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The Phytonutrient Content and Yield of Brassica Microgreens Grown in Soilless Media with Different Seed Densities

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Abstract: Microgreens are increasingly valued by consumers for their phytonutritional benefits. There is limited information to growers on the influence of growth media and seed density on antioxidant properties of Brassica microgreens. Therefore, the study was conducted to determine the effect of seedling media (Hygromix, Promix, and TS1) and seed density (4, 8, and 12 seeds per seed cavity) on morphological parameters, yield, color, antioxidant components, and their activities in radish (*Raphanus sativus)*, cabbage (*Brassica oleracea*), and rocket (*Eruca sativa*) microgreens. Fourteen days after seeding, Promix at a seed density of 12 per cavity improved yield of radish, cabbage, and rocket microgreens. Irrespective of the seed density, all three Brassica microgreens grown in TS1 had higher leaf nitrogen, phosphorus, and calcium content. Interaction effects of Hygromix x seed densities were more pronounced on the antioxidant properties (DPPH and FRAP). These differences could be due to the physical properties of growth medium. Vitamin C and total glucosinolate were improved on Brassica microgreens grown in Hygromix at a seed density of 4 per cavity. Twelve seeds per cavity in Promix growth medium improved Brassica microgreens yield, whereas phytochemicals were most likely improved by growing media, Hygromix followed by TS1 and mineral content improved in TS1 followed by Hygromix at low seed density of 4 per cavity.

Keywords: vitamin C; leaf chlorophyll; minerals; total phenols; total glucosinolate; water-holding capacity

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1. Introduction

Microgreens are grown and sold as green salads. The historical roots of this ingredient can be traced back to San Francisco during the latter part of the 1980s. Subsequently, their usage has gained traction as a culinary component in gourmet restaurants and upscale food markets [1]. Microgreens, also known as vegetable confetti, have become popular due to their high phytochemical content found in their two cotyledons and earliest leaves, compared to mature plants. Nowadays, microgreens are valued for their diverse flavors, colors, textures, and nutritional benefits [2,3].

Microgreens can be grown from a wide range of species, making them highly unique and increasingly in demand. These plants can be grown using either huge greenhouses as well as soilless or hydroponic systems, or in smaller quantities for domestic consumption [4]. They are composed of delicate cotyledons and the initial sets of leaves, with their height ranging from 2.5 to 8 cm, depending on the type of plant [5]. They are harvested when they are small with a growing period of 7 to 28 days after seeding [6]. Young tender stems and leaves are harvested while the roots remain in the growth medium. Microgreens are deemed safer than sprouts because the roots are not usually consumed [7–9]. The harvesting maturity of microgreen leaves varies depending on the growth rate and market

preference. According to Alloggia et al. [10], microgreens can be cultivated soilless or in soil, in small spaces, including regulated rooms with artificial illumination and there is no need for weed, insect, or fertilizer control. However, microgreen cultivation requires large amounts of seed and low yields and substrates hold in water, oxygen, and nutrients for the plant and provide support and anchoring it.

It is well-known that Brassica vegetables, such as broccoli, cabbage, and radish, contain high concentrations of glucosinolates that have anti-inflammatory and anti-cancer properties because of isothiocyanates and indoles formed during breakdown [11]. Furthermore, this family contains phenolic compounds, carotenoids, tocopherols, and ascorbic acid [12]. Most Brassica microgreens contain flavonol glycosides, such as kaempferol, quercetin, and isorhamnetin [13]. As a result, *Brassicaceae* microgreens can serve as functional foods [10]. Hence, managing various plant secondary metabolites, stimulated by environmental and agronomical stresses, could lead to improved profiles and concentrations of phytochemicals [14].

Kyriacou et al. [15] showed the potential impact of substrates on phytochemical biosynthesis in microgreens. Fast-growing substrates (such as peat moss) contribute to nitrate accumulation, especially in *Brassicaceae*. Optimizing the seed density is crucial for the successful production of microgreens, but information regarding seed densities per surface area is lacking [16]. However, the seed density of radish microgreens grown on sphagnum peat and coco coir growing media profoundly influenced the yield of fresh shoots. The highest fresh yield was obtained by sowing radish microgreens at a density of eight seeds per cell (roughly 109 g of seeds per cell) [17]. Producing and marketing high-quality microgreens commercially are challenging [18]. Numerous studies reported challenges of the low yield that microgreens growers are faced with [19,20]. Growers experiment with the seed density and adjust the sowing rates accordingly, while others rely on visual estimation [21]. Therefore, this study aims to evaluate the impact of growing media and seed density on harvest microgreens (radish, cabbage, and rocket) yield, quality, and phytonutrient content.

2. Materials and Methods

2.1. Study Site and Microclimate

The study was conducted at the Tshwane University of Technology (25.7322° S, 28.1619° E), Staatsartillerie Rd, Pretoria West, Pretoria. The study was conducted in a temperature-controlled greenhouse with a pad and fan cooling system. The microclimate in the greenhouse was from 13 to 33 °C in November 2020, during the summer season. The evaluation of photosynthetically active radiation (PAR) in the greenhouse and open areas was conducted using a spectroradiometer (StellarNet Inc. Carlson Cir, Tampa, FL, USA), and varied from 900 to 1500 (μmol $m^{-2}s^{-1}$) [22].

2.2. Plant Material and Growing Media

Three commercial enriched fertilizer seedling media (Hygromix, Promix and TS1), (Hygrotech, Pyramid, Pretoria, South Africa), were filled in 200 cavity polystyrene seedling trays. Untreated cabbage, rocket, and radish seeds were sown at different seed densities (4, 8, and 12 seeds per cavity) on three growth media (Hygromix, Promix and TS1). The experimental layout was a completely randomized design with three replications. Hygromix (Hygrotech SA Pty. Ltd., Pretoria, South Africa) is comprised of peat moss, vermiculite, and polystyrene; Promix Organic (Fast Grow Hydroponics, Randburg, South Africa) is comprised of the turbo grow, fine dolomite lime, worm casting, bone meal, rock phosphate, diatomaceous earth, powdered kelps, cocopeat, and perlite; while TS1 Fine 876 (Greenhouse Technologies, South Hills, South Africa) is a cocopeat. Growing media's physical and chemical properties were analyzed at NviroTekLabs, Hartbeespoort, South Africa (Table 1).

Table 1. Physical and chemical properties of the analyzed seedling growing media.

Chemical Property				
Composition	**Unit**	**Promix Organic**	**Hygromix**	**TS1 Fine 876**
Calcium	mg/L	3.90	61.16	156.32
Magnesium	mg/L	3.62	40.17	16.57
Potassium	mg/L	87.73	32.77	105.18
Nitrogen	%	0.76	0.93	1.26
Iron	µg/L	650.00	450.00	1520.00
Zinc	µg/L	22.00	47.90	275.60
Sulphate (SO_4)	mg/L	9.84	101.55	337.44
Phosphate (PO_4)	mg/L	2.73	113.31	78.25
Manganese	µg/L	20.00	80.00	180.00
Copper	µg/L	35.20	107.30	8.74
Nitrate	mg/L	18.59	56.17	42.71
Ammonium	mg/L	0.66	1.21	3.77
Chlorine	mg/L	105.23	10.14	8.74
Bicarbonate	mg/L	26.56	35.42	40.48
Carbonate	mg/L	0.00	0.00	0.00
Boron	µg/L	25.30	80.50	88.80
pH	-	6.84	6.18	5.43
Electrical conductivity (EC)	mS/m	58.90	82.90	129.00
C:N	-	33.2:1	28.5:1	42.7:1
Physical property				
Dry matter	%	40.65	87.63	53.51
Moisture	%	59.35	12.37	46.49
Air-filled porosity (AFP)	%	13.62	10.91	26.62
Ash	%	56.62	54.44	7.45
Water-holding capacity (WHC)	%	74.6	47.0	55.9

Each seed cavity had a volume of 70 mL, and the irrigation was performed manually once per day, using a hosepipe with a fine nozzle that releases fine water droplets. Accordingly, a total volume of 75 L of water was used daily to irrigate 16,200 cavities with each cavity having a 70 mL capacity.

