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PERFORMANCE AND QUALITY OF HEMP MICROGREENS UNDER SUBSTRATE AND WATERING CONDITIONS

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Abstract

Microgreens are a sustainable and innovative food source, highly valued for their nutritional content, short cultivation time, and potential to address global food security challenges. The experiment was conducted in 2024 in a controlled environment growth chamber using monoecious hemp seeds. It evaluated the effects of three substrates (perlite, peat and vermicompost) and two types of watering (distilled water and water from a recirculating aquaculture system – RAS water) on the biometric traits, yield, and nutritional quality of hemp microgreens. The results showed that the Peat x RAS water variant produced the highest fresh matter yield (12.75 g/100 cm²) and the largest leaf area index (LAI – 332.25 cm²/100 cm²), as well as the highest protein content (23.72%). The Vermicompost x distilled water combination resulted in the highest total fiber content (18.62%), while the Perlite x distilled water variant had the highest content of total soluble solids (7.2°BX). These findings highlight the essential role of substrate and watering in optimizing biometry, yield, and nutritional properties, further establishing hemp microgreens as a sustainable and innovative choice for modern diets.

Key words: *hemp microgreens, biometric traits, matter yield, nutritional quality.*

INTRODUCTION

Microgreens are tender, immature greens that are generally larger than sprouts and smaller than baby greens, recognized as 'functional food' (Lenzi et al., 2019). They have gained significant attention recently due to their high nutraceutical value and ability to meet dietary nutrient adequacy (Di Gioia et al., 2021). This is attributed to their rich bioactive phytochemical content, which includes polyphenols, vitamins, minerals, and proteins (Dayakar Rao et al., 2017). Microgreens are considered specialty crops for their intense flavors, attractive colors, and rich bioactive compounds, making them highly valued in culinary applications (Lenzi et al., 2019).

Different species of microgreens contain varying levels of essential nutrients and bioactive compounds, making them a valuable addition to a health-promoting diet. For instance, amaranth microgreens contain chlorophyll a (0.25 mg/g), chlorophyll b (0.20 mg/g), carotenoids (0.023 mg/g), anthocyanins (9 mg/100 g), and ascorbic acid (0.031 mg/g), which contribute to their antioxidant activity (Sarker and Oba, 2019; Rocchetti et al., 2020). Red beet microgreens are rich in polyphenols (313.8 mg/100 g), betaxanthins (432.7 mg/100 g), and betacyanins (226.7 mg/100 g), offering both antioxidant and gastrointestinal benefits (Rocchetti et al., 2020). Quinoa microgreens are notable for their tocopherols (65 μg/g), β-carotene (738 μg/g), and fatty acids, including α-linolenic acid (35.1%) and linolenic acid (11.36%), which enhance their antioxidant properties (Pathan and Siddiqui, 2022). Spinach microgreens contain chlorophylls (44 μg/g), lutein (54.2 μg/g), β-carotene (44 μg/g), phenols (632.3 μg/g), and ascorbic acid (130.5

µg/g), contributing to their strong antioxidant activity (Petropoulos et al., 2021).

The cultivation of microgreens involves selecting appropriate seeds, growth methods, and substrates to optimize their nutritional value and yield (Gunjal et al., 2024). These young, immature greens are typically harvested between 7 and 21 days after germination, and in some cases up to 28 days, depending on the species, cultivar, and growing conditions. At this developmental stage, microgreens are characterized by high concentrations of vitamins, minerals, antioxidants etc. (Kyriacou et al., 2019; Rouphael et al., 2021; Gunjal et al., 2024; Popa et al., 2024). Scientific literature indicates that microgreens typically contain higher levels of essential phytonutrients compared to their mature counterparts (El-Nakhel et al., 2020; Pannico et al., 2020; Paraschivu et al., 2021). Various growing methods, including indoor, outdoor, and controlled environments like greenhouses, are employed to enhance their growth (Di Gioia and Santamaria, 2015). In terms of value addition, microgreens are increasingly being incorporated into various food products, such as functional beverages, gluten-free baked goods, and ready-to-eat chutney powders, to enhance their nutritional profile and appeal (Sharma et al., 2021; Kaur et al., 2022; Nivedha and Lakshmy Priya, 2018). These applications not only improve the sensory qualities of the products but also provide significant health benefits due to the high content of bioactive compounds in microgreens (Gunjal et al., 2024). Substrate selection is a critical factor influencing the growth, yield, and nutritional quality of microgreens. Different substrates affect water retention, aeration, nutrient availability, and root development, ultimately impacting plant metabolism and biochemical composition. Organic substrates, such as peat and vermicompost, are known to enhance nutrient accumulation, whereas inert media like perlite may influence secondary metabolite synthesis, as previously highlighted by Kyriacou et al. (2019). Understanding the role of substrates in optimizing microgreen production is essential for maximizing both nutritional value and consumer acceptance in sustainable agricultural systems.

