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Research article

Enhancing sunflower microgreen yield and quality through foliar application of various nutrient solutions

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Abstract

Importance of the work: Sunflower microgreen production commonly involves the use of growing media without an initial nutrition input, leading to lower yields and compromised quality.
Objectives: To enhance the sunflower microgreen yield and quality based on the foliar application of various nutrient solutions and a hydroponic solution with additional nutrient.
Materials & Methods: Foliar nutrient solutions, namely hydroponic nutrient solution (HNS), HNS + ammonium sulfate (HNS-AS), HNS + urea (HNS-U), HNS + monosodium glutamate (HNS-MSG) and distilled water (DW) were tested on sunflower microgreens based on a completely randomized design with three replications. The yield and quality parameters of the sunflower microgreens were measured.
Results: The HNS-U treatment induced the highest fresh weight, while the dry weight and water contents were not significantly different among the treatments. The HNS-AS treatment had the highest contents of chlorophyll a and carotenoids, while the HNS-MSG treatment had the highest contents of chlorophyll b and total chlorophylls. The DW treatment produced the highest ratios of chlorophyll a-to-b and chlorophyll a-to-total chlorophyll, the HNS treatment promoted the highest ratios of chlorophyll a-to-carotenoid and total chlorophyll-to-carotenoid and the HMS-MSG treatment stimulated the highest ratios of chlorophyll b-to-total chlorophyll and chlorophyll b-to-carotenoid. The protein and ammonium contents of the sunflower microgreens were highest in the HNS-MSG treatment. The sunflower microgreens treated with HNS had the highest nitrate content. However, nitrite levels were not affected by the different foliar applications. Furthermore, the sunflower microgreens treated with HNS-MSG contained four detectable amino acids: cysteine, phenylalanine, tyrosine and tryptophan.
Main finding: The foliar application of HNS-MSG showed potential for enhancing the yield and quality of sunflower microgreens.

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Introduction

Microgreens offer an excellent option for urban agriculture, thriving in home gardens or compact indoor spaces, providing there is sufficient access to light (Halloran and Magid, 2013). A diverse array of vegetables has been cultivated specifically for microgreen production, including broccoli, Chinese kale, purple radish, radish, rat-tailed radish, red cabbage, fenugreek, green pea, lentil, black sesame, buckwheat, morning glory, red roselle, sunflower and mung bean (Kowitcharoen et al., 2021). Microgreens, characterized by their distinctive single pair of fully developed leaves, constitute the youthful seedlings of herbs or tender, immature green vegetables; typically, they are ready for harvest within 7–21 d after germination (Bhaswant et al., 2023). These tiny greens are not only rich in flavor but are also packed with vitamins, minerals and antioxidants (Turner et al., 2020). The microgreens market has experienced substantial growth, finding applications in restaurants as edible embellishments for a variety of cuisines. Additionally, consumers appreciate incorporating them fresh into salads, soups, sandwiches and other dishes. These tiny greens contribute to improving dishes by enhancing flavor, introducing vibrant color, improving texture and supplementing their nutritional value (Li et al., 2021). In the context of microgreen consumption in Thailand, sunflower microgreens are a popular choice among customers (Chunthawodtiporn et al., 2023). Microgreens are abundant in protein, vitamin C, total phenols and fiber, as well as showcasing heightened antioxidant activity and being recognized for their appealing sensory qualities, positioning them as an excellent option for inclusion in salads (Dhaka et al., 2023).

Currently, there has been strong emphasis on investigating foliar application of fertilization because plant leaves can absorb nutrients in addition to the roots (Kannan, 2010). Foliar nutrition plays a crucial role by enabling rapid and straightforward nutrient absorption, penetrating the stomata or leaf cuticle, and accessing the cells (Nandhini and Suganthi, 2017). A variety of nutrient solutions have been developed for plant cultivation in diverse agricultural practices. Notably, Hoagland's nutrient solution has become widely popular and is recommended for integrated agricultural production to improve both crop yield and quality (Maneetong et al., 2013; Schwabe et al., 2013; Li and Cheng, 2015).