2.3. Data Collection on Morphological Parameters

Fourteen days after sowing, microgreens were harvested at the first true leaf stage without roots by cutting the stem above the growing medium surface using scissors. Data was collected on plant height, stem diameter, fresh mass, and the dry mass was measured. The leaf area (cm^2) was measured using a Leaf area meter (LI-3100C Leaf Area Meter, LI-COR Environmental, Nebraska, USA). The harvested microgreens were weighed using AJ—precision balance scale. Stem diameter was measured in millimeters using a digital Vernier Caliper device (Han Dynasty. American, Joseph R Brown). Plant height was measured using a measuring ruler (mm). Microgreens were dried in a drying oven (Germany, Amenities) at 65 °C for 48 h for the leaf dry mass determination.

2.3.1. Determination of Leaf Color, Soluble Solid Content (SSC), Titratable Acidity (TA) and Ascorbic Acid

Microgreens' leaf color was measured using a CR400 chromameter (Konica Minolta, Osaka, Japan) at harvest [23]. Leaf color values were measured at three points on the microgreen variety, one to the right and one to the left of the main vein (lower region) and closest to the tip of the leaf. The CIE color system was utilized to measure the color changes. The system included the lightness L^*, which ranged from no reflection ($L^* = 0$, black) to perfect diffuse reflection ($L^* = 100$, white). The presence of positive a* values indicated the intensity of red, whereas positive b* values indicated the intensity of yellow. These numerical values were then transformed into the saturation variable or chroma (C^*) and a measure of chromaticity known as the hue angle (h°). The C^* signifies the saturation of color, which ranges from low to high values, while the h° value is defined as a color wheel

with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180°, and blue at 270°. The color changes were measured in the *L**, a*, b*, *C**, and *h*° color spaces, according to [24].

Soluble solids content (SSC) was determined by grinding 5 g of freshly harvested leaves with a mortar and pestle. The resulting mixture was then filtered through a three-layered cloth and the filtrate was analyzed for percentage soluble solid concentration (%SSC) using a hand-held refractometer (Atago Co. in Tokyo, Japan) [25].

The titratable acidity (TA) of 10 mL of the extracted 5 g sample was determined by adding three drops of 1% phenolphthalein solution into a 100 mL beaker. The provided solution was gradually titrated using a 25 mL burette containing 0.1 N NaOH until a neutral state was achieved, detected by the indicator changing from colorless to pink [26].

The 2,6-dichlorophenolindophenol (DCIP) titration method was used to establish the ascorbic acid content in a sample [27]. A 25 mL burette was utilized to dispense the DCIP dye into the sample solution slowly until equivalence was attained, as indicated by a pink coloration. The ascorbic equation was applied to compute the amount of ascorbic acid, and the outcomes were presented in milligrams of ascorbic acid per 100 g.

$$\mathrm{AA} = \frac{\mathrm{Ts}(\mathrm{Std} \times \mathrm{V\ std} \times \mathrm{V\ total} \times 100\mathrm{g})}{\mathrm{V\ aliquot} \times \mathrm{T\ std} \times \mathrm{V\ sample}}$$

AA = Ascorbic acid, Ts = DCIP dye added, Std = 0, 01 Ascorbic acid/1 mL Ascorbic acid, V std = mL of Ascorbic acid, V total = Total volume (mL metaphosphoric acid and mL of sample), V aliquot = mL of metaphosphoric acid, T std = Standard 3, 1, V sample = mL of sample.

2.3.2. Determination of Total Phenolic

Following the approach described by [28], a revised methodology was employed to determine the total phenolic content across three replicates. The extracted sample was diluted using Folin–Ciocalteau reagent, adding 1000 µL of 10-fold, followed by introducing 7.5% Na_2CO_3 into the mixture, which was subsequently incubated for 2 h. The mixture was subjected to vortexing and then incubated in a dark environment at room temperature for 2 h to facilitate the development of color. Following this, the mixture was analyzed with a microplate reader with a 760 nm wavelength (specifically the Zenyth 200rt instrument developed by Biochrom Ltd., Cambridge, UK). Gallic acid was used as the standard, and the results expressed in terms of mg/100 g of gallic acid equivalent.

2.3.3. Determination of the Antioxidant Power [Ferric Reducing Ability of Plasma (FRAP)]

The measurement of antioxidant power, as discussed by [29], relied on the ability of antioxidant compounds to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in the formation of a blue complex (Fe^{2+} TPTZ) with increased absorption at 593 nm. The FRAP reagent used for this assay consisted of a mixture of acetate buffer (pH 3.6), TPTZ solution (10 mmol), and $FeCl_3$ solution (20 mmol). The reagent (190 µL) and sample solution (10 µL) were added to a 96-micro-plate reader and incubated in the dark at 37 °C for 30 min. The resultant absorbance was measured at 593 nm, and a standard curve using Trolox was constructed to determine the reducing antioxidant capacity, which was reported as µM TEAC/g of microgreens.

2.3.4. Determination of the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity

The radical-scavenging capacity was determined using the DPPH assay, following the methodology described by [30]. A microgreen sample weighing 0.1 g was combined with 1 mL of 80% methanol and subjected to centrifugation at 4°C for 5 min at a force of 3000× *g*. The resultant sample concentrations were then diluted to varying levels ranging from 0 to 10 mg/mL, after which 100 µL of each concentration was mixed with 200 µL of DPPH solution (13 µL DPPH/mL methanol). After incubating for 20 min at a temperature of 25 °C, the alteration in color was assessed at a wavelength of 517 nm utilizing a 96-well multi-plate reader (manufactured by BMG LABTECH GmbH, SpectroStar Nano, located in

Ortenberg, Germany). The percentage inhibition was calculated using the formula below, and then the result was used to calculate the IC_{50} (mg/mL).

$$\%\text{inhibition} = \frac{\text{Control} - \text{sample}}{\text{control}} \times 100$$

2.3.5. Determination of ABTS-Based Scavenging Activity (2,2-Azinobis, 3-Ethyl-benzothiazoline-6-sulfonic Acid)

The method described by [30] was utilized to determine the radical-scavenging ability of ABTS. The ABTS radical cation was produced by combining the ABTS stock solution, which had a concentration of 7 mM, with potassium persulphate at a 1:1 ratio of 4.9 mM. The resulting mixture was then incubated for 12–16 h at a temperature of 25 °C. Following this, 40 µL of the test sample was introduced to 200 µL of $ABTS^+$ at varying concentrations of 0–10 mg/mL. The mixture was then incubated at 37 °C for 10 min, with no exposure to light, utilizing a 96-well multi-plate reader (BMG LABTECH GmbH, SpectroStar Nano, Ortenberg, Germany). The resulting reduction in absorption at 734 nm was measured and the result was presented as IC_{50} (mg/mL).

