

Article

Practices of Organic Medium Enclosed Trough and Amaranth Species Variation Enhanced Growth, Nutritional Composition and Bioactive Compounds

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Abstract: Organic Medium Enclosed Trough (OMET) system is a new non-drainable growing technique. The study set out to investigate the interactive effects of OMET and Amaranth species variation on some growth attributes, yield, bioactive compounds, and nutrients. Three Amaranth species, *A. caudatus*, *A. cruentus*, and *A. tricolor*, were grown in OMET and non-OMET growing conditions. Growth attributes, irrigation water, bioactive compounds, and nutrients were determined. Non-OMET growing conditions and *A. cruentus* showed the highest total phenolics and flavonols followed by *A. caudatus* and *A. tricolor*, whilst tannins were predominant in *A. caudatus* followed by *A. cruentus* and *A. tricolor*. The use of unsupervised PCA showed major metabolic variation based on Amaranth species and the use of supervised OPLS-DA showed clear metabolic variation based on OMET and non-OMET. OMET and *A. cruentus* showed the highest plant height, stem diameter, biomass, and some nutrients (%protein, Ca, P, Cu, Se, Zn), followed by *A. caudatus* based only on growth and biomass, and *A. tricolor* based only on some nutrients (Mg, K, Mn, and Fe). The use of the OMET system on *A. cruentus* and *A. tricolor* can be recommended to mitigate climate change effects on nutritional composition using relatively low levels of irrigation water.

Keywords: Amaranth; OMET; bioactive compounds; amino acids; minerals; PCA



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1. Introduction

Amaranthus spp. is one of the commonly consumed leafy vegetables from the Amaranthaceae botanical family. In South Africa, tender young leaves are cooked to be part of daily meals as relish [1]. Given its nutritional status, predominated by macro and micronutrients, it falls under the category of recommended indigenized leafy vegetables with the potential to combat food and nutritional insecurity [1,2]. Amaranths consists of 60 species, including *A. cruentus*, *A. caudatus*, and *A. tricolor*, which differ based on their morphological attributes, such as type of leaf venation or blades, count of stamens in the inflorescence, as well as the presence of red spots on their leaf surfaces [3]. This further corroborates that there are nutritional composition and secondary metabolites variations in different Amaranth species. For example, Sarker and Oba [4] reported leaves of different *A. cruentus* accessions to be rich in dietary fiber, protein, fat, carbohydrates, energy, and ash. In fact, Amaranth has sulphur-containing amino acids, which are normally limited in other protein rich crops such as grain legumes, thus making Amaranth ideal for supporting human dietary needs. Moreover, Mateos-Maces et al. [5] reported that phytochemical composition in *A. cruentus* were predominated by total phenolic compounds equipped with phenolic acids (hydroxycinnamic and hydroxybenzoic acid) and flavonoids. However, Amaranth is one of the forgotten leafy vegetables, often produced in a small scale farming, which limits its commercial distribution. Different cultivation practices have been reported as

part of the strategies to enhance cultivation of different Amaranth species. These include the use of organic fertilizers, biostimulants, regulating the planting date, and irrigation schedules [6–8].

The Organic Medium Enclosed Trough (OMET) system is a non-drainable growing technique characterized by sandwiching growth medium to form an enclosed trough using a polyethylene plastic [9]. The OMET system design offers a micro-climate system to the growing seedling, and utilizes less water, eliminates water or nutrient seepage, and requires no weeding. Therefore, OMET can be regarded as a green-smart growth technique to reduce the effect of climate change. The OMET system has been reported to improve growth and yield in okra [10]. However, there is still a gap of information on its impact on growth, yield, nutritional composition, and bioactive compounds in Amaranth species (*A. caudatus*, *A. cruentus*, and *A. tricolor*).

2. Materials and Methods

2.1. Experimental Site, and Treatments of the OMET System

This trial was established in a greenhouse setting in 2021 and was repeated in 2022 between November and December at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10" S, 29°44'15" E). The site is located 2126 m above sea level. The temperature and relative humidity in the greenhouse ranged from 18 °C to 24 °C and 40% and 50%, respectively. A randomized complete block design was used to layout a 2 × 3 factorial trial consisting of two growing conditions (OMET and non-OMET) and three Amaranth species (*A. caudatus*, *A. cruentus*, and *A. tricolor*) in triplicates. The OMET system was prepared on greenhouse benches; a layer of growth medium (25 cm thick) was sandwiched using high density polyethylene (HDPE) plastic (flexible, translucent/waxy, weatherproof, good low temperature toughness (−60 °C), easy to process by most methods, low cost, and with good chemical resistance) to form an enclosed trough [10]. The growth medium comprised of steam pasteurized loam soil, sand, and Hygromix growth medium (Hygrotech, Pretoria North, South Africa) at a ratio of 2:1:1. Holes for planting the seedlings under OMET were made on the top enclosing sheet with row spacing of 20 cm × 20 cm between seedlings. The non-OMET system (control) was prepared in the same way but without plastic.

2.2. Establishment of Seedling on the OMET System

Seeds of the three Amaranth species, namely *A. caudatus*, *A. cruentus*, and *A. tricolor*, were obtained from the Agricultural Research Council, Vegetables, Industrial, Medicinal Plants (ARC-VIMP), Pretoria, South Africa. Seedlings for each Amaranth species were germinated on seedling trays filled with Hygromix® growth medium for up to four weeks until the seedlings were at the four-leaf stage. Then, uniform seedlings were transplanted on the holes for OMET and non-OMET treatment per specie. Irrigation of seedling were performed only when the status of 'dry' was shown on a Xylux moisture tester and the volume of water per irrigation was 250 mL tap water, as described by Mpai et al. [11]. Fertilisation with Multi feeder (Multisol 'P') (active ingredients being nitrogen, phosphorus, potassium, magnesium, zinc, iron, copper, manganese, and boron) was applied at weeks 4 and 8 for each Amaranth species. From the preliminary experiments, Amaranth maximizes its leaf production before the flowering stage, which occurs eight weeks after transplanting. Therefore, the seedling were grown for up to eight weeks (56 days) before flowering to ensure good quality leaves.

2.3. Growth and Yield Data Collection

Growth parameters: Stem diameter and plant height of seedling were collected once per week during the growth period. The stem diameter was measured five centimetres above the soil surface using a digital Vernier calliper; plant height and leaf length were measured using a tape measurer and meter ruler, respectively; time of flowering was recorded as per observation. Fresh mass was measured using a laboratory weighing

balance (TADB 220-4-B, Germany). Thereafter, the leaf samples from each Amaranth species were packaged separately in brown bags and oven dried at 40 °C for 72 h. After drying, they were then grinded into fine powder using a grinder (29105A, SA).

