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Original article

Nutrients and phytochemical density in *Mesembryanthemum crystallinum* L. cultivated in growing media supplemented with dosages of nitrogen fertilizer

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ABSTRACT

Mesembryanthemum crystallinum L. is an annual halophyte that originated from southern Africa. However, little has been reported about factors that modulate the quantity of secondary metabolites in the plant. In this study, the twin effect of different nitrogen concentrations (0.36, 0.6, 0.8 g/L) and growing media (LECA clay, peat, vermiculite and silica sand) on plant growth, chlorophyll contents, minerals, proximate and antioxidant metabolites in hydroponically cultivated *M. crystallinum* was investigated. This is important to determine the dosage of N fertilizer that will optimize the bio-productivity and biosynthesis of secondary metabolites and antioxidants in *M. crystallinum* grown in a hydroponic system. The untreated plant (0 g/L N) was taken as the control. At the end of the experiment, optimum yields in leaf number (9.2), fresh weight (50.40 g), Ca, N, and Protein (34.04 %) were recorded in *M. crystallinum* grown with peat enhanced with different dosages of N-fertilizer. Likewise, chlorophyll level, dry weight, ABTS/TEAC, FRAP, ADF and NDF contents were optimized in LECA clay treated with N-fertilizer. Silica sand with 0.36 g/L dosage of nitrogen fertilizer optimized P, Mn and Zn levels, so also the moisture (9.83 % at 0.8 g/L N), fat (2.38 %, 0 g/L N) and carbohydrates (44.98 and 44.95 %). The highest ash content, Mg and Fe were recorded in the untreated vermiculite as well as polyphenols and K, at 0.6 g/L; Cu and root length (14.60 cm), at 0.8 g/L. In conclusion, different dosages of nitrogen fertilizer and growing media could enhance the growth potential, chlorophyll, phytochemicals, and nutritional properties of *M. crystallinum*.

1. Introduction

Agricultural systems are under stress due to climate change-related issues like elevated temperature, drought and salinization, which affect crop nutrition and potential production (Dai, 2010; Folland et al., 2002; Gomez-Zavaglia et al., 2020; Munaweera et al., 2022). The agriculture industry has recorded increasing utilization of fertilizer to increase crop nutrition and yield throughout time to sustain food supply (Chen et al., 2018; Dobermann et al., 2022; FAO, 2005). However, increased fertilizer use has been shown to have detrimental environmental effects (Chen et al., 2018). Nevertheless, Nitrogen fertilizer is widely used to optimize plants' growth and development (Galloway et al., 2017; Good, 2018). The availability of nitrogen is a crucial environmental component for plant growth (Erisman et al., 2015) as it directly influences plant metabolism, material and nutrient distribution, improved nutrient cycling, balanced plant nutritional composition, and increased enzyme activity (Aczel, 2019).

However, proper planting and nitrogen application patterns require optimal use of water, air, and heat, which are crucial for boosting water and nitrogen use efficiency, and plant development (Liu et al., 2006). For efficient water and nutrient management in assisted agriculture, hydroponic systems are becoming popular (Bello et al., 2019; Treftz and Omaye, 2016). The hydroponic systems are most frequently employed to produce ornamental plants, fodder, vegetables, and field crops on a commercial scale, to meet the rising demand for food in a sustainable way (Eigenbrod and Gruda, 2015; Hirel et al., 2011; Mai et al., 2023).

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Hence, plants produced in hydroponic systems can expand up to 50 % faster than those grown in soil (Viljoen et al., 2021). In addition, hydroponics creates a safe environment for culture, allows multi-layer vertical production of vegetables and could be integrated with automated irrigation and fertilization management (Ragaveena et al., 2021).

The flexibility of hydroponic systems could be exploited to grow wild vegetables including *M. crystallinum* (common ice plant) to ensure food security and nutrient diversity (Viljoen et al., 2021). *Mesembryanthemum crystallinum* L. (Aizoaceae) is an underutilized halophytic coastal plant that could serve as an alternative to spinach (Kang and Joo, 2023; Klak et al., 2007). The species is a perennial succulent that originated in eastern and southern Africa and is now widespread worldwide (Bohnert and Cushman, 2000). *Mesembryanthemum crystallinum* is edible and thrives in high-salt environments, such as seawater-irrigated soils, as well as in extreme dry circumstances (Loconsole et al., 2019). Recent studies have described the potential uses of common ice plant in food fortification, phytoremediation and human health improvement (Agarie et al., 2009; Rodríguez-Hernández and Garmendia, 2022). Its succulent texture leaves behind a taste that is mildly salty and evocative of the ocean when chewed. Because of this, the common ice plant is highly prized in haute cuisine (Calvo et al., 2022) as *M. crystallinum* is widely consumed in some regions, and there is growing interest in its consumption (Mndi et al., 2023; Śliwa-Cebula et al., 2020).

The mechanisms underlying mineral requirements, cultivation protocols and phytochemistry of *M. crystallinum* are poorly understood due to the paucity of research on factors that may enhance the accumulation of nutrients and secondary metabolites in the plant (Kang and Joo, 2023). Several studies have outlined nitrogen levels, xenobiotics, abiotic, and biotic markers as determinants of the health, development, and productivity of a plant (Malgioglio et al., 2022; Shah et al., 2022). Of recent, chlorophyll has been noted as one of the most reliable indicators of plant health and productivity due to its crucial roles in photosynthetic efficiency and bio-productivity (Liu et al., 2019; Wang et al., 2022). However, little information is known about how chlorophyll content can be modulated in *M. crystallinum* by growth conditions. Because halophytes contain polyphenols which are precursors for antioxidant and protective effects against diseases, there is the possibility to obtain healthy secondary metabolites and antioxidant extracts from *M. crystallinum* (Calvo et al., 2022).

Despite the edibility and pharmacological values of *M. crystallinum* as reported by Agarie et al., (2009), He and Qin, (2021), Rodríguez-Hernández and Garmendia, (2022), there is a dearth of information on *M. crystallinum* particularly on the formation of essential minerals under various growing conditions aided with different nitrogen dosages. As a result, knowledge of the possibilities and advantages of nutrition requirements is lacking or incomplete. Therefore, promoting the cultivation of *M. crystallinum* became imperative as the plant has been shown to have nutraceutical and neuroprotective properties in addition to its many aesthetic benefits (Calvo et al., 2022; Marrero-Rodríguez et al., 2021). The main goal of this study was, therefore, to determine how different nitrogen concentrations and growing conditions could enhance growth quality, yield, chlorophyll content, nutrients, secondary metabolites, and antioxidants in *M. crystallinum* and to determine the dosage of N fertilizer that will optimize the bio-productivity and biosynthesis of nutrients and antioxidants in *M. crystallinum* grown in a hydroponic system.

2. Materials and methods

2.1. Greenhouse location

The greenhouse experiment took place at the Horticultural Sciences Department (33°55′56″ S, 18°38′25″ E), Cape Peninsula University of Technology, Bellville campus, South Africa. The greenhouse had mist, a heating bed, and an environmental regulator with the greatest photosynthetic photon flux density of 1020 mol/m^2/s per day averaging at 420 mol/m^2/s. The daily temperature adjustments of the greenhouse were 12–18 °C (night), and 21–26 °C (day) with mean relative humidity of 60 %.