2.3.6. Total Glucosinolate Content

The amount of total glucosinolate was calculated using the spectrophotometer in accordance with the procedure outlined by [11]. The microgreens were used to create a methanolic extract, which was created by homogenizing 0.1 g of defatted seed meal with 80% methanol in a 2 mL container. After being at ambient temperature for an entire night, this homogenate was centrifuged at 3000 rpm for 4 min. After centrifugation, the supernatant was collected and diluted with 80% methanol to make 2 mL. A total of 100 µL of the extract was combined with 100 mL of double-distilled water, 170 µL of concentrated HCL, and 3 mL of 2 mM sodium tetrachloropalladate (58.8 mg of sodium tetrachloropalladate). A spectrophotometer measured absorbance at 425 nm after 1 h of incubation at room temperature. Total glucosinolates was calculated by putting the absorbance of each sample taken at 425 nm into the predicted formula $y = 1.40 + 118.86 \times A425$.

2.4. Statistical Analysis

All the experiments in this study were repeated thrice, and the data were subjected to a two-way ANOVA analysis. Two factors represent the microgreens' growing media and seed density (radish, cabbage, and rocket). The data were analyzed using Statistica version 10 (Statsoft, Inc., Tulsa, FL, USA 2011), and Fisher's protected least significant differences (LSD) test was carried out at a significance level of ($p < 0.05$) to determine the mean differences. If interactions were significant, they were used to explain the results. If interactions were insignificant, the means of main effects were separated using Fisher's protected *t*-test, with the least significant difference [31].

3. Results

3.1. Plant Morphology and Leaf Chlorophyll Content

The results showed that the plant height, stem diameter, and leaf chlorophyll content of the three Brassica microgreens were significantly affected by the interaction of growing media and seed density at $p \leq 0.001$ (Table 2). Promix produced the tallest radish, cabbage, rocket, and cabbage microgreens with a seed density of 12 seeds per cavity. In general, an increase in the seed density increased plant height, irrespective of the growing media (Table 2). This could be explained by closely spaced microgreens competing for sunlight due to the high seed-density planting [32]. Although the plant height increased with an increase in the seed density, the opposite was noticed in the stem diameter, which decreased with an increase in seed-density rate (Table 2). Radish and cabbage grown in Promix and rocket grown in TS1 at 4 seed density had significantly thicker stems. This result agrees with the finding of [33] that lettuce grown in low plant densities had a thicker stem.

Table 2. Interaction effect of growth media and seed density on plant height, leaf chlorophyll content, and stem diameter of Brassica microgreens.

Treatments	Plant Height (mm)			Leaf Chlorophyll (spad)			Stem Diameter (mm)		
	Radish	**Cabbage**	**Rocket**	**Radish**	**Cabbage**	**Rocket**	**Radish**	**Cabbage**	**Rocket**
TS1 × 4D	37.8 ± 5.2 ^{c}	38.65 ± 3.87 ^{d}	22.29 ± 4.28 ^{e}	34.56 ± 2.24 ^{a}	41.56 ± 7.02 ^{a}	34.56 ± 2.23 ^{a}	1.08 ± 0.09 ^{b}	1.13 ± 0.13 ^{b}	1.18 ± 01.4 ^{a}
TS1 × 8D	37.3 ± 4.8 ^{c}	40.44 ± 3.77 ^{cd}	37.80 ± 5.12 ^{c}	31.86 ± 3.05 ^{bc}	38.98 ± 7.85 ^{a}	29.52 ± 5.17 ^{bcd}	1.07 ± 0.10 ^{b}	1.09 ± 0.10 ^{d}	1.01 ± 0.27 ^{bc}
TS1 × 12D	38.0 ± 3.4 ^{bc}	48.15 ± 5.13 ^{a}	39.93 ± 7.08 ^{b}	32.02 ± 3.22 ^{b}	38.44 ± 4.92 ^{ab}	29.91 ± 3.65 ^{bcd}	0.98 ± 0.05 ^{e}	1.10 ± 0.10 ^{c}	1.08 ± 0.09 ^{b}
H × 4D	22.3 ± 4.3 ^{d}	26.48 ± 4.56 ^{f}	24.02 ± 4.51 ^{d}	29.52 ± 5.17 ^{cde}	29.38 ± 8.38 ^{d}	31.86 ± 3.05 ^{b}	1.01 ± 0.27 ^{c}	0.89 ± 0.13 ^{f}	1.07 ± 0.10 ^{bc}
H × 8D	24.0 ± 4.5 ^{d}	27.48 ± 3.32 ^{f}	38.02 ± 3.36 ^{c}	29.02 ± 5.97 ^{cde}	34.50 ± 7.51 ^{c}	29.02 ± 5.97 ^{cd}	0.84 ± 0.90 ^{f}	0.97 ± 0.08 ^{d}	0.84 ± 0.09 ^{d}
H × 12D	24.7 ± 5.1 ^{d}	32.99 ± 1.74 ^{e}	40.38 ± 7.08 ^{b}	31.04 ± 5.38 ^{bcd}	33.69 ± 6.35 ^{c}	28.92 ± 3.44 ^{cd}	0.80 ± 0.12 ^{g}	0.93 ± 0.10 ^{e}	1.00 ± 0.10 ^{bc}
P × 4D	39.9 ± 7.1 ^{bc}	41.93 ± 7.24 ^{bc}	24.67 ± 5.09 ^{d}	29.91 ± 3.65 ^{bcde}	39.03 ± 4.58 ^{ab}	32.02 ± 3.22 ^{ab}	1.18 ± 0.14 ^{a}	1.31 ± 0.14 ^{a}	1.02 ± 0.14 ^{b}
P × 8D	40.4 ± 7.1 ^{b}	43.52 ± 7.33 ^{b}	37.34 ± 4.75 ^{c}	28.92 ± 3.44 ^{de}	36.40 ± 5.53 ^{bc}	31.04 ± 5.38 ^{bc}	1.00 ± 0.10 ^{d}	1.10 ± 0.13 ^{c}	0.80 ± 0.12 ^{d}
P × 12D	47.7 ± 6.2 ^{a}	47.97 ± 4.77 ^{a}	47.72 ± 6.24 ^{a}	27.65 ± 2.78 ^{e}	33.60 ± 7.67 ^{c}	27.65 ± 2.78 ^{d}	1.02 ± 0.14 ^{c}	1.08 ± 0.17 ^{c}	0.98 ± 0.05 ^{c}
LSD 0.05	2.41 ***	3.05 ***	1.52 ***	2.46 ***	3.0 ***	2.61 ***	0.01 ***	0.02 ***	0.09 ***

Values in a column followed by the same letter are not significantly different ($p > 0.05$), using Fishers' protected *t*-test. LSD: least significant difference; *** significant at 0.1%, respectively. H = Hygromix, P = Promix; 4D, 8D, and 12D are for seed density at 4, 8, and 12 seeds per cavity, respectively.