2.4. Determination of Total Irrigation Water

Irrigation water was recorded as the weekly sum of irrigation water. The amount used for irrigation was constant (250 mL). Details of calculation of irrigation water were similar to those described by Mpai et al. [11]. The only variation was the day interval for irrigation/week over eight weeks as the plant grows. The Xylux moisture tester was used to guide when to irrigate (when the moisture status was 'dry').

2.5. Determination of Bioactive Compounds and HPLC-MS-QTOF Untargeted Metabolites

Extraction of the Amaranth samples for bioactive compounds (total phenols, total flavonoids, and tannins) and HPLC-MS-QTOF untargeted metabolites profiling were performed. A total of 200 mg of dried amaranth sample were homogenized with 2 mL methanol: HCL: distilled water (80:0.5:19.5 *v/v/v*) to allow a 1:10 ratio of sample and a solvent in a thermostatic shaking water bath at 70 °C [3] for 15 min following the method described by Mpai et al. [12] without any modifications. Prior to the biochemical analysis, the extracts were centrifuged for 15 min, dried under N₂ gas, and re-suspended and filtered as described by Mpai and Sivakumar [13]. Total phenol content was determined following the Folin–Ciocalteu method, as described by Mpai et al. [12]. The results were calculated and reported as mg/100 g using Gallic acid standard curve. Total flavonoids were carried out following an aluminium chloride method [14]. The results were calculated using a catechin standard curve. The Folin–Ciocalteu method [15] was used to determine the tannin content of the three Amaranth species' leaves. Tannin content was expressed as mg GAE/100 g DW.

Chlorophyll a and b was performed using fresh leaves of Amaranth species (0.2 g) and the procedure adopted was similar to that described by Managa et al. [16]. Chlorophyll was extracted using 1.5 mL of acetone-hexane mixture (4:6 *v/v*). After shaking and centrifugation at 10,000 rpm for 15 min, 250 µL of the supernatant was transferred into a 96 well micro plate and the absorbance was read at 470, 646, and 662 nm wavelength. The content of Chl a + b gives the total chlorophyll content, and it was expressed in mg/kg on a fresh weight basis [16].

2.6. Determination of Protein

The percentage protein was estimated using the micro Kjeldahl method as described by Helrich [17]. In each Amaranth species, two grams (2 g) of each sample was mixed with 10 mL of concentrated sulphuric acid H₂SO₄ in a heating tube. One tablet of selenium catalyst was added to the tube and the mixture was heated inside a fume hood. The digest material was transferred into a 100 mL volumetric flask and made up with distilled water. A ten millilitre (mL) portion of the digest was mixed with equal volume of 45% NaOH solution and poured into a Kjeldahl distillation apparatus. The mixture was distilled, and the distillate was collected into a 4% boric acid solution, containing three drops of indicator. A total of 50 mL distillate was collected and titrated as well. The sample was duplicated three times, and the average value was taken. The nitrogen content was calculated and converted to percentage protein by using a protein conversion factor of 6.25. This was given as:

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

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

2.7. Determination of Minerals or Trace Elements

Mineral estimation was performed using oven dried leaf samples of the Amaranth species following the method described by Mathipa et al. [18]. A total of 10 g dried materials

were digested in 40 mL of 4% nitric acid (HNO₃), followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, and then incubated in a 95 °C water-bath for 90 min, allowed to cool down at room temperature, filtered, decanted into 50 mL tubes and then covered with a foil. Then, selected nutrient elements were analysed using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

2.8. Determination of Amino Acids

Amino acid analysis was performed using dried leaves of three Amaranth species, according to Mpai et al. [12], using fresh leaf samples (100 mg) that were vortexed with 6 N HCl 0.5 mL. The outcome mixture was held in an oven at 110 °C for 18 h and then left to cool. After cooling, the mixture was centrifuged and filtered. The resulting filtrate was dried using a speed vac and reconstituted in borate buffer (70 µL) for derivatisation following the method by Mpai et al. [12].

2.9. Data Analysis

The study adopted a two-factorial analysis (OMET and Amaranth species). Mean separation for significant treatments was achieved through Duncan multiple range test (DMRT) at the significance level of 5% ($p \leq 0.05$) using the Statistix 10.0 computer statistical software.

3. Results

3.1. Effect between Growth Conditions and Species on Growth, Flowering, Irrigation Water and Yield Attributes of Three Amaranth Species

The results of the effects of the OMET system on growth of stem diameter and plant height of the three Amaranth species are shown in Figures 1 and 2. There were significant ($p \leq 0.05$) differences in the growth of stem diameter and plant height of the three Amaranth species grown under OMET and non-OMET conditions. The stem diameter and plant height in *A. cruentus* grown in an OMET and non-OMET system outperformed all other studied treatments over the growing period. However, stem diameter and plant height in *A. caudatus* grown in an OMET system were higher than that of non-OMET and *A. tricolor* species grown in OMET and non-OMET.

Table 1 shows the flowering time of the three Amaranth species grown on OMET and non-OMET growing conditions. The *A. caudatus* and *A. tricolor* grown under the non-OMET flowered early at week four and five, respectively. In contrary, the same species grown under OMET and *A. cruentus* (OMET and non-OMET) flowered a week later at week six.

Table 1. Time of flowering in three Amaranth species grown under OMET and non-OMET system.

Treatment	Amaranth Species	Time of Flowering (Weeks)							
		1	2	3	4	5	6	7	8
OMET system	<i>Amaranthus caudatus</i>					X			
	<i>Amaranthus cruentus</i>						X		
	<i>Amaranthus tricolor</i>						X		
non-OMET system	<i>Amaranthus caudatus</i>				X				
	<i>Amaranthus cruentus</i>						X		
	<i>Amaranthus tricolor</i>					X			

The amount of water used was constant (250 mL per irrigation day) and increased in the intervals of irrigation per week as observed in Figure 3. Samples grown in OMET system utilised 2500 mL of irrigation water whereas the non-OMET system utilized 3000 mL irrespective of the species. The OMET system has utilized 500 mL lesser than the non-OMET system.