2.2. Plant collection and nursery preparation

Viable seeds of *M. crystallinum* were collected from a plant population at the CPUT's Granger Bay Campus (33°53′58.2″ S, 18°24′41.4″ E). A total of 1,000 seeds were planted to ensure that at least 160 seedlings were available for the experiment. Seeds were frozen for twelve hours before sowing to disrupt dormancy. The growing medium comprised 70 % sand and 30 % peat which was sifted to eliminate foreign materials. Seeds of *M. crystallinum* were planted in sowing trays filled with the growth media, levelled and watered. The filled trays were covered with a thin layer of sand. The trays were placed in a warm room and were exposed to at least ten hours of light every day, and water was added again. Seed germination was aided by misting the trays with water twice a day, in the morning and afternoon; seeds sprouted in seven to twenty-one days. Seedlings were transplanted to pots (8.8 cm) after four weeks from trays to prevent stunted growth, and plants were watered twice daily. After reaching 2–3 cm in height, plants were placed in a hydroponic system for two weeks to adapt. One hundred and sixty (160) uniform-sized seedlings of *M. crystallinum* were transplanted into 12.5 cm pots filled with sterilized growth media including silica sand, peat, vermiculite, and LECA clay. During this time, plants were given a nutrient medium three times every week. The nutrient medium was made by mixing NUTRIFEED^{TM} growth medium (manufactured by STARKE AYRES Pty. Ltd. in Gauteng, South Africa) with clean tap water at a rate of 2 g/L. The nutritional solution contained Fe (1500 mg/kg), K (130 mg/kg), P (27 mg/kg), Cu (20 mg/kg), Mg (22 mg/kg), Ca (70 mg/kg), N (65 mg/kg), Mn (240 mg/kg), Mo (10 mg/kg), Zn (240 mg/kg), B (240 mg/kg) and S (75 mg/kg).

After 14 days of establishment, the grown seedlings were irrigated for 7 days with clean water to remove contaminants. Four treatments were organized by adding varying quantities of nitrogen fertilizer (0.36 g/L, 0.6 g/L, and 0.8 g/L) and the control received no nitrogen fertilizer (0 g/L) after establishment. Plants were fed with or without nitrogen treatment via spraying from a nutrient solution reservoir.

2.3. Hydroponic set up

The hydroponic systems comprised four similar Nutrient Film Techniques. Each system was labelled the numbers S1, S2, S3 and S4. Four tables were utilized as support surfaces for gutters. Each table had four gutters mounted on it, resulting in sixteen gutters forming the hydroponic system. Each gutter was made to accommodate ten pot-filled substrates. Each gutter was filled with growth media and labelled G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat) accordingly. The gutters were held in place with cape ties. Ten pots having diverse substrates were placed in each gutter. Each table had its own water supply. Each reservoir had a submersible water pump that pumped fertilizer solution through a 20 mL pipe to each gutter. All gutters were sloped with bricks to allow the nutrient solution to circulate seamlessly. All gutters were covered with black plastic polyethene sheets to keep the fertilizer solutions out of direct sunlight and to ensure that algae did not grow on polyethene sheets.

After germination, 160 turgid, robust, and disease-free seedlings (n = 160) of the same height, leaf size, leaf colour, branch number, and width were selected from propagated plants and placed in 12.5 cm pots with varied substrates each of which was prepared for 40 plants. Nitrogen was applied in four different doses (0 g/L, 0.36 g/L, 0.6 g/L, and 0.8 g/L) per reservoir and was fed to 40 plants (n = 40) in each reservoir. Nitrogen was only applied by hand. Fertilizer was administered in the morning to guarantee maximum effectiveness and keep the plants in a climate-controlled greenhouse. Hydroponic systems were operated for 15 min in the morning and afternoon. The pH was maintained at 6.0 in

all treatments. After 12 weeks of nitrogen-treated plant growth, during which time growth metrics were taken, all plants were harvested for different postharvest assays (See Table 1).

2.4. Determination of plant growth

2.4.1. Number of leaves

The number of leaves was counted manually every two weeks to determine new growth in the treated plants.

2.4.2. Root length

The root length was measured with a measuring tape during harvesting to determine variation in the root lengths of treated plants.

2.4.3. Plant weight

The weight of the fresh and dry plant samples was measured on a laboratory scale. Roots, stems and shoots were measured after harvest, and the fresh weights of each sample were documented. The plant material was then dried in a chamber at 35 °C to a constant weight. The amount of water stored inside plant tissues was compared using the difference between fresh and dried weight (Sogoni, 2020).

2.4.4. Determination of chlorophyll content

The chlorophyll concentration was measured with a SPAD-502 chlorophyll meter (Konica-Minolta, Milnerton, South Africa) at the beginning and end of the growing season. This instrument measures red light transmission at a wavelength of 650 nm when chlorophyll absorbs light, and infrared light transmission at a wavelength of 940 nm when there is no absorption (Nkcukankcuka et al., 2021). The device determines a SPAD level using these two transmission values, which serve as a proxy for chlorophyll content. The chlorophyll content was measured in two completely developed leaves from each treatment, and the SPAD-502 m averaged the results to produce a final result. The readings were measured during weeks 2 and 12 of the trial, between 11 a.m. and midday.

2.5. The antioxidant and phytochemical analyses

2.5.1. Preparation of crude extract

After harvesting, the shoot materials were air-dried in a well-ventilated room kept at 37–40 °C under air condition for two weeks. The plant samples were transferred into an oven set at 45 °C for 12 hrs to crispy dry and milled to powder after which 100 mg of the powdered material was macerated in 70 % ethanol (25 mL) for 1 h to extract the shoot material. The mixture was filtered, and the supernatant was used for all analyses after centrifugation at 4000 rpm for 5 min.

2.5.2. Determination of secondary metabolite and antioxidant content

The antioxidant content and secondary metabolites accumulated in the tested samples were evaluated by referenced assays for DPPH (diphenylpicrylhydrazyl), ABTS/TEAC (total antioxidant capacity), reducing antioxidant power of iron (FRAP), total polyphenols and flavonols.

Table 1
Nutrient Film Technique hydroponic systems with different growing media and N fertilizer concentrations.

NFT/ Table	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	0.36 g/L + Peat	0.36 g/L + Vermiculite	0.36 g/L LECA clay	0.36 g/L Silica sand
2	0.60 g/L + Peat	0.6 g/L + Vermiculite	0.6 g/L + LECA clay	0.6 g/L + Silica sand
3	0.00 g/L + Peat	0 g + Vermiculite	0 g + LECA clay	0 g + Silica sand
4	0.80 g/L + Peat	0.8 g/L + Vermiculite	0.8 g/L + LECA clay	0.8 g/L + Silica sand

*NFT = Nutrient Filter Technique hydroponic system.

2.5.3. The ABTS/ TEAC antioxidant content

The ABTS antioxidant content was measured following a procedure used by Jimoh et al., (2019) with minor modifications. Both ABTS (7 mM) solution and $K_2S_2O_8$ (140 mM) solution were mixed to constitute a stock medium. The mixture was left to react at room temperature for 24 h. After 24 hrs, 25 µL each of the plant extracts and Trolox standard was reacted with 300 µL ABTS solution in a dark cupboard. After 5 min of incubation, the absorbance was taken on a microplate reader at 734 nm at 25 °C.

2.5.4. The DPPH antioxidant content

A 0.135 mM DPPH solution that was prepared in a dark bottle yielded the DPPH radical (Sogoni et al., 2021). A 25 µL volume each of plant extracts and graded Trolox standard (0–500 M) were reacted with 300 µL of DPPH solution and incubated for 30 mins after when a Thermo Electron Corporation's Multiskan Spectrum plate reader was used to measure the absorbance at 517 nm. The results were determined as M Trolox equivalent per gram dry weight (M TE/g DW).