Microgreens of Brassica showed discrepancies in leaf chlorophyll responses to growing media and seed density. All three Brassica microgreens had high leaf chlorophyll content at a seeding density of 4 seeds per cavity in TS1. Similarly, cabbage microgreens seeded with 8 and 12 seeds per cavity exhibited comparable chlorophyll content (highest) to those seeded with 4 seeds per cavity in TS1 medium. The chlorophyll content of cabbage microgreens seeded in Promix medium with 4 seed densities per cavity was similar to that of those seeded in TS1 medium with 4, 8, and 12 seed densities. Similarly, rocket microgreens seeded in TS1 and Promix media with 4 seeds displayed comparable levels of chlorophyll content. High leaf chlorophyll content, at low seed density of 4 and 8 per cavity, could be due to less competition for light and mineral nutrients between plants than high-density seeding. The tendency of increased leaf chlorophyll in TS1 followed by Hygromix may be explained by higher N content of the media (Table 3). Generally, the leaf chlorophyll content of microgreens across the growing media tended to decrease with an increase in the seed density, except for cabbage grown in Hygromix, which performed high at seed densities 8 and 12 (Table 2). The chlorophyll pigment is crucial for plants to photosynthesize and influences growth and yield [34]. Chlorophyll synthesis requires elements such as N and P from the growing medium; thus, growth media should influence chlorophyll in plant leaves [34]. The increased tendency of leaf chlorophyll content in TS1 and Hygromix could be explained by high N and P in the growing media compared to Promix. Leaf chlorophyll content is closely linked to leaf N in crops and is an indication of N availability in growth media [35–37]. The TS1 medium showed higher nutrient composition (such as Ca; Mg; and N) followed by the Hygromix medium, with relatively low chlorine (Table 2).

Table 3. Interaction effect of growth media and seed density on total chlorophyll content, fresh and dry mass of radish, cabbage, and rocket microgreens.

Treatment	Fresh Plant Mass (g/m^2)			Dry Plant Mass (g/m^2)		
	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket
TS1 × 4D	500.43 ± 43.23 ^{e}	387.76 ± 22.52 ^{f}	313.14 ± 11.04 ^{e}	119.98 ± 5.06 ^{e}	86.33 ± 5.06 ^{e}	74.62 ± 7.60 ^{d}
TS1 × 8D	1006.7 ± 14.11 ^{c}	532.63 ± 15.41 ^{c}	443.37 ± 50.62 ^{c}	159.49 ± 5.06 ^{c}	109.7 ± 8.77 ^{b}	106.8 ± 6.70 ^{b}
TS1 × 12D	1246.7 ± 83.75 ^{b}	752.12 ± 93.87 ^{b}	462.39 ± 38.35 ^{b}	184.37 ± 4.38 ^{b}	122.9 ± 4.38 ^{a}	109.7 ± 8.77 ^{b}
H × 4D	338.01 ± 8.779 ^{f}	165.34 ± 11.04 ^{h}	114.13 ± 4.389 ^{i}	102.42 ± 6.70 ^{f}	64.38 ± 2.53 ^{g}	59.99 ± 2.53 ^{f}
H × 8D	700.90 ± 128.9 ^{d}	269.24 ± 58.45 ^{g}	146.32 ± 32.94 ^{h}	147.79 ± 14.11 ^{d}	80.48 ± 12.6 ^{f}	67.31 ± 10.13 ^{e}
H × 12D	1062.3 ± 178.8 ^{c}	449.22 ± 88.37 ^{e}	181.44 ± 20.74 ^{g}	181.44 ± 9.13 ^{b}	106.8 ± 9.13 ^{c}	74.62 ± 4.38 ^{d}
P × 4D	579.45 ± 68.99 ^{e}	383.37 ± 142.74 ^{f}	286.80 ± 164.3 ^{f}	121.45 ± 6.70 ^{d}	84.86 ± 9.13 ^{e}	73.16 ± 12.67 ^{d}
P × 8D	955.51 ± 38.35 ^{c}	503.36 ± 128.45 ^{d}	378.98 ± 136.1 ^{d}	158.03 ± 4.38 ^{c}	102.4 ± 11.0 ^{d}	92.18 ± 15.20 ^{c}
P × 12D	1805.6 ± 45.69 ^{a}	803.33 ± 148.02 ^{a}	687.73 ± 105.3 ^{a}	200.46 ± 11.04 ^{a}	124.3 ± 11.0 ^{a}	133.1 ± 9.13 ^{a}
LSD 0.05	5.91 ***	4.72 ***	2.19 ***	5.51 ***	2.9 ***	2.93 ***

Values in a column followed by the same letter are not significantly different ($p > 0.05$), using Fishers' protected *t*-test. LSD: least significant difference; *** significant at 1%, 0.1%, respectively. H = Hygromix, P = Promix; 4D, 8D, and 12D are for seed density at 4, 8, and 12 seeds per cavity, respectively.

3.2. Fresh and Dry Plant Mass

The results show that increasing the seed density leads to significantly increased fresh and dry mass of microgreens (Table 3). Further, regardless of substrate, radish microgreens produced much higher fresh yields than cabbage and rocket microgreens. It has been noted that lower yield at low seeding density is due to the low number of microgreens per unit of area. The costs of seeds should be considered at high-density planting of microgreens to optimize yield. High seed density of microgreens was reported to increase fungal disease incidences, reducing quality of microgreens [37,38]. However, no fungal diseases were observed in this study at high seed-density planting. Regardless of microgreens variety, Promix had the highest fresh and dry mass, followed by TS1 and Hygromix growth media. The production trends increase with increasing plant population per unit area [39]. Biomass yield tends to be low on TS1 followed by Hygromix as compared to Promix. This may be due to the low water-holding capacity in TS1 (55.9%) and Hygromix (47%) compared to the high water-holding capacity of Promix (74.6%) (Table 3). Soilless media composition

considerably affects plant growth, development, shoot nutrients, quality, and yield [40]. The ideal growth medium should have fundamental properties that will improve nutrient availability and plant growth and provide an anchor for the plants. These properties include high substrate stability, sufficient air–water balance around the root system, and suitable pH and cation exchange capacity [41]. In this study, the Promix medium composition has been shown to have ideal fundamental physical and chemical properties to promote a higher yield of Brassica microgreens, followed by TS1 and Hygromix media. The Promix medium had the highest water-holding capacity of 74.6%, less dry matter of 40.65%, and a pH of 6.84 (Table 1). This agrees with the findings of [42] that fresh and dry leaves of sprouts obtained were related to the physical and chemical characteristics of the coir-dust medium, and its higher water-holding capacity and cation exchange capacity [43]. Another previous study has shown that a pH between 5.5 and 7.0 is generally considered neutral, and most vegetables prefer a soilless medium containing neutral to moderately acidic [42]. The pH in the Promix medium could be another factor that improved the high fresh and dry mass of Brassica microgreens since the pH medium had shown the highest pH level of 6.84, which was between the recommended ranges. However, further studies need to be conducted to evaluate the effect of soilless pH on microgreen's performance.