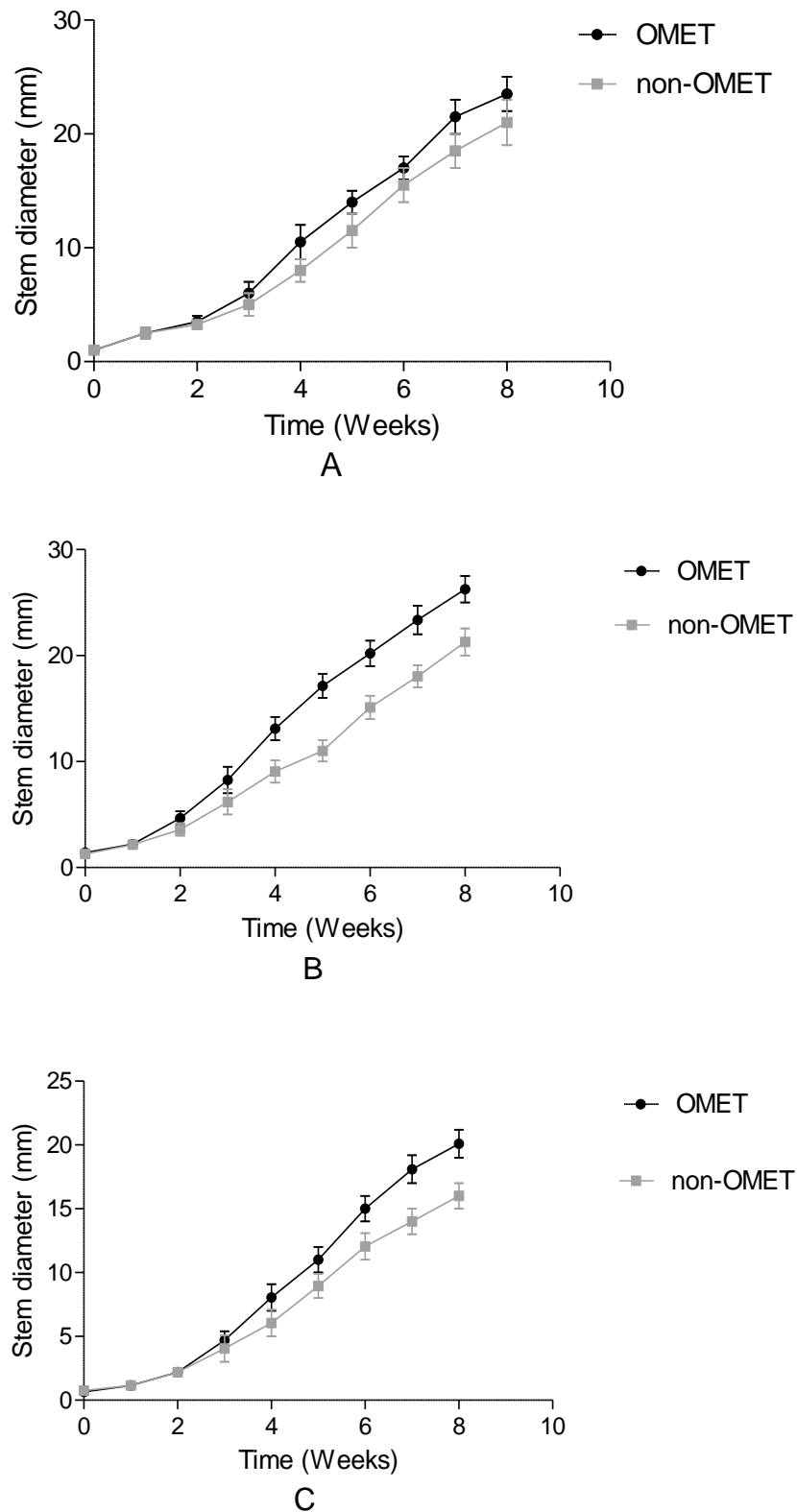


Figure 1. The effects of OMET system on stem diameter in (A) *Amaranthus caudatus*, (B) *Amaranthus cruentus*, and (C) *Amaranthus tricolor* from week 0 to 8 after transplanting. Results are expressed as the mean values \pm standard error ($n = 12$).

