stress levels and sandy loam, in different environments, during season two (2018/19); 35 means severe water stress, 75 means moderate water stress, and 100 means no water stress (control). $LSD_{0.05}$ is the least significant difference.

3.3. β -Carotene

Table 2 presents the treatment effect on the β -carotene, vitamin C, vitamin E, total flavonoids, and total phenols of African horned cucumber fruit under different growing environments. For the greenhouse, shade, and open-field environment, the results illustrated that there was a significant ($p \leq 0.05$) difference between the interaction of water stress levels and soil types. β -carotene ranged from 1.5 to 17 mg 100 g⁻¹ DW. In addition, the results demonstrated that the severe water stress combined with sandy loam slightly

decreased β -carotene from 1.7 to 1.5 mg 100 g⁻¹ DW, whereas the treatment of severe water stress combined with loamy soil increased it from 1.5 to 1.7 mg 100 g⁻¹ DW. For the shade-net environment, β -carotene ranged from 1.5 to 1.6 mg 100 g⁻¹ DW. In addition, the results showed that water stress levels (moderate and severe water stress) combined with both soils slightly decreased β -carotene from 1.6 to 1.5 mg 100 g⁻¹ DW, whereas moderate water stress treatment combined with loamy soil increased it from 1.5 to 1.6 mg 100 g⁻¹ DW. Under the open-field environment, β -carotene increased from 1.5 to 1.6 mg 100 g⁻¹ DW. The treatment of water levels (moderate and severe water stress) indicated a decrease from 1.6 to 1.5 mg 100 g⁻¹ DW, whereas no-water-stress treatment (control) combined with both soils expressed an increase from 1.5 to 1.6 mg 100 g⁻¹ DW.

Table 2. Treatment effect on biochemical constituents of African horned cucumber fruit harvested from different growing environments.

Treatment	β -carotene (mg 100 g ⁻¹ DW)	Vitamin C (mg 100 g ⁻¹ DW)	Vitamin E (mg 100 g ⁻¹ DW)	Total Flavonoids (CE g ⁻¹ DW)	Total Phenols (GAE g ⁻¹ DW)
Greenhouse					
W1S1	1.6(0.0)	26.6(0.2)	11.7(1.1)	0.66(0.03)	3.1(0.1)
W2S1	1.6(0.0)	24.3(0.2)	29.8(0.1)	0.56(0.03)	5.2(0.2)
W3S1	1.7(0.01)	23.5(0.5)	24.4(13.4)	0.25(0.1)	4.5(0.1)
W1S2	1.5(0.01)	23.8(1.9)	9.3(5.5)	0.26(0.1)	4.4(0.1)
W2S2	1.6(0.01)	30.3(0.9)	31.7(0.5)	0.55(0.02)	5.8(0.2)
W3S2	1.5(0.01)	23.2(0.9)	35.1(0.5)	0.21(0.0)	4.2(0.0)
Grand mean	1.6	25.3(0.1)	23.7(0.9)	0.4	4.5
LSD0.05	0.020	1.528	12.95	0.060	0.196
<i>p</i> -value	0.001	0.001	0.204	0.001	0.001
Shade net					
W1S1	1.5(0.0)	33.1(0.5)	18.1(16.9)	0.75(0.01)	4.2(0.1)
W2S1	1.6(0.1)	30.2(0.4)	16.9(3.7)	0.84(0.0)	5.3(0.1)
W3S1	1.5(0.0)	28.2(0.0)	11.3(5.8)	0.54(0.1)	3.6(0.0)
W1S2	1.5(0.0)	31.7(16.8)	10(3.4)	0.63(0.02)	4.3(0.3)
W2S2	1.5(0.1)	22.6(0.1)	12.5(2.9)	0.77(0.03)	4.4(0.1)
W3S2	1.5(0.0)	27.2(0.1)	14.3(1.1)	0.49(0.0)	3.5(0.1)
Grand mean	1.5	28.8	13.9	0.670	4.2
LSD0.05	0.009	12.58	19.35	0.038	0.205
<i>p</i> -value	0.001	0.658	0.29	0.009	0.001
Open field					
W1S1	1.6(0.0)	17.0(0.7)	10.7(0.5)	0.73(0.03)	6.4(0.01)
W2S1	1.5(0.0)	19.0(0.4)	11.8(2.7)	0.47(0.02)	4.1(0.1)
W3S1	1.5(0.01)	16.6(0.6)	8.3(3.0)	0.85(0.02)	4.8(0.1)
W1S2	1.6(0.01)	18.7(0.6)	13.4(3.2)	0.42(0.02)	5.4(0.1)
W2S2	1.5(0.0)	27.5(0.9)	13.5(0.4)	0.41(0.02)	5.1(0.0)
W3S1	1.5(0.01)	15.5(0.7)	9.7(0.3)	0.65(0.04)	3.1(0.2)
Grand mean	1.5	19.03	11.2	0.59	4.8
LSD0.05	0.009	1.231	6.079	0.057	0.207
<i>p</i> -value	0.001	0.001	0.809	0.001	0.001