3.3. Mineral Content

Leaf N content of radish improved significantly in TS1 across the seed density treatments and in Hygromix at 4 seed density compared to other treatments (Table 4). TS1 in all the seed densities (4, 8, and 12) and Hygromix at 4 and 8 seed density and Promix at 4 seed density had high leaf N content in cabbage. Cabbage microgreens showed a decreased tendency of leaf N content with an increase in the seed density. Rocket microgreens were high in leaf N content on TS1 at seed densities of 4 and 8, and Promix at the lowest seed density of 4. The accumulation of N content in all Brassica microgreens may be due to the higher N content of TS1 followed by Hygromix compared to Promix (Table 1). High N in media could increase leaf growth and chlorophyll content while its decrease may also be detrimental to productivity [42]. Calcium leaf content of radish was significantly high on TS1 across all the seed density treatments, followed by Hygromix at 4 seed density per cavity compared to other treatments (Table 4). The calcium content of cabbage microgreens was significantly high on TS1 at a seed density of 4, although it did not differ significantly with TS1 at a density of 8 and 12 and Hygromix at a seed density of 4. Microgreens grown in TS1 generally had a high Ca content, followed by Hygromix, which could explain why they outperformed Promix (Table 1). There was a decrease in N and Ca content with an increase in seeding density, which may be explained by the high number of microgreens per cavity resulting in competition for nutrition uptake. Leaf Mg content in radish and cabbage was significantly high when grown in Hygromix at a low seed density of 4. Growth medium TS1 across all the seed density treatments resulted in low cabbage and rocket Mg content compared to other treatment combinations. High leaf Mg content in Promix was only found at a seed density of 4. The Mg content of Brassica microgreens was generally high in Hygromix due to its high Mg content of growth medium (Table 1). Promix generally improved the leaf K content of radish, cabbage, and rocket microgreens compared to TS1 and Hygromix. The K content of radish microgreen was significantly high at a seed density of 12, followed by Promix at a seed density of 4 and 8. Cabbage and rocket microgreens had significantly high K content when grown in Promix at a seed density 4. Leaf K content of rocket grown in Promix at a seed density of 4 did not differ significantly from Promix at a seed density of 12 and TS1 at a seed density of 4 (Table 2). This could be linked to the high levels of K present in both Promix and TS1 growing media (Table 1). The phosphorus content of radish microgreen was high in Promix at a seed density of 12. The phosphorus content of cabbage microgreens was significantly high across growth media and seed densities, except for TS1 at a seed density of 8, which performed the lowest (Table 4). The phosphorus content of Brassica microgreens was improved in the low P content medium of Promix compared to TS1 and Hygromix. Plants deficient in phosphorus are stunted

in growth and often have an abnormal dark green color [44]. Rocket microgreens had significantly high P content on Hygromix at a seed density of 4, although it did not differ from other treatment combinations except for Hygromix at 12 seed density. Furthermore, higher K, Ca, and Mg in media are other factors that can be found and equally be the cause of the accumulation of nutrients in the plant and better stimulate plant growth and development [45].

3.4. Leaf Color

Among the different treatments, the highest L^* value expresses the brightness of the leaves, while the lowest value indicates lower brightness on the color wheel, and the h° value is the indication of a color change, especially the change from green to yellow [46]. Hygromix at the seed density of 4 significantly increased the brightness (L^*) of radish leaves, even though it did not differ significantly from the TS1 × 12D (Table 5). However, there was a decreased tendency of L^* with an increase in the seed density at Hygromix and Promix. On cabbage microgreens, TS1 at a low seed density of 4 seeds per cavity (4D) had significantly high L^* values, which did not differ significantly with TS1 × 12D, H × 4D and H × 12D treatments. Promix at seed densities of 8 and 12 (P × 8D and P × 12D) performed poorly compared to other treatments in improving the brightness of cabbage microgreen leaves. Rocket microgreen had significantly higher L^* values at H × 12D treatment than the other growing media and seed density combinations. Generally, Promix performed poorly in improving the lightness of Brassica microgreens across the seed densities. Higher saturation of microgreen color is indicated by significantly higher chroma (C) values [47]. Radish grown in TS1 at a seed density of 12 (TS1 × 12D) had a high C value, although not significantly different from H × 4D compared to other treatment combinations (Table 5). Cabbage microgreens had high C value in all the growth media (Promix, Hygromix and TS1) at the seed density of 12 seeds per cavity, including TS1 × 8D. By contrast, rocket microgreens grown in Promix at a seed density of 12 (P × 12D) had a high C value and performed similarly with H × 12D compared to other treatment combinations. The h° value was related to the yellow color. Radish microgreens grown in TS1 × 4D, TS1 × 8D, and TS1 × 12D, cabbage microgreens from TS1 × 4D, and P × 4D, and rocket microgreens from TS1 × 4D and TS1 × 12D showed high value of h° (Table 5). The previous study found that N content is linked with chlorophyll content, the deficiency of N may lead to the loss of green color in leaves, and at the same time high N content may affect the accumulation of N content in leaves [48]. However, the high h° value on TS1 growth medium, which had relatively high leaf N and chlorophyll, could not be explained in this study.

Table 4. Interaction effect of growth media and seed density on mineral compositions of radish, cabbage, and rocket microgreens.

Treatment	N (%)			Ca (%)			Mg (%)			K (%)			P (%)		
	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket
TS1× 4D	2.03 ± 0.10 ab	2.61 ± 0.17 a	2.51 ± 0.13 a	2.45 ± 0.26 c	3.67 ± 0.16 bc	3.56 ± 0.37 ab	0.72 ± 0.04 abc	0.67 ± 0.01 a	0.88 ± 0.12 ab	1.31 ± 0.20 a	2.32 ± 0.21 a	1.52 ± 0.22 a	0.28 ± 0.00 g	0.33 ± 0.01 g	0.23 ± 0.01 c
TS1 × 8D	2.19 ± 0.02 a	2.17 ± 0.14 ab	1.88 ± 0.00 ab	1.84 ± 0.04 d	2.32 ± 0.12 d	2.66 ± 0.06 cd	0.69 ± 0.02 bcd	0.59 ± 0.01 b	0.70 ± 0.02 ab	1.45 ± 0.05 a	2.10 ± 0.03 ab	1.34 ± 0.10 b	0.33 ± 0.01 f	0.36 ± 0.00 fg	0.23 ± 0.00 c
TS1×12D	2.30 ± 0.04 a	2.09 ± 0.00 ab	1.87 ± 0.12 b	1.48 ± 0.04 e	1.74 ± 0.21 e	2.16 ± 0.13 d	0.65 ± 0.03 cd	0.64 ± 0.00 a	0.72 ± 0.08 ab	1.51 ± 0.00 a	1.94 ± 0.00 abc	1.23 ± 0.03 bc	0.36 ± 0.00 e	0.37 ± 0.00 f	0.24 ± 0.01 c
H × 4D	1.99 ± 0.64 abc	2.45 ± 0.09 a	1.51 ± 0.14 c	3.28 ± 0.89 b	3.80 ± 0.02 bc	3.04 ± 0.41 bc	0.76 ± 0.44 ab	0.76 ± 0.05 a	0.91 ± 0.33 a	1.02 ± 0.36 b	1.92 ± 0.09 abc	1.13 ± 0.20 c	0.50 ± 0.10 a	0.84 ± 0.07 a	0.58 ± 0.08 ab
H × 8D	1.09 ± 0.07 c	2.07 ± 0.47 ab	1.23 ± 0.19 c	2.49 ± 0.04 c	3.51 ± 1.00 d	2.56 ± 0.53 cd	0.25 ± 0.00 e	0.75 ± 0.28 a	0.62 ± 0.32 ab	0.63 ± 0.02 c	1.73 ± 0.35 bc	0.95 ± 0.21 d	0.42 ± 0.00 c	0.77 ± 0.04 b	0.50 ± 0.10 ab
H × 12D	1.51 ± 0.15 cd	1.72 ± 0.31 b	1.46 ± 0.09 c	2.66 ± 0.15 c	3.30 ± 0.75 c	2.82 ± 0.38 c	0.29 ± 0.02 e	0.65 ± 0.23 a	0.53 ± 0.37 b	0.67 ± 0.02 c	1.52 ± 0.30 cd	0.87 ± 0.29 d	0.46 ± 0.02 b	0.69 ± 0.02 d	0.51 ± 0.09 ab
P × 4D	1.35 ± 0.06 d	1.96 ± 0.40 ab	2.28 ± 0.71 ab	3.20 ± 0.27 b	4.40 ± 0.61 a	4.14 ± 0.69 a	0.62 ± 0.00 d	0.67 ± 0.00 a	0.70 ± 0.01 ab	0.55 ± 0.07 c	1.11 ± 0.08 d	0.87 ± 0.02 d	0.39 ± 0.06 d	0.73 ± 1.06 c	0.64 ± 0.01 a
P × 8D	1.29 ± 0.02 d	1.64 ± 0.00 b	1.54 ± 0.12 c	3.32 ± 0.16 b	3.67 ± 0.51 bc	3.54 ± 0.14 ab	0.72 ± 0.04 abc	0.76 ± 0.02 a	0.74 ± 0.02 ab	0.60 ± 0.05 c	1.12 ± 0.00 d	0.73 ± 0.16 e	0.38 ± 0.02 de	0.67 ± 0.07 d	0.51 ± 0.14 ab
P × 12D	1.61 ± 0.36 bc	1.54 ± 0.05 b	1.44 ± 0.11 c	3.90 ± 1.00 a	3.95 ± 0.35 b	3.43 ± 0.34 b	0.80 ± 0.10 a	0.86 ± 0.02 a	0.75 ± 0.02 ab	0.74 ± 0.03 c	1.13 ± 0.05 d	0.69 ± 0.04 e	0.47 ± 0.03 b	0.64 ± 0.04 e	0.43 ± 0.02 bc
LSD 0.05	0.5 **	0.72 **	0.63 **	0.22 **	0.3 ***	0.61 ***	0.09 **	0.27 *	0.35 *	0.2 **	0.48 **	0.14 ***	0.02 ***	0.03 ***	0.2 *