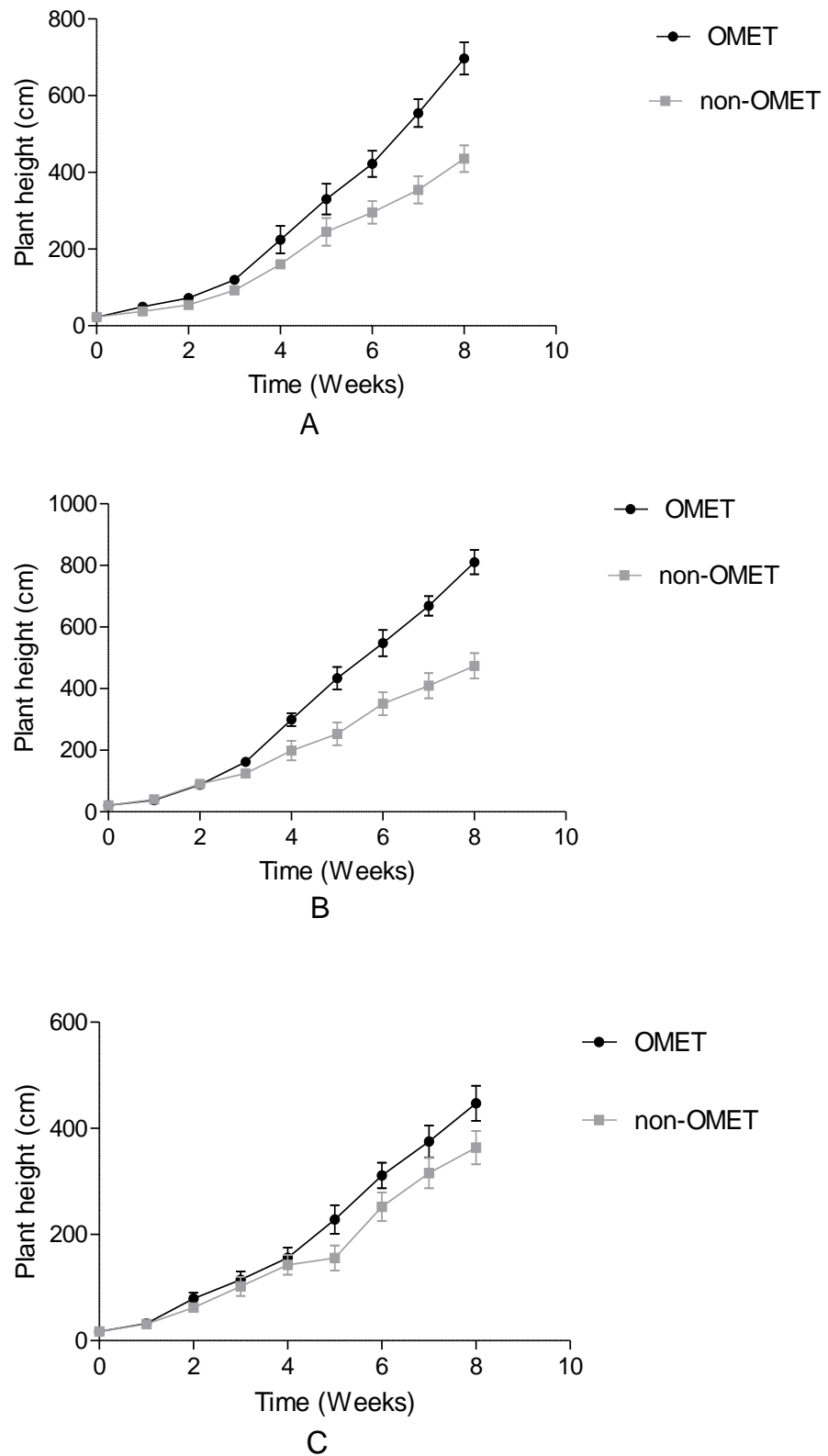


Figure 2. The effects of OMET system on plant height of (A) *Amaranthus caudatus*, (B) *Amaranthus cruentus*, and (C) *Amaranthus tricolor* over 8 weeks after transplanting. Results are expressed as the mean values \pm standard error ($n = 12$).

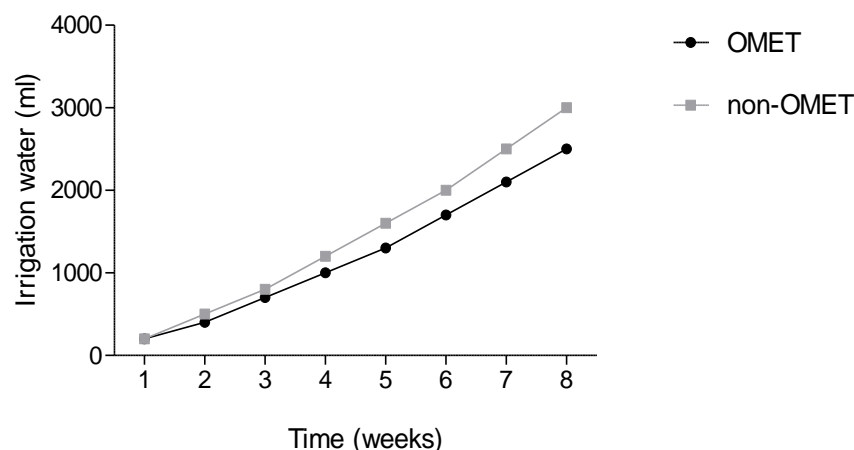


Figure 3. Cumulative amount of water utilised weekly/plant over a period of 8 weeks under OMET and non-OMET system.

The OMET system and species interaction significantly ($p \leq 0.01$) affected the plant fresh biomass. Overall, OMET grown *A. cruentus* (ACr.) measured the highest biomass (103 g), followed by OMET grown *A. caudatus* (Aca.) (69 g). In contrary, non-OMET grown *A. tricolor* (ATr.) measured the lowest biomass (34 g). There was no significant difference between the OMET grown *A. tricolor* and non-OMET grown *A. cruentus* (36 g) (Figure 4).

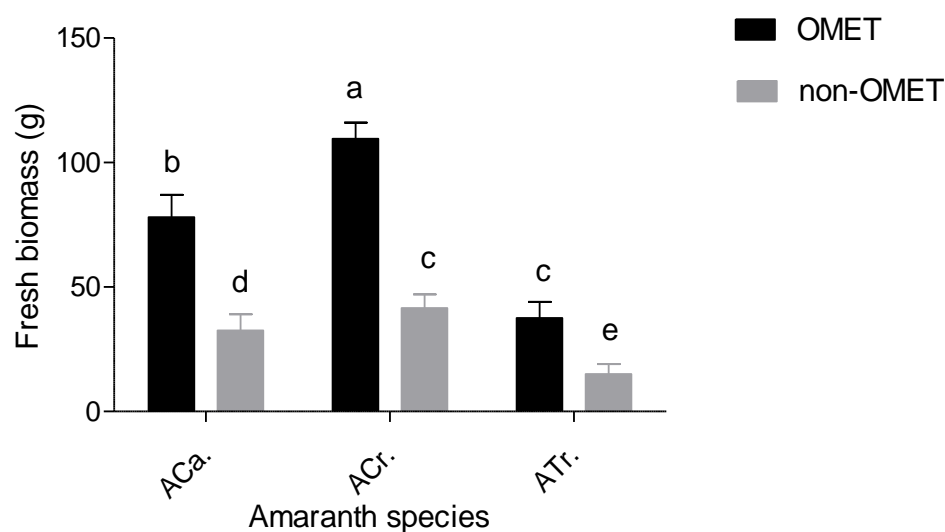


Figure 4. The effects of OMET system on fresh biomass of three Amaranth species. Results are expressed as the mean values \pm standard error ($n = 12$). Bars in the same column marked with different letters indicate significant difference at $p \leq 0.05$. Aca. = *Amaranthus caudatus*; ACr. = *Amaranthus cruentus*, ATr. = *Amaranthus tricolor*.

3.2. Interactive Effect between Growth Condition and Species on Bioactive Compounds and HPLC-MS-QTOF Metabolites

3.2.1. Bioactive Compounds

Growing of Amaranth species on a non-OMET were found to increase the contents of total phenols and total flavonoids, such that samples of *A. cruentus* (262.45 mg GAE/100 g DW and 473.28 mg CA/100 g DW) outperformed all other studied species, followed by *A. caudatus* (259.68 mg GAE/100 g DW and 453.34 mg CA/100 g DW) and lastly *A. tricolor* (257.24 mg GAE/100 g DW and 447.59 mg CA/100 g DW) (Table 2). The non-OMET further enhanced the accumulation of tannins and samples of *A. caudatus* were found to be the highest (9.60 mg GAE/100 g DW), followed by those of *A. cruentus* (8.78 mg GAE/100 g

DW) and *A. tricolor* (6.89 mg GAE/100 g DW). These total tannins found in non-OMET treatment (control) were two-fold higher than those found in OMET treated samples, as shown in Table 2. The results in Table 3 illustrate that the OMET system maintained chlorophyll content in all the three Amaranth species. In the present study, the highest chlorophyll (Chl a + b) was recorded in OMET grown *A. caudatus* (48.085 mg/kg), followed by the non-OMET and OMET grown *A. caudatus* and *A. cruentus*, respectively. The lowest Chl a + b concentration was obtained in non-OMET grown *A. tricolor* (30.946 mg/kg) (Table 3). Total chlorophyll content under OMET grown Amaranth species was higher compared to that of the same Amaranth species grown under non-OMET system.

Table 2. The interactive effects of OMET system and Amaranth species on total phenolics, flavonoids and tannins.