W1 means no water stress (control); W2 means moderate water stress; W3 means severe water stress. S1 is loamy soil, and S2 is sandy loam soil. Numbers in brackets represent the standard deviations of the mean. LSD_{0.05} is the least significant difference of means. The *p*-values in bold are lower than 0.05. Note that only season two results are presented, due to logistical costs, as analysis could not be done for both seasons one treatments.

3.4. Vitamin C

For vitamin C, the results showed that there was a significant ($p \leq 0.05$) difference between the interaction of different water levels and soil types under the greenhouse and open-field environment. However, there was no significant ($p > 0.05$) difference between

different water levels and soil types in the shade-net environment (Table 2). Under the greenhouse environment, vitamin C content ranged from 23.2 to 30.3 mg 100 g⁻¹ DW. The results illustrated that treatment of severe water stress combined with sandy loam decreased vitamin C from 30.3 to 23.2 100 g⁻¹ DW, whereas moderate water stress treatment combined with sandy loam increased it from 23.2 to 30.3 100 g⁻¹ DW. For the shade-net environment, vitamin C content ranged from 22.6 to 33.1 100 g⁻¹ DW. Our results revealed that severe water stress treatment combined with sandy loam decreased vitamin C from 33.1 to 22.6 100 g⁻¹ DW, whereas no-water-stress (control) treatment combined with loamy soil increased it from 22.6 to 33.1 100 g⁻¹ DW.

Regarding the open-field environment, vitamin C content ranged from 15.5 to 27.5 100 g⁻¹ DW. The results of the study indicated that the treatment of severe water stress combined with sandy loam decreased vitamin C content from 27.5 to 15.5 100 g⁻¹ DW, whereas moderate water stress treatment and sandy loam increased it from 15.5 to 27 100 g⁻¹ DW. It is worth to note that the treatment of severe water stress combined sandy loam soil under the open-field environment indicated the lowest vitamin C content (15.5 100 g⁻¹ DW), whereas the no-water-stress (control) treatment combined with loamy soil under the shade-net environment obtained the highest vitamin C content (33.1 100 g⁻¹ DW).

3.5. Vitamin E

The results of the study revealed that there was no significant ($p > 0.05$) difference for vitamin E content from the interaction between different water levels and soil types under all growing environments (greenhouse, shade net, and open field). For the greenhouse environment, vitamin E content ranged from 9.3 to 35.1 100 g⁻¹ DW. In addition, the results demonstrated that no-water-stress (control) treatment combined with sandy loam decreased vitamin E content from 35.1 to 9.3 100 g⁻¹ DW, whereas the treatment of severe water stress and sandy loam increased it from 9.3 to 35.1 100 g⁻¹ DW (Table 2). Under the shade-net environment, the no-water-stress treatment (control) combined with sandy loam decreased vitamin E content from 18.1 to 10.0 100 g⁻¹ DW, whereas no water stress (control) and loamy soil increased it from 10.0 to 18.1 100 g⁻¹ DW. On the other hand, open-field vitamin E content ranged from 8.3 to 13.5 100 g⁻¹ DW. Results delineated that treatment of severe water stress combined with loamy soil decreased vitamin E content from 13.5 to 8.3 100 g⁻¹ DW, whereas the severe water stress and sandy loam increased it from 8.3 to 13.5 100 g⁻¹ DW (Table 2).