Hemp (*Cannabis sativa* L.) is a versatile multi-purpose crop cultivated in various agro-ecological conditions and processed for multiple uses, including textile fibers, paper, paint, biofuels, timber, biodegradable plastics, hempcrete, human food and animal feed, as well as for medicinal purposes (Popa et al., 2021; Adam and Isopescu, 2022; Pannico et al., 2022). Hemp plants synthesize hundreds of biologically active secondary metabolites, including terpenoids, cannabinoids, glycosidic compounds, polyphenols, fatty acids, simple acids, amino acids, enzymes, steroids, pigments, and vitamins (Kuddus et al., 2013). These findings suggest that hemp microgreens can be a valuable addition to the diet, offering significant health benefits through their rich content of bioactive compounds.

Given the growing interest in optimizing microgreen production for enhanced nutritional and visual quality, this study aims to evaluate the impact of different growing substrates-perlite, peat, and vermicompost-combined with two watering regimes (distilled water and RAS water) on the yield, nutritional composition, biometric traits, and color parameters of hemp (*Cannabis sativa* L.) microgreens. Specifically, the research seeks to determine how substrate choice influences key nutritional components such as protein, fiber, ash content, total soluble solids, and oxalic acid concentration, as well as biometric traits and color attributes (*L*, *a*, *b* values).

MATERIALS AND METHODS

Biological Material and Experimental Design

The research was conducted at University of Life Sciences Iasi (IULS), using monoecious hemp seeds provided by Agricultural Research and Development Station Secuieni (ARDS Secuieni), the owner of the biological material. Monoecious hemp microgreens were cultivated under controlled conditions to evaluate the influence of different substrates and watering regimes on yield, nutritional composition, biometric traits, and color parameters. The experiment was organized using a bifactorial design (3 × 2), involving three substrate types (perlite, peat and vermicompost) and two watering regimes (distilled water and water from a recirculating aquaculture system - RAS water).

Substrate and Watering Treatments

In this experiment, perlite was used as a growing substrate due to its properties as an inert medium with very good aeration and high drainage capacity, promoting microgreens root development and preventing excess moisture.

The choice of peat was based on its high water retention capacity and moderate aeration, providing a stable growing environment that enhances nutrient availability and sustains root hydration throughout microgreens development.

Vermicompost was selected as a growing substrate due to its biologically active nature, resulting from the decomposition of organic matter by earthworms, which enhances microbial diversity and ensures a steady supply of essential nutrients, thereby promoting optimal microgreens growth and development.

The experimental variants were watered with distilled water and RAS water. The latter was sourced from a recirculating aquaponic system and contains natural nutrients, including nitrogen, phosphorus, and potassium, which may influence the metabolism and growth of microgreens.

Microgreens Growth Conditions

The seeds were uniformly sown in trays filled with the selected substrates. Subsequently, the trays were placed in a growth chamber under controlled environmental conditions: temperature 22 ± 2°C, relative humidity 70%, and a photoperiod of 16 h light / 8 h dark (Figure 1). Watering was performed manually once daily to maintain a consistent substrate moisture level. The microgreens were harvested 15 days after sowing.

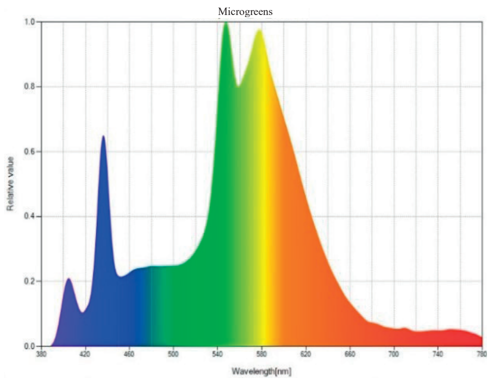

Figure 1. Spectral composition of light in the climate-controlled chamber

Yield and Biometric Traits

At harvest, the following measurements were taken for microgreens: fresh matter yield (g/100 cm²), dry matter yield (g/100 cm²), microgreens length (cm), and leaf area index (LAI).

Fresh matter yield (g/100 cm²) was determined using an analytical balance.

Dry matter yield (g/100 cm²) was determined after drying samples in an oven at 60°C for 48 hours.

Microgreens length (cm) was measured using a ruler.

Leaf Area Index (LAI) was measured using the LI-3100C area meter (LI-COR, Lincoln, NE, USA), with results expressed in cm² per 100 cm².

Nutritional and Biochemical Analysis

The nutritional composition of the microgreens, including **protein, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), fiber** and **energy** content, was analyzed using a Near-Infrared Reflectance (NIR) DA 7250 Analyzer (Perten, Sweden), which enables a rapid and precise assessment of macronutrient content. All compounds were expressed as percentages, except for energy, which was reported in $MJ \cdot kg^{-1}$.

Titratable acidity (TA) was determined using the titrimetric method. Following the homogenization of hemp microgreens samples in distilled water, titration was performed with NaOH until reaching a pH of 8.1. The results were expressed as a percentage of oxalic acid.