Amaranth Species & Treatment		Total Phenolic (mg GAE/100 g DW)	Total Flavonoids (mg CA/100 g DW)	Total Tannins (mg GAE/100 g DW)
<i>A. caudatus</i>	OMET	210.24 ± 0.033 d	391.26 ± 0.046 d	4.09 ± 0.042 d
	non-OMET	259.68 ± 0.046 b	453.34 ± 0.036 b	9.60 ± 0.022 a
<i>A. cruentus</i>	OMET	209.65 ± 0.035 d	382.60 ± 0.047 e	3.98 ± 0.043 d
	non-OMET	262.45 ± 0.028 a	473.28 ± 0.021 a	8.78 ± 0.031 b
<i>A. tricolor</i>	OMET	207.70 ± 0.0036 e	364.24 ± 0.038 f	3.61 ± 0.038 d
	non-OMET	257.24 ± 0.040 c	447.59 ± 0.042 c	6.89 ± 0.040 c

DW = Dry weight, different letters in the same column indicate significance difference at $p \leq 0.05$.

Table 3. The interactive effects of OMET system and Amaranth species on chlorophyll concentration (mg/kg).

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

Chl a = Chlorophyll a; Chl b = Chlorophyll b; Chl a + b = Chlorophyll a + b; different letters in the same column indicate significance difference at $p \leq 0.05$.

3.2.2. HPLC-MS-TOF Metabolites

To summarise the obtained results from the HPLC-MS-Q-TOF of untargeted metabolites profiling, the chemometric analysis approach was applied to observe metabolite variations and similarities within samples treated with OMET and non-OMET in three Amaranth species. With the use of unsupervised principal component analysis (PCA) (Figure 5A), two main clusters were observed and separated based on the Amaranth species. In fact, samples of *A. tricolor* grown on OMET (TR1) system showed a holistic distinctive metabolome profile from samples of *A. caudatus* (CA1) and *A. cruentus* (CR1), irrespective of their growth condition (OMET system). However, there was no clear clustering between samples of *A. caudatus* and *A. cruentus* grown on either OMET or non-OMET. Therefore, a supervised OPLS-DA plot was generated, and results obtained therein showed good model statistics with predictive ability (Q2 cum value: 62%) that was above 50% (Figure 5B). Two clear clusters were now observed to separate 1: *A. tricolor* (TR1) and *A. caudatus* (CA1) grown on OMET, and 2: *A. cruentus* grown on OMET (CR1) and the other three species grown on non-OMET. The OPLS-DA model (Figure 6) was generated to demonstrate metabolites biomarker responsible to the OMET and non-OMET clusters irrespective

of the studied Amaranth species. The tentative identification based on molecular mass (mz), fragmentation, chemical formula, and retention time (rt) were used to identify compounds that contributed significantly to variation between OMET and non OMET (Table 4). The compounds responsible for metabolome variation between OMET and non OMET were detected as 6-Feruloylglucose 2,3,4-trihydroxy-3-methylbutylglycoside, a fatty acyl glycoside of mono and di-saccharides (rt: 15.658; mz: 474.08758, and MSE: 474.08758:246 475), and Apigenin 7-O-glucoside (rt: 17.63; mz: 421.28; MSE: 268.03810:269.04341), respectively. Other compounds that showed significant contribution variations were showed at rt: 8.093; mz: 218.10211, MSE: 218.10655:4843 219, which were identified to be pantothenic acid. The 'Apigenin 7-O-glucoside' is a flavonoid compound that plays a role in improving the adaptation of crops to abiotic stress such as drought. Further studies on its quantification are necessary.

Table 4. Tentative identification of HPLC-Q-TOF-MS untargeted metabolites of Amaranth species.

Retention Time (min)	Exact Mass (g/mol)	Mass Generated ESI (-) TOF MS (g/mol)	Fragmentation	Chemical Formula	Tentative Structural Assignment
15.658	474.4557	474.08423	474.08758:246 475	C ₂₁ H ₃₀ O ₁₂	6-Feruloylglucose 2,3,4-trihydroxy-3-methylbutylglycoside
8093	219.23	218.10211	218.10655:4843 219	C ₉ H ₁₇ NO ₅	Pantothenic acid
21.15	468.46	467.15897	467.13629:1292 468	C ₂₂ H ₂₈ O ₁₁	6-O-(4-Hydroxybenzoyl)-ajugol
17.63	420.41	420.3132	268.03810:269.04341	C ₂₁ H ₂₄ O ₉	Apigenin 7-O-glucoside

3.3. Interactive Effect between Growth Condition and Species on Nutritional Composition

3.3.1. Protein and Nutritive Minerals

The % protein content of the three Amaranth species is shown in Table 5. The highest protein content was recorded in OMET grown *A. cruentus* (28.6%), followed by OMET grown *A. caudatus* and non-OMET grown *A. cruentus* (24.1%). The lowest protein content was recorded in non-OMET grown *A. tricolor* (20.3%), not significantly different from the % protein recorded in non-OMET grown *A. caudatus* (21.4%).

Table 5. The effect of OMET system on total protein % found in three Amaranth species.

Amaranth Species & Treatment	Protein (%)	
<i>A. caudatus</i>	OMET	24.1 ± 0.039 b
	non-OMET	21.4 ± 0.044 d
<i>A. cruentus</i>	OMET	28.6 ± 0.46 a
	non-OMET	24.1 ± 0.040 b
<i>A. tricolor</i>	OMET	22.7 ± 0.036 c
	non-OMET	20.3 ± 0.042 d
<i>p</i> -value	0.04	

Different letters show significance difference at $p \leq 0.05$.

Table 6A represents the contents of macro-elements (Ca, Mg, K, and P) and Table 6B shows micro-elements (Cu, Mn, Fe, Se, and Zn) in three Amaranth species grown under the OMET and non-OMET growing system. The OMET grown *A. cruentus* had the highest Ca (130 mg/kg) and P (34.7 mg/kg) contents, while the highest Mg (82.5 mg/kg) and K (276 mg/kg) contents were found in the OMET grown *A. tricolor*. Non-OMET grown *A. cruentus* recorded the lowest concentrations of Ca (88.3 mg/kg), Mg (56.6 mg/kg), and K (217 mg/kg) in comparison to all other studied Amaranth species grown under both OMET and non-OMET. The same trend was observed in OMET grown *A. tricolor* that had

the highest concentration of P. In contrast, the non-OMET grown *A. tricolor* had the lowest concentration of P (21.4 mg/kg) when compared to the other species under both treatments. There was no significant difference Ca (104 mg/kg DW) measured in *A. caudatus* in both treatment and OMET grown *A. tricolor*.