3.6. Total Flavonoids

Table 2 illustrates that there was a significant ($p \leq 0.05$) difference in total flavonoids, depending on the interaction of different water levels and soil types under varying growing environment (greenhouse, shade net, and open field). For the greenhouse environment, total flavonoids ranged from 0.21 to 0.66 CE g⁻¹ DW. In addition, the results illustrated that the treatment of severe water stress combined with sandy loam reduced total flavonoids from 0.66 to 0.21 CE g⁻¹ DW, whereas treatment of no water stress (control) combined with loam soil increased it from 0.21 to 0.66 CE g⁻¹ DW. Under the shade-net environment, our results showed that total flavonoids ranged from 0.49 to 0.84 CE g⁻¹ DW. The results indicated that severe water stress treatment combined with sandy loam reduced total flavonoids from 0.84 to 0.49 CE g⁻¹ DW, whereas no-water-stress (control) treatment increased it from 0.49 to 0.84 CE g⁻¹ DW. For total flavonoids content in the open-field environment, the results showed that it ranged from 0.41 to 0.85 CE g⁻¹ DW. In addition, the results illustrate that water stress and sandy loam decreased total flavonoids from 0.85 to 0.41 CE g⁻¹ DW, whereas severe water stress and loamy soil increased it from 0.41 to 0.85 CE g⁻¹ DW (Table 2). The observed trend shows that the combination of severe water stress and loamy soil under the open-field environment obtained the highest total flavonoids content, at 0.85 CE g⁻¹ DW, whereas the lowest content was observed on treatment combined.

3.7. Total Phenols

The results indicate that there was a significant ($p \leq 0.05$) difference on the total phenolic content of African horned cucumber between interaction of different water levels and soil types under varying growing environment (greenhouse, shade net, and open field). The greenhouse environment total phenols ranged from 3.1 to 5.8 GAE g⁻¹ DW. Our results illustrated that the treatment of no water stress (control) combined with loamy soil decreased total phenols content from 5.8 to 3.1 GAE g⁻¹ DW, whereas the severe water stress treatment combined with sandy loam increased it from 3.1 to 5.8 GAE g⁻¹ DW.

For the shade-net environment, total phenols content ranged from 3.5 to 5.3 GAE g⁻¹ DW. The study results showed that the combination of severe water stress treatment and sandy loam reduced total phenols content from 5.3 to 3.5 GAE g⁻¹ DW. Under the open-field environment, total phenols content ranged from 3.1 to 6.4. In addition, the results of the study indicated that severe water stress treatment combined with sandy loam decreased total phenols content from 6.4 to 3.1 GAE g⁻¹ DW, whereas treatment combination of no water stress (control) and loamy soil increased it from 3.1 to 6.4 GAE g⁻¹ DW. For the open-field environment, our results showed that total phenols ranged from 3.1 to 6.1 GAE g⁻¹ DW. In addition, the results showed that severe water stress treatment combined with sandy loam decreased total phenols from 6.1 to 3.1 GAE g⁻¹ DW, whereas no-water-stress level (control) combined with loamy soil increased it from 3.1 to 6.4 GAE g⁻¹ DW.