Total Soluble Solids (TSS) were quantified using a digital refractometer, with results expressed in °Brix, in accordance with Irimia (2013) and the OECD standards (2018).

Color Parameters

Leaf color was assessed using a HunterLab colorimeter, applying the CIELAB scale, which measures lightness (L*), where higher values indicate a lighter green shade and lower values correspond to darker tones; the red-green axis (a*), where negative values denote increased greenness; and the yellow-blue axis (b*), where higher values represent a more yellowish hue.

Statistical Analysis

All data were analyzed using one-way and two-way ANOVA to evaluate the effects of substrate and watering treatment. Post-hoc comparisons were performed using Tukey's HSD test ($p < 0.05$) to identify significant differences among variants. Results are presented as mean ± standard error (SE). Statistical analyses were conducted using SPSS v.25 (IBM Corp.).

Additionally, Principal Component Analysis (PCA) and Pearson correlation analysis were applied to assess the effects of substrate type variation and watering regime on the yield, biometric traits, nutritional composition and color parameters of hemp microgreens.

RESULTS AND DISCUSSIONS

Substrate type significantly influenced the yield and biometric traits of hemp microgreens (Table 1). Microgreens grown on peat exhibited the highest fresh matter yield (12.27 ± 0.4 $g \cdot cm^{-2}$), dry matter yield (1.55 ± 0.09 $g \cdot cm^{-2}$), and length (8.78 ± 0.32 cm). In contrast, those cultivated on vermicompost recorded the lowest values for fresh matter yield (4.97 ± 0.46 $g \cdot cm^{-2}$) and dry matter yield (0.65 ± 0.03 $g \cdot cm^{-2}$). However, length did not significantly differ between microgreens grown on vermicompost (7.04 ± 0.55 cm) and those cultivated on perlite (6.68 ± 0.15 cm), while peat resulted in a significantly higher length (8.78 ± 0.32 cm).

The Leaf Area Index (LAI) was also significantly higher in peat-grown microgreens (307.73 ± 30.52 $cm^2 \cdot cm^{-2}$) compared to those cultivated on perlite (209.09 ± 9.01 $cm^2 \cdot cm^{-2}$) and vermicompost (129.14 ± 8.17 $cm^2 \cdot cm^{-2}$). These findings confirm that peat provided the most favorable conditions for biomass accumulation in hemp microgreens, while vermicompost resulted in significantly lower fresh matter yield and dry matter yield, despite similar length to perlite.

Watering regime had a limited effect on the biometric traits of hemp microgreens, while its influence on yield was significant only for dry matter yield. Differences in fresh matter yield, length, and LAI did not reach statistical significance (Table 1). Microgreens watered with RAS water exhibited slightly higher fresh matter yield (9.41 ± 0.22 $g \cdot cm^{-2}$) and dry matter yield (1.13 ± 0.02 $g \cdot cm^{-2}$) compared to those watered with distilled water (8.47 ± 0.38 $g \cdot cm^{-2}$ fresh matter yield, 0.92 ± 0.05 $g \cdot cm^{-2}$ dry matter yield). Among these parameters, only dry matter yield was significantly influenced by the watering regime, with higher values recorded under RAS water application.

Length did not differ significantly between watering regimes, with values ranging from 7.39 ± 0.33 cm in microgreens watered with RAS water to 7.62 ± 0.08 cm in those watered with distilled water. Similarly, LAI was slightly higher in microgreens watered with RAS water (228.09 ± 5.05 $cm^2 \cdot cm^{-2}$) compared to those receiving distilled water (202.54 ± 11.83 $cm^2 \cdot cm^{-2}$), but the difference was not statistically significant.

These results indicate that the watering regime had a significant effect on dry matter yield but did not significantly influence fresh matter yield and biometric traits.

Table 1. Effect of substrate and watering regime on yield and biometric traits of hemp microgreens

Treatment	Fresh matter yield ($g \cdot cm^{-2}$)	Dry matter yield ($g \cdot cm^{-2}$)	Length (cm)	LAI ($cm^2 \cdot cm^{-2}$)
Substrate				
Perlite	9.58 ± 0.23 b	0.88 ± 0.03 b	6.68 ± 0.15 b	209.09 ± 9.01 b
Peat	12.27 ± 0.40 a	1.55 ± 0.09 a	8.78 ± 0.32 a	307.73 ± 30.52 a
Vermicompost	4.97 ± 0.46 c	0.65 ± 0.03 b	7.04 ± 0.55 b	129.14 ± 8.17 b
Significance	*	*	*	*
Watering				
Distilled water	8.47 ± 0.38	0.92 ± 0.05	7.62 ± 0.08	202.54 ± 11.83
RAS water	9.41 ± 0.22	1.13 ± 0.02	7.39 ± 0.33	228.09 ± 5.05
Significance	ns	*	ns	ns

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. LAI - Leaf Area Index; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the biometric traits and yield of hemp microgreens (Table 2). The highest fresh matter yield was observed in microgreens grown on peat with RAS water (12.75 ± 0.38 g), followed by those on peat with distilled water (11.8 ± 0.74 g). The lowest fresh matter yield values were recorded in microgreens cultivated on vermicompost, regardless of watering regime (4.57 ± 0.34 g – 5.37 ± 0.59 g). Similarly, dry matter yield followed a comparable trend, with the highest values in peat-grown microgreens (1.7 ± 0.07 g) and the lowest in vermicompost (0.59 ± 0.02 g).