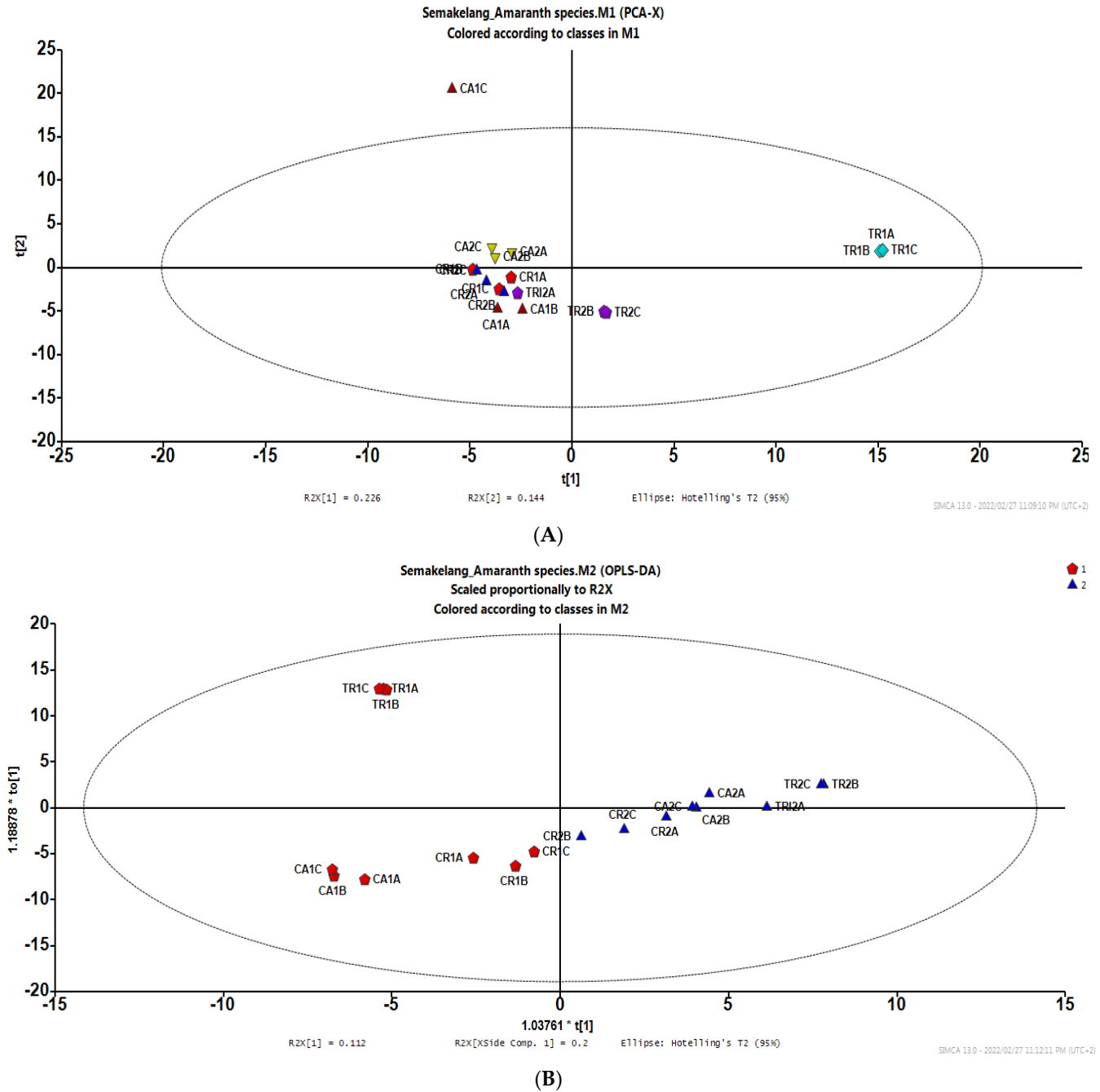


Figure 5. HPLC-MS-QT of untargeted metabolites profiling of *Amaranthus caudatus*, *Amaranthus cruentus*, and *Amaranthus tricolor*, (A) unsupervised PCA and (B) supervised PCA (1 = OMET ad 2 = non-OMET). Red triangles = OMET grown *A. caudatus*. yellow triangles = non-OMET grown *A. caudatus*. Red pentagon = OMET grown *A. cruentus*. Dark Blue triangles = non-OMET grown *A. cruentus*. Light Blue rhombus = OMET grown *A. tricolor*. Purple pentagon = non-OMET grown *A. tricolor*.

(3.41 mg/kg) when compared to the rest of the species grown both under OMET and non-OMET systems. The OMET grown *A. caudatus* contained the second highest concentration of Fe (2.62 mg/kg) and Se (8.03 mg/kg). The non-OMET grown *A. tricolor* measured the lowest Cu (0.78 mg/kg DW), Se (6.36 mg/kg DW), and Zn (0.95 mg/kg DW) when compared to the other Amaranth species grown under both treatments. There was no significant difference in Fe measured in all Amaranth species grown under both treatments, except for OMET grown *A. tricolor*, which had an outstanding Fe concentration (3.41 mg/kg DW). The highest Fe concentration of *A. tricolor* (3.41 mg/kg DW) was higher than the Fe concentration reported by Yahaya et al. [19] in *A. caudatus* (1.06 mg/kg DW), Roselle (1.2 mg/kg DW), and Kenaf (1.17 mg/kg DW).

3.3.2. Amino Acid Composition

The total of 16 free amino acids were quantified in the three Amaranth species grown under OMET and non-OMET system (Table 7). The results revealed that Amaranth species contained both essential and non-essential amino acids. There was a significant difference ($p \leq 0.05$) between amino acid concentration among the same Amaranth species grown under OMET and non-OMET. Essential amino acids threonine, lysine, valine, isoleucine, leucine, and phenylalanine were detected in all Amaranth species grown under both OMET and non-OMET system. The levels of phenylalanine in OMET grown *A. cruentus* (2.54 mg/kg) (Table 7) were higher than the amount reported in the leaves of *A. cruentus* (0.66 mg/kg) [20]. The OMET grown *A. cruentus* also showed a slightly higher amount of leucine (2.21 mg/kg) than the amount reported in *A. cruentus* (1.55 mg/kg) [20]. The highest concentration of valine (1.45 mg/kg) detected in OMET grown *A. cruentus* was shown to be lower than the amount reported in *A. cruentus* (1.51 mg/kg) [20]. Moreover, the highest quantity of isoleucine (1.29 mg/kg) and threonine (1.15 mg/kg) detected in OMET grown *A. cruentus* showed to be both higher than the amount reported in leaves of *A. cruentus* (isoleucine (0.83 mg/kg) and threonine (0.85 mg/kg) [20]. The lysine concentration was 2.37 and 2.25 mg/kg in OMET grown *A. cruentus* and OMET grown *A. caudatus*, respectively (Table 7); this was higher than the concentration reported in the leaves of *A. cruentus* (1.73 mg/kg) [20].