3.8. Micro-Nutrients

Table 3 presents the micronutrient concentration of African horned cucumber. Significant ($p \leq 0.05$) interactions were observed for manganese and zinc, under the open environment, whereas for the shade, significant interactions were observed for iron and zinc. For the open-field environment, significant interactions were found under zinc only. The greenhouse zinc content ranged from 7.7 to 12.7 µg g DW. In addition, results illustrated that treatment of no water stress (control) combined with loam soil presented a decreased zinc content from 12.7 to 7.7 µg g DW, whereas there was a double increase in zinc content from treatment combination of no water stress (control) and sandy loam, from 7.7 to 12.7 µg g DW (Table 3). For the shade-net environment, zinc content ranged from 6.4 to 8.8 µg/g DW. The results demonstrated that treatment of severe water stress combined with sandy loam decreased zinc content from 8.8 to 6.4 µg g DW, whereas no-water-stress (control) treatment combined with sandy loam increased it from 6.4 to 8.8 µg g DW. Under an open-field environment, zinc content ranged 5.1 to 7.9 µg g DW. The lowest zinc content was observed from combination of no water stress (control) and loamy soil at 5.1 µg g DW, while treatment of moderate water stress and loamy soil presented an increase at 7.9 µg g DW. Under the shade-net environment, iron ranged from 1.4 to 1.8 µg g DW. The lowest iron content was observed from treatment of no water stress and sandy loam at 1.4 µg g DW, whereas treatment combination of moderate water stress and sandy loam illustrated higher content, at 1.8 µg g DW.

Table 3. Treatment interaction effect of irrigation water regimes, soil types, and environment on micro-nutrients (µg g DW) of African horned cucumber fruit.

Treatment	Moisture (g)	Copper	Iron	Manganese	Zinc
Greenhouse					
W1S1	193(36)	0.9(0.0)	1.8(0.1)	0.8(0.0)	7.7(1.2)
W2S1	179(40)	0.7(0.1)	2.0(1.5)	0.9(0.1)	9.3(0.8)
W3S1	78(42)	0.8(0.4)	1.6(0.2)	1.0(0.1)	10.1(1.8)
W1S2	95(35)	0.7(0.4)	2.8(1.8)	1.1(0.1)	12.7(1.5)
W2S2	152(5)	0.5(0.0)	3.8(0.2)	0.9(0.2)	8.6(2.0)
W3S2	129(22)	0.5(0.4)	0.5(0.1)	0.9(0.1)	10.6(0.6)

Table 3. Cont.

Treatment	Moisture (g)	Copper	Iron	Manganese	Zinc
Grand mean	138	0.689	2.1	0.942	9.8
LSD0.05	98.4	0.42	1.81	0.1854	2.228
<i>p</i> -value	0.15	0.99	0.06	0.01	0.01
Shade net					
W1S1	162(24)	0.7(0.2)	0.9(0.1)	0.8(0.0)	7.2(1.1)
W2S1	140(30)	0.8(0.3)	2.7(0.3)	0.9(0.1)	7.1(2.2)
W3S1	83(4)	0.6(0.1)	2.7(0.9)	0.9(0.1)	7.2(0.5)
W1S2	157(5)	0.6(0.1)	1.4(0.6)	0.8(0.0)	8.8(0.0)
W2S2	146(5)	0.8(0.3)	1.8(0.2)	0.8(0.1)	12.7(0.6)
W3S2	79(25)	0.6(0.1)	1.7(0.5)	0.8(0.1)	6.4(0.8)
Grand mean	127.7	0.7	1.9	0.811	8.23
LSD0.05	35.9	0.4	0.7	0.1475	2.177
<i>p</i> -value	0.9	0.89	0.03	0.59	0.01
Open field					
W1S1	146(50)	0.5(0.0)	2.4(0.8)	0.5(0.1)	5.1(0.9)
W2S1	220(21)	0.8(0.1)	2.6(0.3)	0.7(0.2)	7.9(0.4)
W3S1	29(16)	0.6(0.2)	1.8(1.3)	0.7(0.1)	7.7(0.2)
W1S2	162(6)	0.7(0.5)	1.3(0.2)	0.8(0.1)	6.8(0.4)
W2S2	155(4)	0.6(0.1)	2.1(0.8)	0.7(0.0)	6.9(0.1)
W3S1	80(19)	0.7(0.1)	0.6(0.2)	0.6(0.1)	7.5(1.4)
Grand mean	137	0.7	1.8	0.7	6.98
LSD0.05	40	0.341	1.211	0.231	1.153
<i>p</i> -value	0.03	0.20	0.61	0.181	0.03

W1 means no water stress (control); W2 means moderate stress; W3 means severe water stress. S1 means loamy soil, and S2 means sandy loam. Values are average over treatments mentioned. Numbers in brackets represent the standard deviations of the mean. LSD_{0.05} is the least significant difference of means. The *p*-values in bold are lower than 0.05. Note that only season two results are presented, due to logistical costs, as analysis could not be done for season-one treatments.