Values in a column followed by the same letter are not significantly different ($p > 0.05$), using Fishers' protected *t*-test. LSD: least significant difference; *, **, *** significant at 5%, 1%, or 0.1%, respectively. H = Hygromix, P = Promix; 4D, 8D, and 12D are for seed density at 4, 8, and 12 seeds.

Table 5. Interaction effect of growth media and seed density on leaf color of radish, cabbage, and rocket microgreens.

Treatment	*L**			*C*			*h*°		
	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket
TS1 × 4D	25.78 ± 0.01 cd	26.9 ± 4.27 a	15.85 ± 3.88 d	24.55 ± 1.30 e	19.26 ± 0.47 b	18.17 ± 2.67 c	120.60 ± 0.45 a	123.64 ± 1.52 a	121.37 ± 1.09 a
TS1 × 8D	23.90 ± 3.89 bc	21.94 ± 3.65 bc	21.34 ± 2.80 bc	25.77 ± 2.69 de	21.77 ± 1.55 a	24.87 ± 3.67 bc	120.54 ± 0.73 a	120.37 ± 0.30 bc	118.39 ± 0.58 b
TS1 × 12D	30.31 ± 8.56 ab	24.78 ± 3.77 ab	21.54 ± 1.42 bc	31.06 ± 4.25 a	22.03 ± 0.75 a	24.77 ± 1.67 bc	118.55 ± 0.26 ab	121.18 ± 1.26 abc	119.42 ± 2.12 ab
H × 4D	30.9 ± 6.91 a	23.95 ± 1.62 ab	22.37 ± 3.77 bc	30.71 ± 4.81 ab	20.87 ± 1.17 b	22.17 ± 2.67 bc	116.28 ± 1.46 b	118.77 ± 1.80 bc	114.27 ± 1.73 e
H × 8D	26.21 ± 1.77 bc	18.99 ± 4.09 cd	24.05 ± 1.42 b	28.37 ± 2.47 bc	20.32 ± 1.12 b	23.47 ± 1.67 bc	115.85 ± 0.26 b	118.46 ± 1.35 c	114.98 ± 0.69 de
H × 12D	21.86 ± 1.41 c	24.62 ± 3.24 ab	31.7 ± 7.35 a	25.35 ± 1.78 de	21.78 ± 1.18 a	29.07 ± 4.67 ab	117.33 ± 1.28 b	118.96 ± 0.95 bc	115.09 ± 0.59 cde
P × 4D	24.18 ± 1.40 cd	20.32 ± 4.23 c	16.75 ± 2.13 d	27.69 ± 1.68 cd	19.85 ± 1.15 b	22.47 ± 2.67 bc	116.66 ± 1.46 b	122.01 ± 2.12 ab	115.94 ± 2.03 cde
P × 8D	24.02 ± 7.16 cd	15.53 ± 4.70 e	20.39 ± 6.39 c	26.74 ± 4.52 cde	18.99 ± 1.19 b	25.07 ± 6.67 bc	117.57 ± 1.07 b	119.56 ± 1.59 bc	117.35 ± 3.39 bcd
P × 12D	21.63 ± 1.84 d	16.14 ± 3.19 de	16.02 ± 4.27 d	25.44 ± 0.40 de	21.65 ± 1.15 a	35.57 ± 22.67 a	117.19 ± 0.89 b	119.66 ± 0.56 bc	117.52 ± 2.68 bc
LSD 0.05	4.21 **	3.44 **	3.05 ***	2.48 **	1.51 *	8.52 *	2.9 *	3.11 *	2.52 **

Values in a column followed by the same letter are not significantly different ($p > 0.05$), using Fishers' protected *t*-test. LSD: least significant difference; *, **, *** significant at 5%, 1%, or 0.1%, respectively. H = Hygromix, P = Promix; 4D, 8D, and 12D are for seed density at 4, 8, and 12 seeds per cavity, respectively.

3.5. Antioxidant and Antioxidant Activities in Microgreens

3.5.1. Ascorbic Acid Content

Ascorbic acid is an essential bioactive phytochemical, commonly referred to as vitamin C, and is a crucial nutrient for human body functioning [4]. Within the category of antioxidants, ascorbic acid serves as a protective mechanism for plants under stressful conditions. Additionally, ascorbic acid plays a crucial role in regulating redox homeostasis, cell division, cellular expansion, and cell wall growth [47]. Hygromix with 4 seed density (H × 4D) treatment showed a significantly high level of ascorbic acid compared to other treatments in all Brassica microgreens, radish (6.51 mg/100 g FW), rocket (4.38 mg/100 g FW), and cabbage (5.20 mg/100 g FW) (Table 6). Various findings have been exploring the influence of different factors on the ascorbic acid accumulation in leafy vegetables grown in a soilless medium i.e., environmental factors, different growing media, light, and other related possible factors [23]. It is revealed that the lower pH in the growing medium leads to higher ascorbic acid content [45]. The ascorbic acid has been identified as a notable antioxidant that could protect plants from oxidative stress. This protection is achieved through the breakdown of reactive oxygen species formed due to regular oxygen metabolism and abiotic stress [49]. There was a noticeable trend of ascorbic acid accumulation when decreasing the seed density in the different growing media (Table 4). Another study revealed that increasing the seed density created shaded leaves, causing a reduction in ascorbic acid. An increase in plant density was reported to decrease the ascorbic acid concentration [50], and a similar response occurred in this study. The high ascorbic acid concentration was observed in microgreens grown with the lowest seed density.