Regarding biometric traits, microgreens cultivated on peat exhibited the greatest length (8.83 ± 0.35 cm), while those grown on perlite had the shortest (6.2 ± 0.38 cm). Vermicompost-grown microgreens had intermediate values (6.94 ± 0.24 cm – 7.13 ± 0.9 cm), without significant differences compared to perlite or peat.

Leaf area index (LAI) was also highest in peat-grown microgreens watered with RAS water (332.25 ± 34.98), while vermicompost treatments resulted in the lowest values (120.86 ± 15.52 – 137.44 ± 4.34).

These results indicate that peat substrates, particularly in combination with RAS water, promoted the highest fresh and dry matter yield, as well as greater leaf area development in hemp microgreens. However, length and LAI were primarily influenced by substrate type rather than the watering regime, as differences between watering treatments within the same substrate were not statistically significant.

Table 2. Combined effect of substrate and watering regime on yield and biometric traits of hemp microgreens

Treatment	Fresh matter yield ($g \cdot cm^{-2}$)	Dry matter yield ($g \cdot cm^{-2}$)	Length (cm)	LAI ($cm^2 \cdot cm^{-2}$)
Perlite x Distilled water	9.05 ± 0.53 c	0.78 ± 0.04 bc	7.17 ± 0.17 ab	203.58 ± 9.08 bc
Perlite x RAS water	10.11 ± 0.32 bc	0.98 ± 0.04 b	6.20 ± 0.38 b	214.59 ± 21.14 abc
Peat x Distilled water	11.80 ± 0.74 ab	1.40 ± 0.17 a	8.73 ± 0.33 a	283.19 ± 44.30 ab
Peat x RAS water	12.75 ± 0.38 a	1.70 ± 0.07 a	8.83 ± 0.35 a	332.25 ± 34.98 a
Vermicompost x Distilled water	4.57 ± 0.34 d	0.59 ± 0.02 c	6.94 ± 0.24 ab	120.86 ± 15.52 c
Vermicompost x RAS water	5.37 ± 0.59 d	0.72 ± 0.05 bc	7.13 ± 0.90 ab	137.44 ± 4.34 c
Significance	*	*	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. LAI - Leaf Area Index; RAS water - water from recirculating aquaculture system.

Substrate type had a significant influence on the nutritional composition of hemp microgreens (Table 3). Microgreens grown on peat exhibited the highest protein content (22.72 ± 0.09%), followed by those on vermicompost (21.47 ± 0.22%) and perlite (20.11 ± 0.17%). Ash content was highest in microgreens grown on perlite (13.88 ± 0.20%) and lowest in vermicompost (13.11 ± 0.14%). Regarding fiber content, peat-grown microgreens showed the highest NDF content (25.25 ± 0.17%), whereas perlite-grown microgreens had the lowest (21.17 ± 0.80%). Acid detergent fiber (ADF) did not significantly differ among substrates. Fiber content was highest in microgreens grown on vermicompost (16.76 ± 0.24%) and lowest on peat (15.95 ± 0.14%). Energy content was highest in perlite-grown microgreens (11.27 ± 0.04 $MJ \cdot kg^{-1}$), with peat showing the lowest values (11.02 ± 0.04 $MJ \cdot kg^{-1}$). These results suggest that peat substrates provide optimal conditions for protein and NDF accumulation, whereas perlite favors higher ash and energy content.

The watering regime significantly influenced certain nutritional properties of hemp microgreens (Table 3). Microgreens watered with RAS water showed significantly higher protein content (22.29 ± 0.18%) compared to those watered with distilled water (20.57 ± 0.04%). Ash, NDF, and ADF contents were not significantly influenced by watering regime, despite slight numerical differences between treatments. Fiber was significantly greater under distilled water conditions (17.30 ± 0.12%) compared to RAS water (15.57 ± 0.10%). Energy content was also not significantly influenced by the watering regime.