4. Discussion

This study investigated the effect of different water stress levels and varying substrates on the nutrient concentration of African horned cucumber fruit grown in three different environments (greenhouse, shade net, and open field). Previous studies conducted by [11,12] have evaluated the nutrient concentration of leafy vegetables grown under different water stress levels. In addition, studies conducted by [1,2] focused on iron and zinc. However, these studies did not evaluate biochemical constituents, such as crude protein, total soluble sugars, total flavonoids, total phenols, and vitamins. Therefore, the findings of this research study serve as a benchmark for the biochemical constituents of African horned cucumber fruit.

4.1. Bio-Chemical Constituents

Ref. [4] determined the mineral constituents and phytochemicals of crops harvested from different locations. Ref. [13] recommended that it is crucial to note the effect of water, irrigation and rainfall received by crops on the mineral constituents, such as β -carotene, total phenols, vitamins, total flavonoids, and micro- and micro-nutrients, so that growers can make an informed decision, to ensure that quality produce is supplied to their target market.

4.2. Total Soluble Sugars

Fruit sugar content is affected by a number of factors, including climate, water supply, and soil type. Total soluble sugars in fruits have a variety of health benefits, including provision of glucose, preventing colorectal cancer, and variety of diseases [14]. Fruit intake

is currently recommended by most dietary practitioners for improvement of health and disease prevention. The findings of this study demonstrated that the treatment affects the total soluble sugars of African horned cucumber fruit grown under varying environment. When plants were subjected to severe water stress under shade-net conditions, total soluble sugars increased, but they decreased under no-water-stress treatment. This implies that, when plants are exposed to different water levels, there is variation in fruit nutrient content.

Refs. [10,15] reported a significant difference in total soluble sugars of kiwi fruit harvested from different sites, due to variation in temperatures and rainfall patterns.

High total soluble sugar content was expected from open-field fruit under moderate water stress, as reported by [14], on pomegranate trees. These authors concluded that active osmoregulation caused by water stress was responsible for sugar variation in fruits, since there is imbalanced fluid movement within plant cells. Similarly, this study's findings unveiled that fruits harvested from water stress treatment had a higher total soluble sugar content, when compared to the other treatments. Therefore, a relatively high total soluble sugar level in fruit is crucial for human nutrition, especially when the °Brix level is above 5. However, the values obtained from this study are slightly higher, making it an important fruit for the fresh and juice market. This suggests that the fruit is valuable and should be considered for commercialization, as the fruit shows potential benefits for human nutrition.

4.3. Crude Proteins

Crude proteins are important in human nutrition because they aid in cell formation, nutrient storage, pH balance, and immune system improvement, and they serve as a messenger [9]. Previous studies have often reached conflicting findings regarding crude protein content of crops harvested from different treatments and growing conditions. For example, [16] presented their findings on crude protein of potatoes harvested under different regions that experience varying weather conditions and treated with varying level of fertilizers. They concluded that potatoes harvested from regions with moderate temperatures subjected to moderate nitrogen fertilizers resulted in higher significant crude protein content, when compared to other treatments, due to high enzyme activities within cells, caused by different nitrogen content. For this study, shade-net conditions expressed high crude protein content, compared to the other growing environment. Perhaps the growing environment of the shade net favored higher crude proteins in moderate and no-water-stress treatments, as compared to the water stressed treatment.

When the surrounding conditions (adequate sunlight and water) are favorable, cells can carry out chemical reaction at an optimum rate, but at a lower rate under stress environment, such as excessive radiation and water stress. These results agree with the fact that excessive temperatures negatively affect protein activities (denature) and have other general destructive effects on plant cells, as reported by [17], who found higher crude protein content in fruits harvested from protected structures, but low in those harvested from open-field conditions. This advocates that African horned cucumber, if grown under optimum environmental conditions may have several health benefits in human nutrition and may also be a potential solution for a hunger and health issues globally.