2.5.5. Ferric reducing antioxidant power (FRAP)

The FRAP analysis was run according to the procedure used by Ngxabi et al., (2021). The FRAP reagent was prepared by mixing 3 mL of 2,4,6- tripyridyl-s-triazine (10 mM in 0.1 M HCl) with 30 mL of acetate buffer (0.3 M, pH 3.6), 3 mL $FeCl_3{\cdot}6H_2O$ (iron (III) chloride hexahydrate) diluted with distilled water (6 mL). A 10 µL volume of each of the plant extract and graded concentrations of L-ascorbic acid standard (0 to 1000 µM) was reacted with 300 µL of the FRAP solution in a 96-well plate. The 96-well microplate containing the mixture was incubated at room temperature. After 30 min of incubation, the absorbance was then read at 593 nm in a microplate reader. The FRAP antioxidant content of the tested extracts was extrapolated from the standard equation and expressed as µM ascorbic acid equivalents (AAE) per gram dry weight (µM AAE/g DW).

2.5.6. The flavonol content

The flavonol content of the tested extract was extrapolated from graded quercetin standard (0, 5, 10, 20, 40, and 80 mg/L) dissolved in 95 % ethanol as described by Bulawa et al., (2022). An equal volume (12.5 µL) of crude extracts and 0.1 % HCl were reacted in 95 % ethanol and 225 µL of 2 % HCl. After 30 min of incubation at room temperature, the absorbance was read at 360 nm. The flavonol content of the crude extract was estimated in milligrams of quercetin equivalent per gram of dry weight of the plant (mg QE/g DW).

2.5.7. The polyphenolic content

The assay for total polyphenols was performed as described by Ingarfield et al., (2023). A 125 µL volume of 10 % Folin-Ciocalteu reagent was reacted with 25 µL plant extract in a 96-well plate. Thereafter, 100 µL of 7.5 % Na_2CO_3 (sodium carbonate) was added, and the mixture was incubated for 2 h at room temperature. The absorbance was then measured at 765 nm in a Multiskan Spectrum plate reader. The polyphenol levels of the tested extracts were determined from graded concentrations (0 to 500 mg/L) of gallic acid standard. The polyphenol content was represented as mg GAE /g.

2.5.8. Nutritional analysis

Shoots of *M. crystallinum* cultivated in different media treated with various nitrogen concentrations were harvested near flowering. The harvested plant samples were kept in a labelled brown paper bag (16 x 24 cm). The marked brown paper bags containing the Shoots were laid on a table in a cool room to air dry after they were oven-dried at 40 degrees Celsius. The crispy dried plant materials were powdered using a capacity standard coffee grinder (Mellerware- Aromatic Coffee Mill &

Grinder) and the full feed analysis was carried out at the Department of Agriculture and Rural Development's analytical laboratory, KwaZulu Natal, South Africa.

2.5.9. Macronutrients and micronutrient analysis

The elemental compositions of the harvested plant materials from replicated treatments were analysed as described by (Adegbaju et al., 2019) with an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The analytical lab of the Department of Agriculture and Rural Development in KwaZulu Natal was the site of the ICP-OES's operation.

2.6. Proximate analysis

2.6.1. Crude fibre content

This crude fibre content was determined following the procedure of (Tshayingwe et al., 2023) with minor modifications. About 2 g of the powdered samples were boiled for 30 min with a digestion tablet in 100 mL of 1.25 per cent concentrated H_2SO_4 before being filtered under pressure. The digested residue was rinsed with 100 mL of 1.25 % NaOH solution after being washed repeatedly with boiling water till a clear mixture was found. Once the residue had been made, it was dried at 100 °C, cooled in a desiccator, and weighed (F1). The residues were then cooled in a desiccator, incinerated for 5 h at 550 °C in a muffle furnace, and then reweighed (F2). The estimated crude fibre was determined from the equation below.

$$\%\ \text{Crude fibre} = \frac{\text{F1} - \text{F2}}{\text{Original weight of the pulverised sample}} \times 100$$

2.6.2. Moisture content

A procedure described by Jimoh et al., (2020) was slightly modified to determine the moisture content of the tested plant samples. Empty ceramic vessels were dried for an hour at 105 °C in an oven, then left to cool, weighed and labelled W1. *Mesembryanthemum crystallinum* samples that had been ground into about 1 g each were put in a vessel and dried in an oven at 105 °C until a constant weight was obtained. The container and its contents were cooled in a desiccator and reweighed (W3). The equation below was used to calculate the percentage moisture content.

$$\%\ \text{Moisture content} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

2.6.3. Crude fat content

The crude fat content was calculated following the laboratory procedures from the Association of Official Analytical Chemists (AOAC, 2016). One gram of the pulverized plant was added to 100 mL of diethyl ether and shaken for 24 h on an orbital shaker. After extraction, The mixture was filtered, and the collected filtrate was transferred into a clean beaker that had previously been weighed (W1). After shaking the ether extract for an additional 24 h on an orbital shaker, the extract was diluted with 100 mL of diethyl ether to equilibrate, and the filtrate was also collected in beaker W1. Before being reweighed in the beaker (W2), the ether filtrate was dried in a steam bath to concentrate and later oven-dried at 55 °C. The crude fat in the tested plant was calculated as given in the equation below.

$$\%\ \text{Crude fat content} = \frac{\text{W2} - \text{W1}}{\text{original weight of the pulverised sample}} \times 100$$

2.6.4. Ash content

The protocol described by Mndi et al., (2023) was adopted to determine the ash content of the treated plant samples. Ceramic crucibles were dried in an oven for an hour at 105 °C, labelled with a heat-resistant marker, and then weighed (W1) after desiccation. Afterwards, 1 g of powdered plant was measured and placed into pre-weighed ceramic crucibles (W2). To ash the samples absolutely, the crucibles with their contents were heated to 250 °C for an hour, then 550 °C for 5 h in a muffle furnace. The samples were weighed (W3) after chilling in a desiccator. The equation below was used to determine the percentage of ash in the plant material that was tested.

$$\%\ \text{Ash content} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

2.6.5. Crude protein

The crude protein was estimated by heating 2 g of pulverised plant materials with 20 mL of concentrated H_2SO_4 to a clear mixture using a digestion tablet as a catalyst as reported by Idris et al., (2019). After digestion, the tested plant materials were filtered, and the extracts were distilled, then dissolved in 250 mL distilled water and transferred into a 500 mL round-bottomed flask containing 50 mL of 45 % NaOH where the aliquot underwent further distillation. A flask containing 250 mL of 2 % boric acid was filled with 150 mL of the distillate and a few drops of methyl indicator were added. The gaseous ammonia being liberated was trapped by submerging the end of the condenser in the liquid. The resulting liquid was back-titrated against 0.01 M HCl when turned violet. The percentage of nitrogen was from the equation below:

$$\%\text{Nitrogen} = \frac{[(\text{ml std acid} \times \text{N of acid}) - (\text{ml bank} \times \text{N of base})] - (\text{ml std base} \times \text{N of base}) \times 1.4007}{\text{original weight of the pulverised sample}}$$

The crude protein was calculated by multiplying the nitrogen value by a constant factor of 6.25, where N = normality (USDA, 2018).