Table 3. Effect of substrate and watering regime on the nutritional composition of hemp microgreens

Treatment	Protein (%)	Ash (%)	NDF (%)	ADF (%)	Fiber (%)	Energy (MJ·kg^{-1})
Substrate						
Perlite	20.11 ± 0.17 c	13.88 ± 0.20 a	21.17 ± 0.80 b	40.17 ± 0.11	16.60 ± 0.07 a	11.27 ± 0.04 a
Peat	22.72 ± 0.09 a	13.83 ± 0.06 a	25.25 ± 0.17 a	40.08 ± 0.15	15.95 ± 0.14 b	11.02 ± 0.04 b
Vermicompost	21.47 ± 0.22 b	13.11 ± 0.14 b	23.33 ± 0.88 ab	40.42 ± 0.15	16.76 ± 0.24 a	11.13 ± 0.05 ab
Significance	*	*	*	ns	*	*
Watering						
Distilled water	20.57 ± 0.04	13.41 ± 0.14	24.06 ± 0.83	40.44 ± 0.14	17.30 ± 0.12	11.10 ± 0.03
RAS water	22.29 ± 0.18	13.80 ± 0.12	22.45 ± 0.53	40.00 ± 0.12	15.57 ± 0.10	11.17 ± 0.04
Significance	*	ns	ns	ns	*	ns

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the nutritional composition of hemp microgreens (Table 4). Protein content varied significantly across treatments, with the highest values recorded in microgreens cultivated on peat with RAS water (23.72 ± 0.19%) and vermicompost with RAS water (23.13 ± 0.43%). In contrast, the lowest protein content was observed in microgreens grown on vermicompost with distilled water (19.80 ± 0.12%) and perlite with RAS water (20.03 ± 0.33%).

Ash content also varied significantly, with the highest value recorded in peat with RAS water (14.17 ± 0.13%) and the lowest value in vermicompost with distilled water (12.90 ± 0.19%).

The highest NDF values were observed in microgreens cultivated on peat with distilled water (25.83 ± 0.17%), while the lowest values occurred in those grown on perlite with distilled water (20.67 ± 1.02%). ADF content was significantly influenced, with the highest value found in vermicompost with distilled water (41.00 ± 0.26%) and the lowest in vermicompost with RAS water (39.83 ± 0.17%). The highest fiber content was observed in microgreens cultivated on vermicompost with distilled water (18.62 ± 0.38%), while the lowest value was recorded in those grown on vermicompost with RAS water (14.90 ± 0.12%).

Energy content ranged from 10.95 ± 0.05 MJ·kg^{-1} (vermicompost with distilled water) to 11.30 ± 0.06 MJ·kg^{-1} (perlite with distilled water and vermicompost with RAS water), confirming the significant combined impact of substrate and watering regime on the nutritional quality of hemp microgreens.

Table 4. Combined effect of substrate and watering regime on the nutritional composition of hemp microgreens

Treatment	Protein (%)	Ash (%)	NDF (%)	ADF (%)	Fiber (%)	Energy (MJ·kg^{-1})
Perlite x Distilled water	20.18 ± 0.09 c	13.83 ± 0.29 ab	20.67 ± 1.02 c	40.33 ± 0.21 ab	16.77 ± 0.08 b	11.30 ± 0.04 a
Perlite x RAS water	20.03 ± 0.33 c	13.92 ± 0.17 ab	21.67 ± 0.99 bc	40.00 ± 0.02 b	16.43 ± 0.13 b	11.24 ± 0.06 ab
Peat x Distilled water	21.72 ± 0.23 b	13.50 ± 0.14 abc	25.83 ± 0.17 a	40.00 ± 0.02 b	16.52 ± 0.09 b	11.05 ± 0.05 bc
Peat x RAS water	23.72 ± 0.19 a	14.17 ± 0.13 a	24.67 ± 0.33 ab	40.17 ± 0.31 ab	15.38 ± 0.22 c	10.98 ± 0.06 c
Vermicompost x Distilled water	19.80 ± 0.12 c	12.90 ± 0.19 c	25.67 ± 1.48 a	41.00 ± 0.26 a	18.62 ± 0.38 a	10.95 ± 0.05 c
Vermicompost x RAS water	23.13 ± 0.43 a	13.32 ± 0.12 bc	21.00 ± 0.89 bc	39.83 ± 0.17 b	14.90 ± 0.12 c	11.30 ± 0.06 a
Significance	*	*	*	*	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; RAS water - water from recirculating aquaculture system.

The type of substrate significantly affected the titratable acidity (TA) and total soluble solids (TSS) content of hemp microgreens (Table 5). The highest TA values, expressed as % oxalic acid, were recorded in microgreens grown on perlite (0.26 ± 0.01%), followed by peat (0.22 ± 0.01%), with the lowest values observed on vermicompost (0.18 ± 0.01%). These findings indicate that perlite may enhance oxalate synthesis, while vermicompost helps to reduce oxalic acid accumulation. TSS content was highest in microgreens grown on perlite and vermicompost (6.50 ± 0.01 °Bx), significantly higher compared to peat (5.60 ± 0.05 °Bx).

These results indicate that vermicompost, despite lowering oxalic acid content, maintained TSS levels comparable to perlite, suggesting its suitability for simultaneously improving nutritional quality and reducing oxalate content.

The watering regime significantly influenced both titratable acidity and TSS content (Table 5). Microgreens watered with distilled water had significantly higher TA (0.27 ± 0.01%) compared to those watered with RAS water (0.17 ± 0.00%), indicating that RAS water provides nutrients that effectively reduce oxalic acid accumulation in hemp microgreens. Similarly, TSS was slightly higher under distilled water conditions (6.40 ± 0.03 °Bx) compared to RAS water (6.00 ± 0.01 °Bx), with significant differences observed between watering regimes.