4.4. β -Carotene

β -carotene, famously known as a major source for Vitamin A, has been reported by [18] as an important compound for human health. It is (i) responsible for the formation and maintenance of teeth, (ii) formation of muscle tissues, and (iii) improvement of eyesight. The grand mean showed that β -carotene content was higher in severe water stress treatment under greenhouse environment, but significantly decreased by the same water stress treatment under open-field environment. In addition, loamy soil seemed to increase β -carotene, whereas sandy loam reduced it.

The fact that carotene is responsible for radiation interception in plants could have been the cause for variation, since there is control of light intensity in the greenhouse, due to cladding material used for protection, as compared to an open-space area. [19] found

that there was variation in β -carotene among some plant varieties subjected to reduced water supply. Their findings are in harmony with those of the current study, whereby varying water levels under different growing conditions significantly altered the β -carotene content of African horned cucumber fruit. β -carotene promotes cell and tissue development, strengthens the immune system, and slows the aging process. Furthermore, it effectively enhances eye vision, skin, nail, and hair function. African horned cucumber fruit contains reasonable amount of β -carotene, which can be converted to vitamin A in the body, to complement it. Therefore, optimum growing environment could serve as strong evidence for mass production and commercialization globally.

4.5. Vitamin C

In the present study, the grand mean showed that vitamin C was higher on fruit grown under greenhouse environment (25.3 mg 100 g⁻¹ DW), as compared to the other growing environments. In addition, vitamin C increased in plants subjected to no water stress (control) under the shade-net environment, but it decreased under severe water stress under the open-field environment. Higher fluctuation in the vitamin C content could be the result of unbalanced turgor pressure in plants, caused by varying irrigation water levels, water holding capacity by a specific substrate, and different growing environments, as reported by [20,21], who mentioned that water and fertilizers stimulate the vitamin C content of cucumber and citrus fruit grown in an open field and semi-protected structure. This was authenticated by [22], when they reported that plants respond to harsh environmental conditions, such as excessive sunlight, heat, and water stress, by producing vitamin C as a defensive mechanism to protect themselves [23].

The mean results also showed that the vitamin C reduction was more on plants subjected to adverse conditions, such as water stress level and open space, as compared to plants that were grown under a protected environment (greenhouse and shade net). The current study findings agree with findings by [24], who reported that plants can tolerate moderate water stress. However, such alteration has a negative impact on the fruit vitamin C content of various fruit crops. Even though vitamin C deficiency is uncommon in today's world, dieticians prescribe vitamin C because it plays a critical role in the production of collagen, iron absorption, wound healing, and bone and tooth health. Determination of optimal conditions that increase African horned cucumber vitamin C content could fill the void in human nutrition, globally, and increase its consumption.

4.6. Vitamin E

For vitamin E, the means illustrated that vitamin E content was greater in the greenhouse environment (23.7 mg 100 g⁻¹ DW), as compared to other growing environments. In addition, the current study findings exhibited that the treatment imposed (water levels and soils types) did not caused significant variation in vitamin E content. However, there was a slight increase on severe water stress treatment under greenhouse conditions, but there was a significant decrease under severe water stress in the open-field environment. Perhaps the evapotranspiration rate, which regulates the osmoregulation, played an important role in the vitamin E variation, since there was a change in stomatal opening and closure, due to alteration in turgor pressure within the guard cells. Carbon dioxide interception is higher when there is balance of solutes movement within the open guard cells, but they close when there is imbalance concentration due to high evapotranspiration rate caused by excessive conditions, such as high wind and radiation, subsequently limiting the ability to synthesis vitamin E due to limited activities in the chloroplast caused by stomatal closure. Closing of stoma not only prevents water loss, but also prevents the plant's ability to synthesize vitamins and other biochemical compounds. The study findings affirm that water stress levels under varying growing environment were the critical contributors of vitamin E content of African horned cucumber fruit, as compared to other factors. These findings agree with [18,21,25], who found significant differences in vitamin E content of fruit such as chilies and peppers subjected to varying water stress, due to the balanced

osmotic flow within plant organs. Vitamin E has a variety of functions in the human body, including preventing free radical damage and acting as an antioxidant. In addition, the vitamin deficiency is associated with stunted development. The values in this study serve as benchmarks required by policymakers for commercialization of this crop, since it has nutritional benefit to humans.