2.6.6. Neutral detergent fibre (NDF)

The following equation was used to calculate the samples' NDF composition, as reported by Idris et al., (2019).

$$\%\ \text{NDF} = \frac{(\text{W1} + \text{W2}) - \text{W1}}{\text{Weight of the sample}} \times 100$$

2.6.7. Non-fibre carbohydrate (NFC)

The amount of non-fibre carbohydrates in the sample was determined using the formula below.

$$\%\ \text{NFC} = 100 - (\%\ \text{Ash} + \%\ \text{Crude fat} + \%\ \text{Crude protein} + \%\ \text{NDF})$$

2.6.8. Total carbohydrate (CHO)

The total CHO content of the tested plant was derived from the equation below according to Bulawa et al., (2022).

CHO = NFC + NDF.

2.7. Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine variability in chlorophyll content, nutrients, phytochemicals, and growth parameters. Means were compared at $P \leq 0.05$ using Fisher's least significant difference obtained from MINITAB 17 statistical software.

Table 2
Effect of different growth media and nitrogen concentration on the number of leaves of *M. crystallinun*.

Growth media	Nitrogen (g/L)	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
LECA clay	0.00	7.1 ± 0.45bc	8.3 ± 0.43c	5.4 ± 0.3f	4.8 ± 0.44f	3.2 ± 0.60d	2.2 ± 0.60d
	0.36	11.2 ± 1.79ab	10.4 ± 0.27ab	10.0 ± 0.30abc	8.6 ± 0.43abc	8.2 ± 0.40ab	9.2 ± 0.50a
	0.60	6.9 ± 1.62bc	8.4 ± 0.4bc	8.2 ± 0.47b-e	7.9 ± 0.50a-f	4.8 ± 1.00a-d	4.4 ± 1.15bcd
	0.80	7.1 ± 0.28bc	8.4 ± 0.22bc	8.1 ± 0.43b-e	7.2 ± 0.60a-f	7.2 ± 7.20ab	7.4 ± 0.50abc
Peat	0.00	7.8 ± 0.36abc	9.4 ± 0.43abc	6.4 ± 0.27ef	5.9 ± 0.40b	4.8 ± 0.68bcd	3.8 ± 0.70 cd
	0.36	12.8 ± 2.28a	8.6 ± 0.6ab	10.2 ± 0.36ab	9.0 ± 0.40b	8.8 ± 0.80a	9.2 ± 0.80a
	0.60	3.2 ± 0.49c	9.5 ± 0.27abc	7.8 ± 0.36cde	7.8 ± 0.50a-e	5.0 ± 0.60bcd	4.4 ± 0.80bcd
	0.80	6.4 ± 0.37bc	7.7 ± 0.26c	6.4 ± 0.26f	6.4 ± 0.20b-f	3.0 ± 0.60d	3.0 ± 0.8a-d
Silica sand	0.00	7.5 ± 0.4abc	8.7 ± 0.30abc	6.5 ± 0.5ef	5.2 ± 0.76ef	4.4 ± 0.83 cd	3.6 ± 0.88 cd
	0.36	5.7 ± 0.67abc	9.7 ± 0.40abc	9.0 ± 0.60a-d	8.2 ± 0.42abc	5.6 ± 0.60a-e	5.2 ± 0.68a-d
	0.60	2.9 ± 0.30c	9.0 ± 0.30abc	8.8 ± 0.68a-d	8.0 ± 0.73a-e	5.4 ± 0.99a-d	5.2 ± 0.90a-d
	0.80	9.1 ± 0.30ab	10.5 ± 0.27a	10.6 ± 0.40a	7.8 ± 0.80a-e	6.6 ± 0.67a-d	5.4 ± 0.5a-d
Vermiculite	0.00	7.8 ± 0.55abc	9.4 ± 0.60abc	6.3 ± 0.26ef	5.3 ± 0.63def	3.4 ± 0.73d	2.8 ± 0.74d
	0.36	9.9 ± 2.74ab	8.8 ± 0.50ab	9.4 ± 0.52a-d	7.9 ± 0.95a-e	6.7 ± 1.05	6.2 ± 1.09d
	0.60	11.1 ± 0.89ab	9.4 ± 0.43abc	10.6 ± 0.60a	9.3 ± 0.37a	8.8 ± 0.95a-d	8.2 ± 1.28ab
	0.80	8.9 ± 0.30ab	10.6 ± 0.43a	10.6 ± 0.52a	8.1 ± 0.65a-d	7.8 ± 0.76abc	7.4 ± 1.27abc

Means without a common letter are significantly different at $P \leq 0.05$; $N = 10$.

3. Results

3.1. Numbers of leaves

The number of leaves obtained from ten replicates (n = 10) in the experimented *M. crystallinum* varied in response to different growing media and nitrogen concentrations as shown in the variability observed in the treated plants (Table 2). At week 2 when the experiment just commenced, peat medium treated with 0.36 g/L produced more leaves than other treatments. This trend was replicated in peat in the 10th week but at week 12, peat and LECA clay supplemented with 0.36 g/L yielded the highest number of leaves whereas in all treatments, the control produced the least number of leaves (Table 2).

3.2. Root length

A significant variability was recorded in the root lengths of the experimented *M. crystallinum*. The longest root determined from ten replicates (n = 10) was recorded in the vermiculite and 0.80 g/L nitrogen treated plants while the shortest roots were produced by peat and silica treated with 0.60 g/L nitrogen and the untreated peat.

3.3. Total fresh and dry weight

The findings showed that different growing media and nitrogen concentrations had a significant effect on the fresh and dry weight of *M. crystallinum* ($P \leq 0.05$). The highest and least fresh weight values were respectively recorded in plants grown with peat treated with 0.60 g/L and the LECA clay control (Table 3). Conversely, plants harvested from LECA clay treated with 0.80 g/L had the highest dry weight values whereas all treatments had the least equivalent dry weight values.

3.4. Chlorophyll content of M. crystallinun leaves

The chlorophyll contents of *M. crystallinum* measured at week 2 (start) and week 12 (end) from ten replicates (n = 10) were significantly impacted by different growth media and nitrogen concentrations. At week 2, the highest chlorophyll concentrations were recorded in *M. crystallinum* cultivated within LECA clay and peat supplemented with 0.6 g/L and 0.8 g/L nitrogen although vermiculite treated with 0.6 g/L N fertilizer had an equivalent effect while the least chlorophyll content was recorded in LECA clay control (Table 4). In the 12th week, LECA clay treated with 0.6 g/L nitrogen produced the highest chlorophyll concentration whereas the least was recorded in the untreated peat (control).

Table 3
Effect of different growth media and nitrogen concentrations on root length, fresh weight, and dry weight of *M. crystallinun*.

Growth media	Nitrogen (g/L)	Root length (cm)	Fresh weight (g)	Dry weight (g)
LECA clay	0.00	4.40 ± 0.60cde	1.53 ± 0.24d	0.42 ± 0.99c
	0.36	11.20 ± 1.79abc	7.11 ± 1.90 cd	1.73 ± 0.33c
	0.60	6.90 ± 1.62bcde	17.45 ± 3.23bcd	8.00 ± 1.46abc
	0.80	11.00 ± 1.88a-d	29.23 ± 5.23abc	12.65 ± 2.46a
Peat	0.00	3.70 ± 0.72de	7.92 ± 2.67bcd	3.84 ± 1.25abc
	0.36	12.80 ± 2.28ab	7.38 ± 2.28bcd	2.43 ± 0.69c
	0.60	3.20 ± 0.49e	50.40 ± 12.80a	12.26 ± 3.78ab
	0.80	5.00 ± 2.02cde	12.09 ± 8.63bcd	3.54 ± 2.28abc
Silica sand	0.00	2.60 ± 0.22e	3.31 ± 0.74 cd	1.25 ± 0.37c
	0.36	5.70 ± 0.67b-e	9.73 ± 2.00bcd	3.31 ± 1.02bc
	0.60	2.90 ± 0.314e	10.74 ± 1.56bcd	4.48 ± 0.75abc
	0.80	4.70 ± 0.67cde	15.24 ± 3.5bcd	6.45 ± 0.63abc
Vermiculite	0.00	6.00 ± 0.58b-e	4.16 ± 2.37 cd	1.44 ± 2.37c
	0.36	9.90 ± 2.74a-e	33.35 ± 5.24ab	4.22 ± 1.27abc
	0.60	11.10 ± 0.89a-d	19.86 ± 6.52bcd	8.22 ± 3.22abc
	0.80	14.60 ± 2.73a	27.50 ± 8.11a-d	9.18 ± 3.12abc

Means without a common letter are significantly different at $P \leq 0.05$, $N = 10$.