Table 5. Effect of substrate and watering regime on titratable acidity and total soluble solids of hemp microgreens

Treatment	TA (% oxalic acid)	TSS (°Brix)
Substrate		
Perlite	0.26 ± 0.01 a	6.50 ± 0.01 a
Peat	0.22 ± 0.01 b	5.60 ± 0.05 b
Vermicompost	0.18 ± 0.01 c	6.50 ± 0.01 a
Significance	*	*
Watering		
Distilled water	0.27 ± 0.01	6.40 ± 0.03
RAS water	0.17 ± 0.00	6.00 ± 0.01
Significance	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. TA - Titratable acidity; TSS - Total Soluble Solids; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the oxalic acid and total soluble solids (TSS) content of hemp microgreens (Table 6). The highest oxalic acid concentration was recorded in microgreens grown on perlite with distilled water (0.33 ± 0.01%), while the lowest levels (0.16 ± 0.01%) occurred in microgreens grown on peat and vermicompost substrates with RAS water. These results suggest that RAS water significantly reduced oxalic acid accumulation in all tested substrates, with organic substrates (peat and vermicompost) showing the greatest reduction, potentially enhancing nutritional safety.

Total soluble solids (TSS) content was highest in microgreens grown on perlite with distilled water (7.20 ± 0.06 °Bx) and lowest in those grown on peat with distilled water (5.00 ± 0.06 °Bx).

These results suggest that the use of RAS water had a significant effect in reducing oxalic acid accumulation in hemp microgreens, regardless of substrate. However, the lowest oxalic acid values were obtained with organic substrates (peat and vermicompost) combined with RAS water, which could represent an effective solution for producing microgreens with reduced oxalate content.

Table 6. Combined effect of substrate and watering regime on titratable acidity and total soluble solids of hemp microgreens

Treatment	TA (% oxalic acid)	TSS (°Brix)
Perlite x Distilled water	0.33 ± 0.01 a	7.20 ± 0.06 a
Perlite x RAS water	0.19 ± 0.01 c	5.80 ± 0.06 c
Peat x Distilled water	0.28 ± 0.01 b	5.00 ± 0.06 d
Peat x RAS water	0.16 ± 0.01 d	6.20 ± 0.06 b
Vermicompost x Distilled water	0.19 ± 0.01 c	7.00 ± 0.06 a
Vermicompost x RAS water	0.16 ± 0.01 d	6.00 ± 0.06 bc
Significance	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. TA - Titratable acidity; TSS - Total Soluble Solids; RAS water - water from recirculating aquaculture system.

Substrate type significantly influenced only the b* (yellowness-blueness) color attribute of hemp microgreens (Table 7). Microgreens grown on peat exhibited the highest b* value (19.34 ± 0.47), followed by vermicompost (18.19 ± 0.82), while perlite resulted in significantly lower b* values (16.84 ± 0.32). This indicates that peat promotes a more vibrant and yellowish-green coloration. However, substrate type had no statistically significant effect on the L* (lightness) and a* (redness-greenness) color attributes.

Watering regime did not significantly influence any of the color parameters (L*, a*, and b*) evaluated (Table 7). Microgreens watered with distilled water and those receiving RAS water exhibited comparable values for all color attributes. Specifically, lightness (L*) was nearly identical between distilled water (37.35 ± 1.77) and RAS water (37.62 ± 1.88). Similarly, no significant differences were observed for redness-greenness (a*), with values of -5.35 ± 0.27 for distilled water and -5.28 ± 0.40 for RAS water. The yellowness-blueness parameter (b*) also remained stable across watering treatments, at 18.14 ± 0.78 for distilled water and 18.10 ± 0.16 for RAS water. These results indicate that watering regime does not significantly affect the visual appearance of hemp microgreens.

Table 7. Effect of substrate and watering regime on the color parameters of hemp microgreens

Treatment	L*	a*	b*
Substrate			
Perlite	33.87 ± 2.10	-4.23 ± 0.72	16.84 ± 0.32 b
Peat	39.65 ± 0.92	-6.01 ± 0.42	19.34 ± 0.47 a
Vermicompost	38.95 ± 2.51	-5.72 ± 0.31	18.19 ± 0.82 ab
Significance	ns	ns	*
Watering			
Distilled water	37.35 ± 1.77	-5.35 ± 0.27	18.14 ± 0.78
RAS water	37.62 ± 1.88	-5.28 ± 0.40	18.10 ± 0.16
Significance	ns	ns	ns

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. L* - lightness-darkness; a* - redness-greenness; b* - yellowness-blueness; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the L* (lightness-darkness) and b* (yellowness-blueness) color attributes of hemp microgreens (Table 8).