4.7. Total Flavonoids

The shade-net grand mean showed higher total flavonoids ($0.67 \text{ CE g}^{-1} \text{ DW}$), relative to greenhouse at ($0.4 \text{ CE g}^{-1} \text{ DW}$). The study findings also remarked that the reduction was more on the severe water stress treatment, relative to moderate and no-water-stress treatment (control). The alteration in total flavonoids could have been caused by turgor pressure within the plant's cells, which subsequently allows the plant to access surrounding atmospheric elements through the epidermal cells, thus allowing the plant to absorb atmospheric elements needed by plants for cellular activities. When the stomata close, plant cellular activities get negatively affected, but they function normally when there is good movement of water within plant organs. However, contradictory findings were noticed by [13,26] on opuntia and red grapes. They determined that total flavonoids significantly increased in fruit harvested from regions with a low rainfall pattern, but decreased in fruit harvested from regions experiencing higher rainfall patterns, due to varying active osmoregulation within plant organs, since plants were trying to cope with stress caused by the environmental conditions. Their findings are consistent with observations made in this current study, whereby severe-water-stress fruit demonstrated a significant increase in total flavonoids when compared to stressed-free fruit. Total flavonoids are well-known in human health for their function in controlling cellular activity, as well as fighting free radicals that cause oxidative stress. The total flavonoids values of African horned cucumber fruit serve as benchmark information required by policymakers; therefore, the crop can be recommended for commercialization, if grown under optimal conditions.

4.8. Total Phenols

In terms of total phenolic content, the study findings outline that open space grand mean exhibited higher total phenols ($4.8 \text{ GAE g}^{-1} \text{ DW}$), relative to the greenhouse ($4.5 \text{ GAE g}^{-1} \text{ DW}$) and shade-net environment ($4.2 \text{ GAE g}^{-1} \text{ DW}$). The study findings showed increased total phenolic content under normal watering on loamy soil from the open-field environment but decreased when subjected to water stress under a similar growing environment. Perhaps variation in water stress and soil types under different growing conditions could have been the major cause in variation of total phenolic content of African horned cucumber fruit since xylem and phloem functions effectively under active-osmoregulation, but solutes uptake decrease when there is lower water movement within the cells caused by higher temperature. For example, [27] found significant differences in several edible fruits such as blackberry and cherry harvested from different locations experiencing varying rainfall patterns. [10] also found a significant difference in total phenols of walnuts' green husks harvested during different periods. They found that fruits harvested earlier have a higher total phenolic content than those which were harvested late, after ripening, due to different metabolites released by plants at different stages of growth.

The current study affirmed that different water stress levels are major triggers of metabolites responsible for this compound, since the plant has to adapt to variation in water levels, as reported by [28], who found a significant total phenolic content in strawberries exposed to different environmental conditions such as water stress and growing conditions. Total phenols are known in human health for their antioxidant properties, which stop free radicals from reacting with other molecules in the body and prevent DNA damage, which is usually caused by a variety of health effects. Therefore, values in this study serve as concrete evidence needed by policymakers in order to consider this crop for commercialization.