Table 7. Total essential and nonessential amino acids (mg/kg) found in three Amaranth species grown both under OMET and non-OMET system.

		Essential Amino Acids					
		Threonine	Valine	Isoleucine	Lysine	Leucine	Phenylalanine
<i>A. caudatus</i>	OMET	0.92 ± 0.024 b	1.09 ± 0.043 c	0.97 ± 0.031 b	2.25 ± 0.05 a	1.66 ± 0.044 b	2.36 ± 0.05 a
	non-OMET	0.66 ± 0.025 d	0.84 ± 0.027 d	0.76 ± 0.026 c	1.3 ± 0.034 b	1.25 ± 0.038 c	1.14 ± 0.035 c
<i>A. cruentus</i>	OMET	1.15 ± 0.034 a	1.45 ± 0.041 a	1.29 ± 0.027 a	2.37 ± 0.05 a	2.21 ± 0.042 a	2.54 ± 0.051 a
	non-OMET	0.93 ± 0.036 b	1.22 ± 0.042 b	1.12 ± 0.039 a	2.19 ± 0.050 a	1.92 ± 0.051 b	1.95 ± 0.043 b
<i>A. tricolor</i>	OMET	0.88 ± 0.021 c	1.08 ± 0.034 c	0.98 ± 0.028 b	1.62 ± 0.036 b	1.66 ± 0.041 b	1.47 ± 0.038 c
	non-OMET	0.43 ± 0.021 e	0.52 ± 0.026 d	0.46 ± 0.022 d	0.8 ± 0.012 c	0.74 ± 0.024 d	0.71 ± 0.023 d
		Non-essential amino acids					
		Arginine	Serine	Glycine	Aspartate	Glutamate	
<i>A. caudatus</i>	OMET	1.12 ± 0.047 b	0.97 ± 0.034 b	1.37 ± 0.05 b	1.2 ± 0.041 c	1.52 ± 0.05 c	
	non-OMET	0.86 ± 0.031 c	0.7 ± 0.030 c	0.95 ± 0.041 c	0.96 ± 0.042 c	1.29 ± 0.049 c	
<i>A. cruentus</i>	OMET	1.37 ± 0.048 a	0.98 ± 0.036 b	1.33 ± 0.044 b	1.33 ± 0.044 b	1.75 ± 0.05 b	
	non-OMET	1.54 ± 0.05 a	1.16 ± 0.045 a	1.46 ± 0.048 a	2.02 ± 0.05 a	2.55 ± 0.05 a	
<i>A. tricolor</i>	OMET	1.12 ± 0.049 b	0.91 ± 0.042 b	1.21 ± 0.047 b	1.53 ± 0.049 b	1.95 ± 0.05 b	
	non-OMET	0.53 ± 0.028 d	0.46 ± 0.022 c	0.59 ± 0.029 d	0.6 ± 0.030 d	0.73 ± 0.032 d	

Values are expressed as means ± standard error; different letters in the same column indicate significance difference at $p \leq 0.05$.

Nonessential amino acids, serine, arginine, glycine, aspartate, and glutamate were detected in three Amaranth species. The glycine, aspartate, and glutamate were identi-

fied as the predominant non-essential amino acids. The highest concentration of glycine in non-OMET grown *A. cruentus* (1.46 mg/kg) was much higher than the amount reported by Manyelo et al. [20] in *A. cruentus* (0.94 mg/kg). Aspartate content was the highest in non-OMET grown *A. cruentus* (2.02 mg/kg). The non-OMET grown *A. cruentus* was predominated by glutamate (2.55 mg/kg). Serine was highest in non-OMET grown *A. cruentus* (1.16 mg/kg) (Table 7), higher than the amount reported in *A. cruentus* (0.90 mg/kg) [20]. Moreover, the lowest concentration of arginine (0.53 mg/kg) was detected in non-OMET grown *A. cruentus* (Table 7), lower than the concentration reported in *A. cruentus* (0.90 mg/kg) [20].

4. Discussion

The quest for improving food security in South Africa entails scouting for strategies that will be adapted to the changing climate and further contains adequate nutrients for human health nourishment. In this study, the interactive effect between Amaranth species and growth condition (OMET and non-OMET) were shown to be crucial factors for manipulating growth, yield, nutrients, and secondary metabolites contents.

The obtained results on the growth and yield section may be attributed to variation of species and OMET system influence. The results can be assimilated with those mentioned in mulching studied due to the similarities of the concepts. Saeed and Ahmad [21] reported that mulch is effective for vegetable growth and yield by improving moisture content of soil and heat energy. Furthermore, reasons related to the potential of OMET to conserve water and nutrients, which causes a moderate availability of water resulting in good nutrient dissolution and absorption by the plants, could also be attributed to the results obtained [22,23]. Recently, Mokgalabone et al. [10] reported the efficacy of OMET on improving growth and yield attributes in okra seedlings. Plant height and stem diameter have a major impact on biomass. Early flowering in *A. caudatus* and *A. tricolor* grown under the non-OMET system could be due to abiotic stress such as water and nutrient stress. The exposure of the plant to a shortened drought period induces early flowering and halts the production of leaves [24]. In this case, the non-OMET system had high rates of water and nutrients loss through evaporation, drainage, and leaching.

Irrigation water used for the OMET system was less than that used for the non-OMET system, irrespective of the studied species. This could be due to the underlying plastic on the OMET system, which is water impermeable, therefore, water loss through seepage/deep drainage was eliminated, unlike the non-OMET system. The high evaporation rate in the OMET system is eliminated by the top plastic covering the growing medium [9].

Furthermore, this can be due to species type in response to different conditions and growing condition factors such as moisture and nutrient availability associated with plant growth and development under each treatment. Although Amaranth is drought resistant, it performs optimally under irrigation [24] and sufficient supply of nutrients, especially nitrogen (N). One of the essential elements, and one which participates directly as an indispensable requirement for normal plant growth, is N. Application of the multi-feeder made N and other vital nutrients readily available for uptake by the plants. Probably due to the potential of OMET system to conserve irrigation water and nutrients, it prolonged the availability and uptake of water, N, and other nutrients by the plants, thus prompting vigorous vegetative growth and high yielding in Amaranth species. The increased yield of Amaranth was probably due to the ability and potential of OMET to supply frequent and enough water, as Amaranth is said to yield optimally under sufficient irrigation. Scharenbroch and Lloyd [25] reported that organic materials increase soil organic matter by directly improving soil properties and ultimately better plant growth and biomass.