3.5.2. Total Phenols Content (TPC)

Total phenol content was significantly higher in radish, cabbage, and rocket microgreens grown under TS1 × 12D, TS1 × 4D, and TS1 × 12D, respectively (Table 6). This research study showed different effects on TPC in different microgreen leaves. The previous study indicated that plant density did not significantly affect the phenolic compound content [51]. The TS1 substrate provided a higher moisture and water retention capacity than any other substrate, which would have induced physiological stress to roots and resulted in a higher accumulation of total phenols [52]. Radish and Rocket microgreens planted in TS1 with 12 seeds accumulated the most TPC, while cabbage microgreens planted with 4 seeds had the highest TPC content. Lombardo et al. [53] found that total phenolic content increases with plant density. Inducing phenylpropanoid biosynthesis may have occurred in response to shading as plant density increased, accounting for the highest TPC value. Nevertheless, Riad et al. [54] found that increasing planting density did not significantly affect cabbage's phenolic content.

3.5.3. Total Glucosinolate Content

The results demonstrated that biosynthesis of glucosinolate content in cabbage microgreens was significantly higher in Hygromix at different seed densities. Radish microgreens had a significantly higher content of glucosinolates when grown in H × 4D, and cabbage microgreens showed higher glucosinolates when grown in H × 12D. Rocket microgreens had significantly higher glucosinolates when grown in H × 4D and H × 8D (Table 6). The variation of total glucosinolate content in plants is widely recognized and influenced by diverse factors such as environmental conditions, climate, soil nutrient availability, plant location and variety, plant density, and the developmental stage [55]. Reduced moisture content in Hygromix could have induced the glucosinolate concentration in all three microgreens. Glucosinolate levels are highest under conditions of water stress.

Table 6. Interaction effect of growth media and seed density on ascorbic acid, total phenols, and total glucosinolate of radish, cabbage, and rocket microgreens.

Treatment	Ascorbic Acid (mg/100 g FW)			Total Phenols (mg GAE/100 g FW)			Total Glucosinolate (µmol/g)		
	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket
TS1 × 4D	3.20 ± 0.58 ^{b}	4.41 ± 0.32 ^{ab}	2.68 ± 0.40 ^{cde}	553.23 ± 24.93 ^{b}	500.83 ± 53.54 ^{a}	332.94 ± 19.50 ^{d}	38.32 ± 0.06 ^{b}	25.95 ± 0.62 ^{bc}	24.81 ± 0.97 ^{d}
TS1 × 8D	2.98 ± 0.89 ^{b}	4.08 ± 0.13 ^{c}	2.63 ± 0.89 ^{de}	546.50 ± 33.67 ^{b}	390.60 ± 26.20 ^{c}	384.46 ± 86.76 ^{b}	39.51 ± 0.13 ^{ab}	25.96 ± 1.72 ^{bc}	23.23 ± 0.44 ^{e}
TS1 × 12D	2.97 ± 0.87 ^{b}	3.62 ± 049 ^{cd}	2.53 ± 0.48 ^{e}	590.07 ± 143.94 ^{a}	341.88 ± 65.28 ^{e}	461.94 ± 75.12 ^{a}	39.27 ± 0.65 ^{b}	25.21 ± 1.16 ^{cd}	27.82 ± 0.65 ^{b}
H × 4D	6.51 ± 2.76 ^{a}	5.20 ± 0.78 ^{a}	4.38 ± 0.56 ^{a}	375.92 ± 54.73 ^{d}	350.71 ± 45.73 ^{de}	312.88 ± 23.54 ^{e}	45.61 ± 0.51 ^{a}	28.10 ± 0.65 ^{abc}	39.83 ± 0.96 ^{a}
H × 8D	2.97 ± 0.47 ^{b}	4.20 ± 0.30 ^{b}	3.28 ± 0.51 ^{bc}	314.11 ± 38.10 ^{g}	358.43 ± 63.35 ^{d}	335.10 ± 35.56 ^{d}	35.03 ± 2.67 ^{b}	28.93 ± 1.58 ^{ab}	39.51 ± 0.75 ^{a}
H × 12D	2.60 ± 1.00 ^{b}	3.46 ± 0.50 ^{cd}	3.17 ± 0.30 ^{bcd}	500.89 ± 25.34 ^{c}	338.78 ± 46.42 ^{f}	316.09 ± 72.22 ^{e}	40.26 ± 0.23 ^{ab}	30.24 ± 1.48 ^{a}	22.87 ± 1.66 ^{e}
P × 4D	2.63 ± 0.64 ^{b}	2.87 ± 1.20 ^{de}	3.77 ± 0.10 ^{b}	343.00 ± 35.56 ^{e}	422.94 ± 57.55 ^{b}	353.76 ± 23.46 ^{c}	35.98 ± 0.31 ^{b}	22.04 ± 0.24 ^{d}	24.57 ± 1.32 ^{d}
P × 8D	2.32 ± 0.46 ^{b}	2.46 ± 1.07 ^{e}	2.98 ± 0.08 ^{cde}	334.92 ± 31.05 ^{f}	317.79 ± 33.00 ^{g}	389.37 ± 72.12 ^{b}	36.22 ± 0.31 ^{b}	26.63 ± 0.44 ^{bc}	26.32 ± 0.24 ^{c}
P × 12D	2.28 ± 0.20 ^{b}	2.07 ± 0.44 ^{e}	2.41 ± 0.50 ^{e}	314.63 ± 39.28 ^{g}	336.21 ± 28.00 ^{f}	333.99 ± 32.46 ^{d}	35.31 ± 0.56 ^{b}	25.72 ± 3.12 ^{bc}	24.02 ± 0.53 ^{de}
LSD 0.05	2.9 *	0.9 ***	0.6 **	6.75 ***	8.92 ***	5.61 ***	6.2 *	3.42 **	1.48 ***

Values in a column followed by the same letter are not significantly different ($p > 0.05$), using Fishers' protected *t*-test. LSD: least significant difference; *, **, *** significant at 5%, 1%, or 0.1%, respectively. H = Hygromix, P = Promix; 4D, 8D, and 12D are for seed density at 4, 8, and 12 seeds per cavity, respectively.

3.5.4. Ferric Reducing Ability of Plasma (FRAP)

Radish, cabbage, and rocket microgreens responded differently to treatments on the FRAP level (Table 7). Radish had a high FRAP level at TS1 × 4D, although not significantly different to H × 8D (Table 7). TS1 irrespective of the seed density had an increased tendency of FRAP compared to other growth media. Cabbage microgreens also showed a significantly high amount of FRAP when grown in H × 8D and H × 4D while rocket was high at H × 8D. Results have indicated that different Brassica microgreens grown in different treatments decreased the FRAP value, specifically when increasing the seed density. This led to shade creation due to the many leaves (Table 7). However, for microgreens grown under P × 4D (less density), the results showed a lower amount of FRAP, which could be related to chemical or physical properties in the growing medium. Electrical conductivity (EC) measures the salts (in the salinity of the medium) of the growing medium. In the present study, TS1 medium had the highest EC, followed by Hygromix with moderate EC and Promix medium with the lowest EC (Table 1). The findings agree with a research study in which a FRAP was significant in soilless media [56]. The highest FRAP value was evident in growth media containing moderate salt concentration [57]. Moreover, lower salt concentrations exhibited significantly lower FRAP values compared to those with moderate salinity. The levels of FRAP found in leafy samples are impacted by various horticultural practices such as the type of substrate used, the growing conditions, the species and cultivar of the plant, the stage of maturity, and the techniques used for extraction. All these factors have the potential to alter the FRAP values [58].