3.5. The antioxidant and phytochemical analyses

3.5.1. ABTS/TEAC content

The ABTS/TEAC content obtained from three replicates (n = 3) in the tested leaf extract of *M. crystallinum* was significantly influenced by the N fertilizer supplemented with different growing media. The peat treated with 0.6 g/L N fertilizer had the highest ABTS/TEAC antioxidant content with a mean value of 12.9 μmol TE/g compared to other treatments (Table 5). The lowest mean value (7.9 μmol TE/g) was recorded in plants grown on vermiculite supplemented with N fertilizer (0.36 g/L).

Table 4
Effect of different growth media and nitrogen concentrations on chlorophyll content of *M. crystallinun.*

Growth media	Nitrogen (g/L)	Week 2	Week 12
LECA clay	0.00	0.71 ± 0.25 cd	1.12 ± 0.02bcde
	0.36	0.80 ± 0.26abcd	1.19 ± 0.02abcde
	0.60	0.91 ± 0.53a	1.27 ± 0.06a
	0.80	0.90 ± 0.34a	1.13 ± 0.02bcde
Peat	0.00	0.77 ± 0.03abcd	1.09 ± 0.01e
	0.36	0.86 ± 0.03abc	1.19 ± 0.02abcde
	0.60	0.90 ± 0.03a	1.19 ± 0.09abcde
	0.80	0.89 ± 0.05a	1.17 ± 0.03abcde
Silica sand	0.00	0.72 ± 0.04bcd	1.14 ± 0.02abcde
	0.36	0.82 ± 0.03abcd	1.23 ± 0.02ab
	0.60	0.88 ± 0.40ab	1.10 ± 0.03de
	0.80	0.88 ± 0.20ab	1.19 ± 0.02abcde
Vermiculite	0.00	0.68 ± 0.03d	1.1 ± 0.02cde
	0.36	0.76 ± 0.03abcd	1.22 ± 0.01abc
	0.60	0.91 ± 0.03a	1.21 ± 0.01abcd
	0.80	0.87 ± 0.3abc	1.1 ± 0.02cde

Means without a common letter are significantly different at $P \leq 0.05$, N = 10.

3.5.2. DPPH content

The DPPH antioxidant content in the leaves of *M. crystallinum* (n = 3) grown in different growing media treated with different concentrations of N fertilizer varied significantly. The highest mean value of DPPH antioxidant content (11.5 µmol TE/g) was obtained in LECA clay treated with 0.36 g/L compared to other treatments whereas the lowest DPPH value of 6.8 µmol TE/g was obtained in extracts of plants that were grown on vermiculite supplemented with 0.6 g/L N fertilizer (Table 5).

3.5.3. FRAP content

Nitrogen fertilizer with different growing media had a significant influence on the FRAP capacity of the leaves of *M. crystallinum* (Table 5). Three growth media namely peat treated with 0.6 g/L N fertilizer, LECA clay supplemented with 0.36 g/L and 0.6 g/L N fertilizer had the highest and equivalent effect on the FRAP antioxidants while the lowest FRAP value was obtained from vermiculite medium treated with 0.6 g/L N fertilizer.

3.5.4. Flavonols content

The flavonol content in the leaves (n = 3) varies significantly at $P \leq 0.05$ with different growing media supplemented with N fertilizer concentrations (Table 5). Plants that were grown with LECA clay supplemented by 0.60 g/L N fertilizer had the highest mean value of flavonols content (2.9 mg QE/g) in their leaves compared to other treatments. The lowest mean value of flavonols content (1.8 QE/g) was obtained from the extract of plants that were cultivated in vermiculite supplemented by 0.36 g/L of N fertilizer (Table 5).

3.5.5. Polyphenol content

Polyphenols in the treated *M. crystallinum* extracts (n = 3) varied significantly at $P \leq 0.05$ compared with each other and the control. Plants that were cultivated with silica sand supplemented with 0.6 g/L of N fertilizer had the highest mean value of polyphenol content (6.3 mg GAE/g) compared to other treatments. The lowest mean value of polyphenol content was yielded by plants that were grown with vermiculite treated with 0.6 g/L N fertilizer.

3.6. Nutritional properties of M. crystallinum

3.6.1. Macronutrients and micronutrients in M. crystallinum

Different growing media and nitrogen concentrations applied to *M. crystallinum* (n = 3) had a varied effect on the accumulation of macroelements in the plant. Plants with the highest potassium content were produced in vermiculite treated with 0.60 g/L while the lowest value of K was recorded in LECA clay supplemented with 0.36 g/L (Table 6). The lowest and highest Ca contents were respectively recorded in silica sand and peat media with corresponding dosages of 0.80 g/L and 0.60 g/L of N fertilizer. The untreated vermiculite (control) produced the highest Mg whereas LECA clay fertilized with 0.80 g/L nitrogen fertilizer had the least Mg content. Like Ca, the lowest and highest nitrogen mineral contents were respectively produced in the untreated silica sand and peat media, however, supplemented with 0.80 g/L N fertilizer. The lowest and highest phosphorus P were respectively obtained from plants grown in the untreated and 0.36 g/L silica sand although the 0.8 g/L treated vermiculite had an equivalent lowest yield of P with silica sand. Similarly, accumulation of sodium varied among treatments with 0.80 g/L nitrogen fertilizer-treated plants yielding the highest Na while the unfertilized silica sand produced plants with the lowest Na content (Table 6).(See Table 7).

Accumulation of micronutrients namely, Mn, Fe, Zn and Cu in tested *M. crystallinum* samples also varied considerably among treatments. The highest concentration of Mn and Zn were recorded in plants grown in silica sand with 0.36 g/L N fertilizer dosage while the lowest Mn value was derived from plants cultivated with peat (0.60 g/L N fertilizer) and Zn, from vermiculite supplemented with 0.36 g/L and 0.60 g/L N fertilizer dosages (Table 6). The lowest and highest Fe and Cu were obtained from peat and vermiculite media at different dosages of N fertilizer (Table 6).

3.6.2. Proximate composition

Proximate materials (ADF, ash, fat, moisture, NDF, protein, NFC and carbohydrate) varied considerably in *M. crystallinum* leaves (n = 3).