Given the response of Amaranth species to OMET and non-OMET, bioactive compounds including total phenols, total flavonoids, and tannins showed a decrease in OMET conditions. These results suggest the efficacy of OMET in keeping water and nutrition (fertilizers) at the plant rooting zones. According to the known carbon-nitrogen theory [26], nitrogen fertilizer enhances the accumulation of nitrogen containing metabolites such as

amino acids and protein, while reducing the production of compounds such as polyphenols (phenols and flavonoids) [27]. On this basis, the OMET grown Amaranth had fewer bioactive compounds and higher contents of amino acids and proteins. Minor drought stress is associated with major causes for crop loss and poor performance. In this case, Amaranth species grown under the non-OMET system developed minor drought, leading to a significant reduction of photosynthesis [28]. These facts are authenticated by the chlorophyll contents on the leaves, which were reduced in non-OMET samples. Some in vivo studies demonstrated that water deficit results in damages of the photosynthesis II (PSII) oxygen-evolving complex [29].

Kwenin and Dzomeku [30] reported a 4.46% protein content in *A. cruentus*, much lower than all the protein content of all the Amaranth species in the present study, ranging between 20.3 and 28.6%. The protein content of *A. cruentus* (28.6%) is considerably higher than common vegetables such as spinach, cabbage, and kale consumed in RSA. Hanif et al. [31] measured a protein content % ranging from 0.9 to 2.1% in cauliflower, carrot, cabbage, lettuce, spinach. There are numerous factors such as nutrient and water conservation potential by the OMET system that influenced the accumulation of protein in all the three Amaranth species. Wijewardana et al. [32] revealed that maintaining a high level of soil moisture especially during the reproductive stage was beneficial to acquiring higher protein content. In this study, the OMET system, in comparison to the non-OMET system, was a moisture conserving growing technique that maintained a higher level of soil moisture content; this might have influenced highest accumulation of protein in OMET grown Amaranth species. Differences in species and treatment are probably attributable to different protein contents. Amaranth vegetables grown under OMET system can be an important source of dietary protein.

In the study of Yahaya et al. [19], Se concentrations for *A. caudatus* was not quantified. Yahaya et al. [19] reported the Zn concentrations of *A. caudatus* (0.048 mg/kg DW), which was lower than the lowest Zn concentration of non-OMET grown *A. cruentus* (1.66 mg/kg DW) in the present study. The low concentrations of all the microelements in the present study were found in non-OMET grown Amaranth species. *A. tricolor* grown on non-OMET system had the lowest concentration of Cu (0.78 mg/kg), Fe (2.47 mg/kg), Se (6.36 mg/kg), and Zn (0.95 mg/kg) than the rest of the Amaranth species grown on both OMET and non-OMET system. In addition, the lowest concentration of Mn was found in non-OMET grown *A. cruentus* (1.17 mg/kg), higher than the concentrations reported by Yahaya et al. [19] for *A. caudatus* (0.079 mg/kg DW). The *A. caudatus* grown on both the OMET and non-OMET systems had average concentrations of the microelements when compared to other species. The OMET system enhanced the concentration of microelements (Table 3).

Minerals are essential for plant growth, development, reproduction, and seed quality. Mineral deficiency and poor minerals uptake and transport due to abiotic stress such as drought especially at the reproductive stage result in yield loss and poor seed quality [33]. Lowered absorption of the minerals could be due to reduced transpiration flow, limited availability of energy for assimilation, and interference in the unloading mechanism [34,35]. The OMET system has successfully increased the concentration of the mineral elements for Amaranth species than the non-OMET system. This could probably be due to its potential to eliminate leaching of nutrients, conserving and reserving them for uptake by the plant roots. The less to non-fluctuating temperatures of the OMET system is conducive for better nutrient absorption. The root exudates become useful for the plant since they are not leached. The non-OMET system, on the other hand, had little influence on the accumulation of mineral elements, probably due to its inability to conserve nutrients. The nutrients are leached during irrigation through deep drainage or seepage since there is no underlying plastic to inhibit drainage, the nutrients become out of reach for plant roots absorption. The mineral accumulation is also influenced by the degree of soil moisture stress. Both macro and micro-elements showed to be higher under OMET grown Amaranth species, subjected to highest soil moisture, compared to non-OMET grown Amaranth

species. It can be concluded that the optimum high moisture content positively enhances elemental composition.

The results clearly showed that both essential and non-essential amino acids are highly available in the OMET grown *A. cruentus* than any other Amaranth species grown under both treatments. Lysine, leucine, and phenylalanine were the three dominating essential amino acids whereas glycine, aspartate and glutamate were the dominating nonessential amino acids in all the three Amaranth species. Furthermore, both non-OMET grown *A. cruentus* and OMET grown *A. tricolor* shows to be the second potential sources of amino acids compared to other Amaranth species with distinct treatments. Based on the amino acid analysis, it can be concluded that OMET grown *A. cruentus* can be regarded as a reliable growing technique for Amaranth species to be greater sources of essential amino acids (Table 4). Therefore, for the enhancement of a balanced diet, the daily consumption of *A. cruentus* needs to be encouraged. The trend or pattern in amino acid composition could relate to possible inherent differences between genotypes, species and/or treatment. Similar trends were observed regarding the concentrations of essential amino acids in *A. caudatus* and *A. cruentus* probably confirm that these species respond almost the same to the same treatment as compared to *A. tricolor*.

5. Conclusions and Recommendations

The climate change lower crop yields and nutritional quality due to drought, heat waves and flooding as well as increases in pests and plant diseases, leading to high rates of malnutrition and food insecurity. The good news is that there are tools in the form of science-based farming practices that buffer farmers from climate damage and help make their operations more resilient and sustainable for the long term, such as OMET system.

In this study, the aim was to investigate the effects of the OMET system on the growth, yield, nutritional and phytochemical composition of Amaranth species. The findings of the study suggest maintaining optimum soil moisture, soil temperature and nutrient conservation (OMET system) is very significant for the accumulation of improved % protein content, amino acids, and mineral content. We found that OMET system reduces the cases of plant stress, which prohibits early flowering of Amaranth species, positively influencing better plant growth and ultimately, higher yields. This study reveals complementary effects between the OMET system and Amaranth species with regards to the accumulation of primary metabolites. It is important to investigate other growing techniques that will improve the yield and quality of indigenous vegetables. Furthermore, investigations in Amaranth species for the development of nutraceutical products will be essential. Inclusion of the Amaranth species in the food database would be a source of income for the government, Department of Agriculture, Forestry and Fishery.

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