3.5.5. DPPH 2.2-Diphenyl-1-picrylhyrazyl Radial Scavenging Activity

The results showed that the DPPH activity of all three Brassica microgreens differed depending on the treatment (Table 7). The DPPH activity was significantly higher in radish, cabbage, and rocket microgreens grown in H × 8D, H × 12D, and H × 12D, respectively (Table 7). Notably, the highest DPPH activities were observed in extracts from radish, cabbage, and rocket microgreens grown in Hygromix growing medium. Similar to the FRAP, salinity and soilless media were noticeably impacted the DPPH radical-scavenging activity. The moderate salt concentration in the soilless media treatment yielded the greatest radical activity, while the highest concentrations resulted in the lowest. These findings agree with [59], who reported that moderate salinity concentration had the highest radical-scavenging activity. It has been suggested that high salt levels may lead to cell rupturing and hinder the absorption of nutrients and water [60]. Additionally, the response of plants to salinity in soilless media varies, as some demonstrate a notable increase in radical-scavenging activity while others display a significant decrease in biological activity [61]. Therefore, the Hygromix medium for commercial production of culinary aromatic microgreens and considering different seed densities for different microgreens is recommended for improved DPPH value.

3.5.6. ABTS-Based Scavenging Activity

The results demonstrated that radish and rocket microgreens grown in TS1 × 12D and TS1 × 4D showed significantly higher ABTS values. Furthermore, significantly higher ABTS values were obtained in cabbage microgreens grown in P × 4D (Table 7). The results of the scavenging activity assays suggest that various treatments used to cultivate radish, cabbage, and rocket microgreens can yield extracts that exhibit free radical-scavenging properties. Additionally, previous results indicate that the plant extracts from various treatments possess compounds that can donate hydrogen to free radicals, reducing these radicals or oxygen species to their inactive states. This process can potentially inhibit cellular damage caused by free radicals in individuals who consume these vegetable crops as a regular part of their diet [61].

Table 7. Interaction effect of growth media and seed density on antioxidants of radish, cabbage, and rocket microgreens.

Treatment	ABTS IC_{50} (µg/mL)			DPPH IC_{50} (µg/mL)			FRAP mMTEAC/g		
	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket	Radish	Cabbage	Rocket
TS1 × 4D	450.03 ± 11.08 ^{e}	496.62 ± 5.60 ^{c}	1275.32 ± 25.37 ^{a}	586.49 ± 33.55 ^{f}	479.06 ± 10.09 ^{g}	1018.75 ± 11.31 ^{c}	6.9 ± 0.2 ^{a}	6.0 ± 0.8 ^{b}	5.8 ± 2.3 ^{b}
TS1 × 8D	457.93 ± 6.229 ^{d}	466.34 ± 4.78 ^{d}	1107.94 ± 81.77 ^{b}	661.48 ± 5.869 ^{e}	667.68 ± 6.521 ^{b}	807.45 ± 11.61 ^{g}	6.6 ± 0.1 ^{b}	5.5 ± 0.4 ^{c}	3.2 ± 0.2 ^{d}
TS1 × 12D	578.95 ± 41.50 ^{a}	463.28 ± 14.1 ^{d}	989.33 ± 54.12 ^{c}	556.91 ± 4.982 ^{g}	525.46 ± 13.22 ^{e}	767.08 ± 18.96 ^{i}	5.6 ± 0.1 ^{c}	5.4 ± 0.1 ^{c}	2.4 ± 0.1 ^{e}
H × 4D	432.14 ± 4.090 ^{f}	435.03 ± 7.18 ^{e}	996.07 ± 20.36 ^{c}	671.67 ± 26.08 ^{d}	433.80 ± 5.508 ^{h}	1037.22 ± 30.22 ^{b}	6.7 ± 0.4 ^{ab}	7.0 ± 1.0 ^{a}	4.4 ± 0.4 ^{c}
H × 8D	466.16 ± 15.33 ^{c}	441.17 ± 9.74 ^{e}	960.20 ± 5.909 ^{d}	864.64 ± 21.42 ^{a}	568.18 ± 17.22 ^{c}	979.83 ± 10.55 ^{e}	4.0 ± 0.2 ^{e}	6.7 ± 0.6 ^{a}	6.8 ± 0.6 ^{a}
H × 12D	461.29 ± 14.14 ^{d}	466.03 ± 8.74 ^{d}	910.52 ± 18.17 ^{e}	800.03 ± 6.238 ^{b}	982.79 ± 31.55 ^{a}	1367.06 ± 12.88 ^{a}	3.1 ± 0.4 ^{f}	5.9 ± 1.7 ^{b}	3.4 ± 0.2 ^{d}
P × 4D	518.35 ± 5.329 ^{b}	523.95 ± 5.90 ^{a}	855.28 ± 5.148 ^{f}	530.87 ± 15.64 ^{h}	544.24 ± 29.05 ^{d}	981.36 ± 23.22 ^{d}	5.6 ± 0.2 ^{c}	5.8 ± 2.6 ^{b}	1.7 ± 0.0 ^{ef}
P × 8D	460.13 ± 25.54 ^{d}	388.35 ± 6.58 ^{f}	836.06 ± 21.09 ^{g}	741.72 ± 17.18 ^{c}	511.81 ± 12.48 ^{f}	784.04 ± 26.21 ^{h}	5.1 ± 0.2 ^{d}	5.4 ± 1.2 ^{c}	1.6 ± 0.2 ^{ef}
P × 12D	371.20 ± 12.94 ^{g}	506.30 ± 0.96 ^{b}	699.62 ± 10.84 ^{h}	561.92 ± 18.54 ^{g}	563.62 ± 2.886 ^{c}	871.09 ± 13.33 ^{f}	4.9 ± 0.2 ^{d}	5.4 ± 4.3 ^{c}	1.2 ± 0.1 ^{f}
LSD 0.05	4.5 ***	6.2 ***	7.5 ***	6.1 ***	5.5 ***	5.8 ***	0.2 ***	0.3 **	0.8 ***

Values in a column followed by the same letter are not significantly different ($p > 0.05$), using Fishers' protected *t*-test. LSD: least significant difference; **, *** significant at 1%, or 0.1%, respectively. H = Hygromix, P = Promix; 4D, 8D, and 12D are for seed density at 4, 8, and 12 seeds per cavity, respectively.

A significantly strong and positive correlation existed between the total phenolic contents and antioxidant activities (ABTS, FRAP and DPPH) of extracts in all tested assays. In ABTS (R = 0.73) and FRAP (R = 0.64), similarly, the total phenolic contents showed a high positive correlation to DPPH (R = 0.42). This observation suggests that the phenolic compounds present in the microgreens somewhat contributed to the antioxidant activities.

4. Conclusions

This study shows that seed densities and growth substrates have a considerable impact on the morphological, nutritional, and phytochemical characteristics of the three Brassica microgreens. However, color, fresh weight, and desirable health-related benefits, such as ascorbic acid and glucosinolates, ensure their value to consumers. Therefore, this study provides information on suitable substrates, seed densities, and nutritional and phytochemical contents for cabbage, radish, and rocket microgreens to benefit marketers and consumers.

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