4.9. Micro-Nutrients

Micronutrients deficiency, including of iron, copper, and zinc, may lead to decreased intellectual ability, development, bone mineralization, and immune response, whereas deficiency in zinc may lead to poor digestion, metabolism, reproduction, and wound healing. According to WHO, the recommended daily nutrients intake of zinc for children between four and six years should range from a minimum of 9.6 $\mu\text{g g DW}$ and above. The study findings showed that zinc grand mean of greenhouse grown fruit was higher, at 9.8 $\mu\text{g g DW}$, relative to shade net at 8.2 $\mu\text{g g DW}$ and open field (7.0 $\mu\text{g g DW}$). This micronutrient is vital for metabolism and reproduction. Its deficiency may lead to poor digestion and bone diseases. The zinc content of the African horned cucumber fruit serves as a benchmark for commercialization of this fruit, since it has the potential to meet human nutritional needs. In addition, the study findings have shown that moderate water stress and sandy loam increase zinc content under greenhouse environment, and this has added to the information needed by potential growers, since they will be able to create suitable growing environment in order to increase vital micronutrients content for the African horned cucumber fruit.

Iron is another micronutrient that is vital for blood health, bone development, and immune system. Shortage of iron may reduce intellectual capacity, slow growth and poor bone development. According to WHO, recommended daily nutrient intake (RNI) of iron by children between the age of one and three should be 5.8. A range of (0.5 to 3.5 $\mu\text{g g DW}$) was observed on the African horned cucumber fruit, which is slightly lesser than the recommended daily intake (RNI) by WHO [29]. However, the study findings showed that the fruit has a high potential of meeting the recommended nutrient intake (RNI) if grown from treatment of moderate water stress level combined with sandy loam soil under greenhouse environment. The study's findings serve as a benchmark on the potential nutritional benefit of African horned cucumber fruit. Other researchers, such as [21], reported that growing environment and temperature as growth factors are able to cause variation in nutrient content of crops.

They have shown that a higher evapotranspiration rate, caused by extreme temperatures, could cause a significant variation in fruit nutrient content, as osmotic balance is directly affected, subsequently causing an abnormal flow rate of water and other soluble nutrients within xylem and phloem. Similar findings were observed in the current study, whereby interaction between irrigation water levels and soil types under different growing environments affected the micro-nutrient content (Zn and Fe) African horned cucumber fruit. Several authors remarked that water levels and soil types affect micro-nutrient content of fruits. For example, [30] found that variation in nutrient content may occur when plants are subjected to water stress. They have also demonstrated that, when the plant is subjected to water stress, stomata close, but they open under normal watering.

Their findings unveiled that when the stomatal opening reduces, there is limited carbon dioxide entry in leaves, subsequently affecting the plant's ability to synthesize its own nutrients. [31] report that less frequencies in irrigation significantly increased nutrient content in tomatoes, when compared to treatment that received more irrigation frequencies [31]. They have shown that there is a direct relationship between stomatal conductance and active osmoregulation under less frequencies, but complications occur when there is over-/under-supply of water in plants, as it negatively affects the xylem functions. It worth noting that Cu and Mn were not significantly affected by treatment imposed. [23] observe that a nutrient element such as Mn depends on environmental factors such as adequate water supply, temperature, and plant genotype. However, in this study, irrigation water levels and soil type under different growing environments did not significantly cause variation in some of African horned cucumber fruit, but they significantly affected the Zn and Fe content.

5. Conclusions and Future Research

Quantification of quality parameters such total soluble sugars and macro- and micro-nutrients contribute to the factors required by policymakers before commercializing a specific crop. Therefore, the outcome of this study has shown that African horned cucumber fruit contain vital biochemical constituents required by humans in both larger and smaller quantities. In addition, this research has provided evidence that the African horned cucumber fruit quality content is significantly affected by treatments. This is useful information to farmers, as quality has become more significant to most consumers worldwide. When grown in the open field, total soluble sugars increased; this is important for the juice-manufacturing industry and for fresh markets, where many fruits are required to meet the demand. Quality parameters such as total flavonoids, total phenols, micro-nutrients and vitamins metabolites seem to be treatment-imposed. This is an important finding, as these factors influence the flavor of fruits. Where the market is geared towards organoleptic quality—in expensive markets, for example—it may be best to grow this crop under a specific growing environment, depending on your target market. The other advantage is that the crop can grow well under protected structures, which eliminate potential damage caused by higher rainfall, hail, and extreme heat in summer.

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