Table 5
The effect of different nitrogen concentrations and growing media on secondary metabolites and antioxidants in *M. crystallinum.*

Media	Nitrogen (g/L)	ABTS/ TEAC (µmol TE/g)	DPPH (µmol TE/g)	FRAP (µmol TE/g)	Flavonols (QE/g)	Polyphenols (mg GAE/g)
LECA clay	0.00	12.9 ± 0.31a	10.5 ± 0.29abcd	28.0 ± 1.01a	2.7 ± 0.08ab	5.6 ± 0.16ab
	0.36	8.0 ± 0.32de	8.0 ± 0.24ef	21.0 ± 0.35bcd	1.8 ± 0.08def	3.8 ± 0.08 fg
	0.60	9.8 ± 0.43b-e	8.9 ± 0.08de	22.4 ± 0.25bc	1.9 ± 0.013ef	4.4 ± 0.10def
	0.80	9.0 ± 0.49cde	10.9 ± 0.17abc	21.0 ± 0.8bcd	2.4 ± 0.13bcd	4.9 ± 0.21bcd
Peat	0.00	9.0 ± 0.33cde	9 ± 0.3cde	21.6 ± 0.75bcd	2.0 ± 0.05cdef	3.8 ± 0.15 fg
	0.36	9.0 ± 0.24cde	8.0 ± 0.29ef	18.0 ± 0.77d	1.8 ± 0.07f	3.5 ± 0.1gh
	0.60	7.9 ± 0.64e	6.8 ± 0.07f	19.0 ± 0.9 cd	2.1 ± 0.1cdef	3.0 ± 0.09 h
	0.80	9.0 ± 2cde	9.0 ± 0.3cde	18.0 ± 0.93d	1.9 ± 0.0.9def	3.5 ± 0.03gh
Silica sand	0.00	12.0 ± 0.43ab	10.0 ± 0.45a-d	22.5 ± 0.61b	2.5 ± 0.09abc	5.1 ± 0.12bcd
	0.36	12.0 ± 0.1ab	11.5 ± 0.42a	26.0 ± 0.09a	2.2 ± 0.08bcdef	4.7 ± 0.17cde
	0.60	11.5 ± 0.61abc	11.2 ± 0.38ab	28.0 ± 0.26a	2.9 ± 0.03a	5.3 ± 0.02bc
	0.80	8.7 ± 1.37de	9 ± 0.41b-e	21.5 ± 0.27bcd	2.1 ± 0.11cdef	5.2 ± 0.15bc
Vermiculite	0.00	11.0 ± 0.34abc	8.2 ± 0.35ef	19.0 ± 0.59bcd	2.3 ± 0.07bcde	5.6 ± 0.22ab
	0.36	10.0 ± 0.29b-e	7.8 ± 0.38ef	22.0 ± 0.95bc	2.3 ± 0.1bcde	5.3 ± 0.15bc
	0.60	10.7 ± 0.16a-d	10 ± 0.53a-d	22.0 ± 0.31bc	2.3 ± 0.06bcd	6.3 ± 0.17a
	0.80	11.0 ± 0.16abc	11 ± 0.56ab	21.0 ± 0.46bcd	1.9 ± 0.09def	4.3 ± 0.18ef

Means without a common letter are significantly different at $P \leq 0.05$, (n = 3).

Table 6
Macronutrients and micronutrients of *M. crystallinum*.

Media	Nitrogen (g/L)	Macronutrients (mg/100 g)						Micronutrients (mg/100 g)			
		K	Ca	Mg	N	P	Na	Mn	Fe	Zn	Cu
LECA Clay	0.00	10288.5 ± 1.5e	1420.5 ± 0.5 h	1249.4 ± 0.6f	3361.5 ± 1.5 *m*	529.3 ± 1.0e	2861.5 ± 1.5c	17.45 ± 0.10d	65.09 ± 0.11 h	9.36 ± 0.04e	0.93 ± 0.03d
	0.36	862.0 ± 2.0p	1549.5 ± 0.5 g	790.5 ± 0.5 l	3881.5 ± 1.5f	662.0 ± 2.8b	978.5 ± 1.5*n*	8.42 ± 0.00 l	49.60 ± 0.50j	8.40 ± 0.10gh	0.11 ± 0.01 g
	0.60	9422.5 ± 2.5i	1630.5 ± 0.5e	891.6 ± 1.6j	4471.5 ± 1.5c	660.9 ± 1.3b	1020.5 ± 0.5 *m*	10.70 ± 0.30i	50.90 ± 0.10i	7.85 ± 0.15i	0.15 ± 0.05 fg
	0.80	7379.0 ± 1.0 *m*	1390.5 ± 0.5i	761.8 ± 1.8 *m*	4397.0 ± 3.0d	550.6 ± 0.9d	2171.0 ± 1.0f	10.85 ± 0.15i	79.60 ± 0.40f	8.00 ± 0.10hi	0.23 ± 0.03ef
Peat	0.00	9919.0 ± 1.0f	1569.0 ± 1.0f	917.5 ± 2.5i	3519.0 ± 1.0 l	549.5 ± 0.7d	3774.5 ± 4.0b	9.75 ± 0.05j	23.15 ± 0.50 *m*	8.75 ± 0.05 fg	0.00 ± 0.00 h
	0.36	9338.5 ± 1.5j	2168.5 ± 1.5b	919.0 ± 1.0i	4677.5 ± 2.5b	529.7 ± 0.4e	1158.5 ± 1.5 k	11.30 ± 0.01 h	20.77 ± 0.03o	15.39 ± 0.41c	0.15 ± 0.05 fg
	0.60	10371.5 ± 1.5d	3039.0 ± 1.0a	959.1 ± 0.9 g	3728.5 ± 1.5j	549.3 ± 1.1d	1678.5 ± 1.5 g	6.15 ± 0.05 *m*	24.04 ± 0.06 l	8.95 ± 0.050ef	0.10 ± 0.00gh
	0.80	7083.0 ± 3.0*n*	2070.5 ± 0.5d	960.5 ± 0.5 g	5439.0 ± 1.0a	489.0 ± 1.4f	2509.0 ± 1.0b	15.95 ± 0.06e	32.55 ± 0.05 k	15.84 ± 0.06b	0.00 ± 0.00 h
Silica Sand	0.00	8958.5 ± 1.5 k	1210.5 ± 0.5 k	868.5 ± 1.5 k	3257.0 ± 3.0*n*	49.8 ± 0.3i	188.5 ± 1.5p	9.30 ± 0.00 k	67.80 ± 0.09 g	7.90 ± 0.00i	0.24 ± 0.04ef
	0.36	8611.0 ± 1.0 l	2099.0 ± 1.0c	939.0 ± 1.0 h	3997.5 ± 2.5e	839.8 ± 0.3a	1029.0 ± 1.0 l	35.80 ± 0.30a	22.05 ± 0.25*n*	17.95 ± 0.15a	0.95 ± 0.05d
	0.60	10849.0 ± 1.5b	1180.5 ± 0.5 l	1578.5 ± 1.5d	3768.0 ± 2.0 h	589.5 ± 0.7c	1429.0 ± 1.0 h	17.30 ± 0.20d	139.05 ± 0.45c	9.15 ± 0.15ef	2.05 ± 0.06c
	0.80	4062.0 ± 2.0o	770.5 ± 0.5p	480.9 ± 0.9*n*	3848.0 ± 2.0 g	339.8 ± 0.3 g	7762.0 ± 2.0a	17.95 ± 0.05c	22.38 ± 0.08*n*	8.2 ± 0.21hi	0.31 ± 0.01e
Vermiculite	0.00	9494.0 ± 6.0 h	1359.0 ± 1.0j	2048.0 ± 2.0a	3178.5 ± 1.5o	489.5 ± 2.1f	409.0 ± 1.0o	9.66 ± 0.05jk	384.75 ± 0.15a	9.32 ± 0.12e	2.15 ± 0.05c
	0.36	10662.0 ± 1.5c	1109.5 ± 0.5 *m*	1450.5 ± 1.5e	3762.5 ± 2.5hi	60.8 ± 1.3 h	1272.0 ± 2.0j	13.75 ± 0.25 g	81.95 ± 0.15e	6.35 ± 0.50j	2.15 ± 0.05c
	0.60	11231.0 ± 0.5a	1049.5 ± 0.5*n*	1589.5 ± 0.5c	3631.5 ± 1.5 k	588.9 ± 1.6c	1332.0 ± 2.0i	15.17 ± 0.07f	107.35 ± 0.05d	6.75 ± 0.60j	2.45 ± 0.05b
	0.80	9859.0 ± 1.0 g	988.5 ± 1.5o	1718.9 ± 1.1b	3759.5 ± 0.5i	50.1 ± 0.2i	2421.5 ± 1.5e	20.48 ± 0.08b	169.47 ± 0.17b	10.54 ± 0.05d	3.11 ± 0.01a

Means that do not share a letter are significantly different at $P \leq 0.05$, (n = 3).