Table 8. Combined effect of substrate and watering regime on the color parameters of hemp microgreens

Treatment	L*	a*	b*
Perlite x Distilled water	36.22 ± 1.09 ab	-4.37 ± 0.32	17.98 ± 0.54 ab
Perlite x RAS water	31.51 ± 3.38 b	-4.08 ± 1.12	15.70 ± 0.30 b
Peat x Distilled water	37.12 ± 0.94 ab	-6.19 ± 0.42	18.59 ± 0.54 ab
Peat x RAS water	42.18 ± 0.94 a	-5.83 ± 0.72	20.10 ± 0.82 a
Vermicompost x Distilled water	38.72 ± 3.71 ab	-5.48 ± 0.62	17.85 ± 1.43 ab
Vermicompost x RAS water	39.17 ± 1.65 a	-5.94 ± 0.23	18.52 ± 0.79 ab
Significance	*	ns	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values aTastați ecuația aici.ssociated to different letters are significantly different according to Tukey's test at p<0.05. L* - lightness-darkness; a* - redness-greenness; b* - yellowness-blueness; RAS water - water from recirculating aquaculture system.

The highest L* value, indicating a lighter green coloration, was observed in microgreens grown on peat with RAS water (42.18 ± 0.94), while the lowest L* value, corresponding to darker green shades, was recorded in microgreens grown on perlite with RAS water (31.51 ± 3.38). The yellowness-blueness parameter (b* values) also varied significantly, with peat-grown microgreens watered with RAS water having the highest value (20.10 ± 0.82), indicating a more pronounced yellowish hue. The lowest b* value was recorded in microgreens grown on perlite with RAS water (15.70 ± 0.30).

In contrast, substrate and watering regime interactions had no statistically significant influence on the redness-greenness (a* values). These results highlight that substrate and watering regime interactions predominantly affect the brightness and yellowish coloration of hemp microgreens.

Substrate selection plays a crucial role in determining the yield, nutritional composition, biometric traits, and color parameters of hemp (*Cannabis sativa* L.) microgreens, as evidenced by the results of the PCA analysis applied to the data obtained.

The PCA analysis returned five principal components (PCs), four of which had eigenvalues greater than 1, indicating that these components explain most of the variability in the dataset. The first two principal components (PC1 and PC2) together explain 68.28% of the total variation in the data, with PC1 having an eigenvalue of 6.39 and explaining 42.59% of the variation, and PC2 having an eigenvalue of 3.85 and contributing 25.69% (Table 9). These results suggest that the first two principal components are the most relevant for describing the differences between experimental variants.

Table 9. Eigenvalues of the correlation matrix showing affinity of different PCA against the traits of hemp microgreens

PC	Eigenvalue	Percentage of variance (%)	Cumulative percentage (%)
1	6.39	42.59	42.59
2	3.85	25.69	68.28
3	2.38	15.86	84.14
4	1.40	9.32	93.46
5	0.98	6.54	100.00

The graphical representation of PC1 and PC2 from the PCA analysis indicates that cultivation in peat with RAS water is most favorable for growth and protein accumulation, while perlite results in higher energy content and titratable acidity (TA). Conversely, vermicompost with distilled water is associated with increased fiber, ADF, and total soluble solids (TSS) content (Figure 2). These findings align with previous studies highlighting that organic substrates provide better nutrient retention and microbial interactions, leading to improved biomass and nutrient accumulation in microgreens (Lenzi et al., 2019; Di Gioia et al., 2021). Particularly, peat promoted higher microgreens length and fresh matter yield, reinforcing the idea that organic substrates optimize water retention and aeration, creating a more favorable microenvironment for microgreens development (Gunjal et al., 2024).

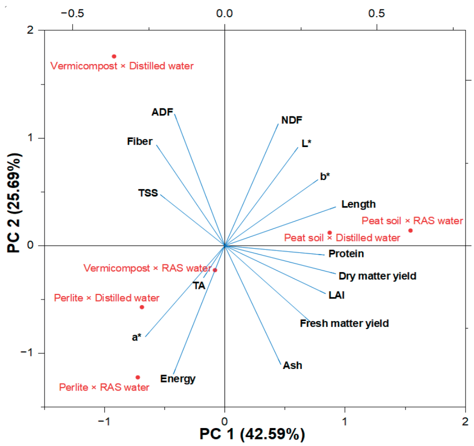

Figure 2. PCA plot showing the association of treatments with hemp microgreens traits

Watering with RAS water significantly increased protein content while reducing oxalic acid levels, a desirable outcome given the anti-nutritional effects of oxalates. These results are also highlighted by the Pearson correlation diagram, which indicates a strong negative relationship. In contrast, watering with distilled water resulted in a strong positive correlation between protein content and oxalate levels (Figure 3). This supports previous findings that nutrient-rich water sources, including those derived from aquaponic systems, enhance nitrogen availability, leading to improved protein synthesis and reduced anti-nutritional compounds in microgreens (Pannico et al., 2022; Kyriacou et al., 2019). Additionally, the results suggest that RAS water provides bioavailable nutrients that optimize metabolic activity, a phenomenon also observed in studies evaluating the impact of hydroponic nutrient solutions on microgreens (Di Gioia and Santamaria, 2015).

The color parameters (L*, a*, b*) varied significantly based on substrate selection, with peat-grown microgreens exhibiting higher L* values, indicating a lighter green hue, while perlite resulted in darker shades. This is consistent with reports stating that substrate composition influences chlorophyll accumulation and carotenoid content, both of which contribute to plant coloration (Meas et al., 2020; Pannico et al., 2022). In the PCA biplot (Figure 2), L*, a*, and b* are positioned prominently along PC1, indicating their significant contribution to variability. The b* values, indicating the yellow-blue spectrum, were highest in peat-grown microgreens, suggesting increased carotenoid accumulation (Petropoulos et al., 2021). The results of this study reveal that peat-based substrates can enhance marketability by producing visually attractive, nutrient-rich microgreens. In contrast, darker microgreens grown on perlite substrate may indicate increased chlorophyll content, potentially enhancing antioxidant properties. However, while substrate type had clear impacts on microgreens characteristics, the watering regime showed a more limited effect. Specifically, watering significantly influenced nutritional composition, but its impact on yield was significant only for dry matter, with limited effects on biometric traits and color parameters. This indicates that substrate characteristics exert a more dominant influence on microgreens structure, supporting previous research suggesting that substrate composition primarily determines microgreen quality (Di Gioia et al., 2021; Sharma et al., 2021). The observed trends in biometric traits and yield align with previous findings showing that peat-based substrates support greater biomass accumulation compared to inert substrates such as perlite (Lenzi et al., 2019).

Overall, the study highlights the importance of selecting appropriate substrates and watering strategies to optimize the nutritional value, yield characteristics, and visual appeal of hemp microgreens. Organic substrates such as peat and vermicompost, combined with nutrient-rich watering sources, provide a promising strategy for enhancing microgreens yield and bioactive compound content (Kyriacou et al., 2019; Pannico et al., 2022).

Future research should focus on optimizing light conditions and biofortification strategies to further enhance hemp microgreens' nutritional quality, as previous studies have demonstrated the significant impact of spectral modifications on microgreens phytochemistry (Lobiuc et al., 2017; Meas et al., 2020; Petropoulos et al., 2021).

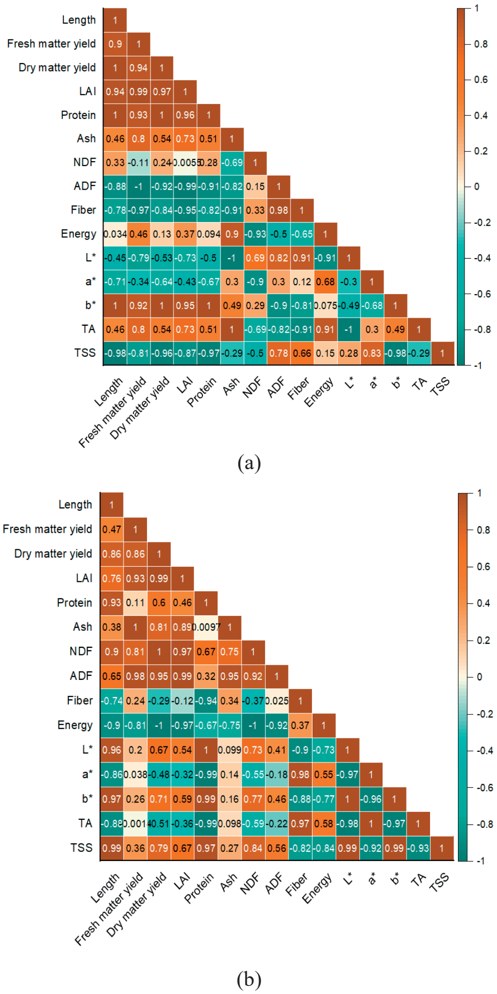

Figure 3. Pearson correlation diagram illustrating the effects of (a) distilled water and (b) RAS water on the linear relationships between the traits of hemp microgreens

CONCLUSIONS

The organic substrate, particularly peat, promoted the greatest biomass development, as reflected by higher fresh and dry matter yield, microgreens length, and leaf area index (LAI), compared to perlite and vermicompost substrates. Therefore, peat can be recommended as the optimal substrate for hemp microgreens cultivation.

Using water from the recirculating aquaculture system (RAS) significantly increased the protein content of microgreens and reduced oxalic acid accumulation, a beneficial aspect from a food safety perspective.

The most favorable substrate-water combination for maximizing yield and nutritional quality (high protein content and low oxalic acid) proved to be peat substrate watered with RAS water.

Color parameters were predominantly influenced by substrate type, with peat producing lighter and more vibrant-colored microgreens (higher L and b values), which is an important attribute for enhancing the commercial appeal of the final product.

Utilizing organic substrates (peat or vermicompost) in combination with nutrient-rich water from recirculating aquaponic systems (RAS) provides a sustainable and efficient approach for enhancing yield, nutritional value, and visual attractiveness of hemp microgreens.

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