LECA clay respectively treated with 0.8 and 0.36 g/L N fertilizer yielded the highest ADF and NDF contents whereas plants with the highest moisture, fat and carbohydrate were harvested from silica sand at different N dosages. The peat medium influenced the accumulation of non-fibre carbohydrates and proteins at different nitrogen dosages while the highest ash material was found in plants grown in the untreated vermiculite.

4. Discussion

Global climate change, including rising temperatures, water scarcity, and extreme weather changes, will continue to pose challenges in already stressed agricultural ecosystems due to a continued population increase resulting in increasing demand for food (Mangal et al., 2022; Nkrumah, 2018; Zulu et al., 2022). As the demand for food supply escalates and is expected to exceed the existing production capacity, it becomes a priority for growers, horticultural researchers, nutritionists and crop scientists to rework the improvement of cultivation methods by determining and implementing efficient systems that will optimize the bioaccumulation of nutrients in plants.

In South Africa, wild plants are not freely used as edible due to little information on many of the species. Notwithstanding, growing these plants could provide rural residents access to nutritious and wholesome food. The introduction of new crops or cultivation of underutilized vegetables could also boost the local economy by providing financial growth opportunities to disadvantaged households through job creation (Garekae and Shackleton, 2020; Nkrumah, 2018; Pereira et al., 2022; Zulu et al., 2022). *Mesembryanthemum crystallinum* is an underutilized vegetable that can be cultivated in high-saline soils with high levels of sodium equivalent to those found in seawater (Agarie et al., 2007; Atzori et al., 2017; Xia & Mattson, 2022). The succulent halophyte is becoming well-known around the world and being utilized as a pre-made salad for both local and commercial uses, as food, medicine, and as a source of soap suggesting that *M. crystallinum* is a plant with diverse medicinal and food values (Loconsole et al., 2019). Therefore, this study closes the information gap on cultivation, vegetative growth parameters, chlorophyll content, pharmacological potential and nutritional benefits of *M. crystallinum* cultivated under different growing media and nitrogen fertilizer treatments.

Nitrogen is one of the main factors restricting plant growth in both natural and agricultural environments because it is needed in the greatest amount of all critical elements for plant growth and development (Ghiasy-Oskoee et al., 2020; Jimoh et al., 2020). The roots of plants primarily take up nitrogen from the growth medium. Nitrogen fertilizer is mostly used in soilless culture to achieve maximum output. Both quality and productivity may be impacted by an inadequate or excessive supply (Nkcukankcuka et al., 2021; Viljoen et al., 2021). In this study, different growth parameters such as leaf number, leaf length, root length, and fresh and dry weight were examined on treated *M. crystallinum*. Plant growth results for *M. crystallinum* that were treated with nutrient solutions containing 0.8 g/L and 0.6 g/L of nitrogen fertilizer combined with vermiculite were the highest. Findings from this study are supported by a recent study by Feng & Zhang, (2021) which found that vermiculite treated with high nitrogen concentration produced the highest vegetative growth values. The air and water-holding ability of vermiculite compared to other growth media may be responsible for this outcome as reported by (Pisa et al., 2020). Furthermore, this study demonstrated that peat yielded the maximum fresh weight when supplemented with 0.6 g/L nitrogen, outperforming LECA-clay, vermiculite and silica sand. This is a result of the key function of peat in nutrient retention, high water-holding capacity, and high humus content which allows nutrient retention and availability to plants (Kitir et al., 2018). Plants grown with unfertilized LECA-clay had the lowest results suggesting that *M. crystallinum* needs nitrogen for optimum

Table 7
Effect of different growing media and different nitrogen concentrations on proximate materials of *M. crystallinum* leaves.

Media	Nitrogen (g/L)	ADF %	Moisture %	Ash%	Fat %	Protein %	NDF %	NFC %	Carbohydrate %
LECA Clay	0.00	11.93 ± 0.07gh	8.47 ± 0.15defg	35.25 ± 0.63d	2.33 ± 0.23ab	20.44 ± 0.55 h	23.72 ± 0.29 fg	18.26 ± 0.44bc	41.98 ± 0.37c
	0.36	20.92 ± 0.69ab	11.20 ± 0.38 g	31.15 ± 0.59f	2.05 ± 0.07aabc	23.62 ± 0.62de	30.83 ± 0.88a	12.35 ± 0.54e	43.18 ± 0.71abc
	0.60	20.39 ± 0.59ab	8.72 ± 0.38cdef	32.94 ± 0.57ef	2.04 ± 0.06abc	28.26 ± 0.34bc	27.07 ± 0.57bcd	9.69 ± 0.39f	36.76 ± 0.48e
	0.80	21.68 ± 0.32a	8.01 ± 0.03 fg	38.08 ± 0.88c	2.06 ± 0.06abc	27.17 ± 0.37c	27.24 ± 1.24bc	5.45 ± 0.64 g	32.69 ± 0.94f
Peat	0.00	11.39 ± 0.39 h	8.77 ± 0.15bcdef	32.23 ± 0.73f	1.89 ± 0.25bcd	21.34 ± 0.65gh	21.62 ± 0.48 h	22.62 ± 0.53a	44.24 ± 0.51ab
	0.36	14.36 ± 0.38ef	8.90 ± 0.08efg	29.00 ± 0.70 g	2.07 ± 0.07abc	28.98 ± 0.29b	22.74 ± 0.74gh	17.21 ± 0.45c	39.95 ± 0.60 cd
	0.60	12.99 ± 0.39eg	9.09 ± 0.2abcd	32.00 ± 1.11f	1.66 ± 0.16 cd	23.67 ± 0.33de	22.89 ± 0.15gh	19.78 ± 0.44b	42.67 ± 0.30bc
	0.80	11.69 ± 0.41hg	9.04 ± 0.26abcd	31.75 ± 0.88f	2.06 ± 0.06d	34.04 ± 0.06a	20.11 ± 0.52i	12.04 ± 0.38e	32.15 ± 0.45f
Silica Sand	0.00	20.26 ± 0.75b	9.46 ± 0.38abc	33.02 ± 0.37ef	2.38 ± 0.13a	19.65 ± 0.70 h	28.36 ± 0.41b	16.59 ± 0.40 cd	44.95 ± 0.41a
	0.36	16.96 ± 0.34c	8.69 ± 0.61cdefg	27.89 ± 0.25 g	2.17 ± 0.07ab	24.96 ± 0.59d	27.03 ± 0.14bcd	17.95 ± 0.26c	44.98 ± 0.20a
	0.60	14.76 ± 0.54de	9.74 ± 0.37ab	36.10 ± 1.00 cd	1.91 ± 0.13bcd	23.87 ± 0.33de	25.50 ± 0.54de	12.62 ± 0.50e	38.12 ± 0.52d
	0.80	11.99 ± 0.69gh	9.83 ± 0.37a	38.89 ± 0.79b	1.88 ± 0.2bcd	23.55 ± 0.55de	20.55 ± 0.46i	15.13 ± 0.50cde	35.68 ± 0.48ef
Vermiculite	0.00	11.70 ± 0.6gh	8.99 ± 0.01abcde	42.02 ± 0.49a	2.08 ± 0.10abc	19.94 ± 0.07 h	24.95 ± 0.06ef	11.01 ± 0.18ef	35.96 ± 0.08ef
	0.36	15.79 ± 0.21 cd	6.80 ± 0.07abcdef	32.65 ± 0.65ef	1.95 ± 0.06abc	23.06 ± 0.43ef	26.31 ± 0.33cde	16.03 ± 0.37 cd	42.34 ± 0.35bc
	0.60	14.83 ± 0.37de	8.83 ± 0.30bcdef	33.11 ± 0.29ef	2.31 ± 0.19cdab	21.95 ± 0.75eg	25.81 ± 0.41cde	16.82 ± 0.41 cd	42.63 ± 0.41bc
	0.80	15.06 ± 0.25de	8.39 ± 0.29defg	34.42 ± 0.57de	0.99 ± 0.05def	22.89 ± 0.59de	26.27 ± 0.29cde	15.43 ± 0.38cde	41.70 ± 0.34c

Means that do not share a letter are significantly different at $P \leq 0.05$. * ADF = acid detergent fibre, NDF = neutral detergent fibre, NFC = non fibre carbohydrate, (n = 3).

growth. This contravenes findings by Xia & Mattson, (2022) that *M. crystallinum* does not require fertilizer for growth.

The significance of chlorophyll as an indicator of sound health and productivity of plants cannot be underestimated (Liu et al., 2019; Wang et al., 2022). The impact of various nitrogen treatments and selected growing media on the chlorophyll content of *M. crystallinum* was examined in this study. The results of this investigation showed that nitrogen application had a positive effect on chlorophyll production in *M. crystallinum*. A combination of LECA clay and 0.6 g/L N supplement had a comparable effect on chlorophyll content with vermiculite (0.6 g/L N), peat (0.8 g/L), LECA clay (0.8 g/L), and peat (0.6 g/L) treatments produced the highest chlorophyll content. This corroborates findings of Trelka et al., (2010) who reported that growing medium had no impact on the amount of chlorophyll in leaves. Nevertheless, Muhammad et al., (2022) agree with the current study that the amount of chlorophyll in leaves increases with increasing N application. These results indicate that *M. crystallinum* can be cultivated in most growing media enhanced with nitrogen treatments to optimize chlorophyll levels. Although growing media are not always necessary for chlorophyll formation (Ngxabi et al., 2021), findings from this study are in tandem with other studies that the higher the nitrogen fertilizer application, the higher the chlorophyll content (El-Banna et al., 2022; Muhammad et al., 2022; Zhang et al., 2022).

The diversity of secondary metabolites in plants reveals their therapeutic potential as phytochemicals exert tremendous influence on the antioxidant properties of plants (Jimoh et al., 2019). However, the accumulation of phytonutrients is modulated by the twin effect of Nitrogen availability and the right growing medium (Song et al., 2019). The findings of this study demonstrated that the accumulation of phytochemicals and antioxidants was significantly influenced by light soilless medium and moderate N fertilizer application. This was supported by the findings of Xia & Mattson, (2022) who recorded lower phenolic acids in *M. crystallinum* grown under different light treatments compared to this study, although higher phenolics were recorded in the same plant subjected to salt and water stress (Mndi et al., 2023). Notwithstanding, this work contradicts earlier research by Ismail-Embong et al., (2021) which claimed that only growing media with a high capacity for retaining water, such as peat and vermiculite, can produce significant levels of secondary metabolites. The study also found no connection between high phytochemical and antioxidant content and fertilizer use as high amounts of ABTS/TEAC and FRAP antioxidants were recorded in the unfertilized LECA clay. This agrees with the findings of Tshayingwe et al., (2023) who reported the highest ABTS/TEAC and FRAP antioxidants in *Trachyandra divaricata* grown with LECA clay. However, 0.36 g/L N fertilizer had an equivalent effect on flavonol metabolites, FRAP, and DPPH antioxidants. Given the highest influence of vermiculite on mean values of polyphenols, this study shows that growing media with a high water-holding capacity could modulate the accumulation of polyphenols in plants.

Recently, Xia & Mattson, (2022) and Mndi et al., 2023) examined the nutritional properties of *M. crystallinum* subjected to stress due to salinity and irrigation intervals. The reported proximate compositions of the tested plant materials were comparable to those found in this research except that the protein content was higher in this study. Generally, the unfertilized treatments (control) in all growth media yielded the least protein content while *M. crystallinum* with the highest protein was recorded in peat medium supplemented with the highest dosage of nitrogen fertilizer. High protein yield may be attributed to the dosages of nitrogen fertilizer applied to the plant although the protein yield varied in other fertilized growth media. The lack of consistency in treatments that received different dosages of N fertilizer may be due to the physico-chemical characteristics of the growth media which may

have influenced the protein yield. However, a similar trend was reported by Bressani et al., (1987) and (Duncan et al., 2018) where fertilizer application did not influence the protein yield in amaranths and wheat. Similarly, macronutrient and micronutrient levels of tested *M. crystallinum* in this study were higher than those reported by Xia & Mattson, (2022) and Mndi et al., (2023), both of which applied salinity to *M. crystallinum*. Undoubtedly, salinity could have modulated the nutrient levels in the plant whereas, the elevated mineral concentrations observed in this study may not be unconnected with the applied dosages of nitrogen fertilizer. However, both studies reported a higher level of Na^+ due to salt treatments.

The influence of nitrogen fertilizer and growing media on the nutritional properties of plants has been reported (Effah et al., 2023; Miceli and Miceli, 2014). Findings from this study suggest that the selected treatments (different growth media and nitrogen fertilizer dosages) could optimize the mineral elements and proximate content of *M. crystallinum*. Since its introduction in 1943, Recommended Dietary Allowances (RDA) has been widely considered the standard reference guide for nutrition, dietetics, and allied health (Institute of Medicine, 2006; Sato et al., 2023). RDA is the recognized scale for measuring nutrient allowances for healthy individuals. Interestingly, all macronutrients (K, Ca, Mg, N, Na) and micronutrients (Mn, Fe, Zn, Cu) profiled in the experimented *M. crystallinum* exceed the RDA threshold for healthy individuals. The daily nutritional needs of adults should contain 20–35 % protein, 20–35 % fat, and 45–65 % carbohydrate to reduce the risk of developing chronic diseases. The acceptable ranges for children are like those for adults, except that infants and younger children need a somewhat higher proportion of fat in their diets (Adegbaju et al., 2019; Institute of Medicine, 2006). Given the high level of minerals and proximate materials recorded in this study, it is apparent that daily minerals and proximate requirements could be guaranteed by consuming *M. crystallinum*.

5. Conclusion

In conclusion, the interactive effects of different dosages of nitrogen fertilizer and growing media could enhance the growth potential, chlorophyll content, phytochemicals, and nutritional properties of *M. crystallinum*. Given the high level of minerals and proximate materials recorded in this study, it is apparent that daily minerals and proximate requirements could be guaranteed by consuming *M. crystallinum*. Further studies are recommended on the characterization of index metabolites in the plant and the potential use of these chemicals in drug production to promote healthy living.

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Declaration of competing interest

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

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