Foliar fertilizers are a well-suited, efficient, cost-effective, and labor-saving approach, especially for microgreen production. The cultivation of microgreens using growing media with minimal nutrients input can indeed impact both the yield and quality of the microgreens. The foliar application of a modified Hoagland's nutrient solution could help to enhance the productivity and quality of microgreen yields. Therefore, the current study aimed to investigate the effects of various foliar applications based on a modified Hoagland's nutrient solution to enhance the yield and quality of sunflower microgreens.

Materials and Methods

Plant materials

Sunflower seeds (*Helianthus annuus* L.) were weighed (50 g per sample) and then immersed in warm water at 50°C for 6 hr. Before planting, plastic trays with dimensions of 33 cm ×25 cm × 5 cm and equipped with drainage holes were lined with tissue paper, which served as a medium for supporting root growth. These trays were thoroughly saturated with distilled water until the tissue paper was completely wet. After sowing the seeds, the trays were placed at room temperature (approximately 28–30°C) and received a daily watering of 100 mL of distilled water per tray for 5 d. Subsequently, the microgreens were treated with 30 mL of foliar application per tray, tailored to each specific treatment, and left for 24 hr. Following this, the microgreens were exposed to red-blue light-emitting diode (LED) light with a photosynthetic photon flux density of 250 μmol/m^2/s for 48 hr before being harvested. After harvest, the microgreens were rinsed with distilled water, packaged in Ziploc bags and stored in a freezer at -20°C prior to plant analysis. This experiment was conducted at the Laboratory of Urban Agriculture Technology, a part of the Division of Agricultural Technology within the Department of Agricultural and Fishery Science, Faculty of Science and Technology at Prince of Songkla University, Pattani campus, Thailand.

Experimental design

The hydroponic nutrient solutions were derived from a modified Hoagland's nutrient solution (Sirinupong, 2017). There were five treatments: 1) hydroponic nutrient solution (HNS) containing 390 mg/L potassium nitrate, 65 mg/L monoammonium phosphate, 500 mg/L calcium nitrate, 250 mg/L magnesium sulfate, 50 mg/L monopotassium phosphate, 4 mg/L manganese sulfate, and micronutrients

(4 mg/L each of boron ethylenediaminetetraacetic acid (EDTA), Mn EDTA, MgO, Cu EDTA, Mo EDTA and Fe EDTA) and 25 mg/L iron chelate; 2) HNS-MSG (HNS + 3.365 mg/L monosodium glutamate); 3) HNS-U (HNS + 2.561 mg/L urea); 4) HNS-AS (HNS + 1.316 mg/L ammonium sulfate); and 5) distilled water (DW) as a control treatment. The experiment was conducted with three replications. A completely randomized design was applied to investigate the impact of the treatments.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). When the ANOVA indicated significance, differences among the means were further evaluated at a 95% confidence using the least significant difference (LSD). Data processing was carried out using the Excel software package (version 7.0, Microsoft Corp.; Redmond, WA, USA).

Measurements of fresh, dry weight and water content

The fresh weight of each sunflower microgreen was determined after delicately patting dry with soft tissue paper to eliminate any surface moisture. Immediately, the weight was recorded of 100 microgreen seedlings. The dry weight of the 100 microgreen seedlings of sunflowers was determined following drying in an oven at 65°C for 48 hr and then cooling in a dry Ziplock bag before being weighed.

Typically, the water content in the sunflower microgreens is expressed as a percentage of the fresh weight that is measured by comparing the weight of a fresh sunflower microgreen sample with the weight of the same sample after it has been dried to remove all water. The formula for calculating the water content percentage is shown in Equation 1:

$$\text{Water content \%} = [(\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight}] \times 100 \quad (1)$$

Determination of chlorophyll a, chlorophyll b and carotenoid Contents

The contents of chlorophyll a, chlorophyll b and carotenoids were determined based on extraction using ethanol following the protocols outlined in the studies of Kukrić et al. (2012) and Chang et al. (2013). In brief, precisely 0.1 g of microgreens was weighed and then finely ground using a mortar and pestle. Then, these ground materials were subjected to extraction with 15 mL of ethanol. Subsequently, the extracted solution was passed through filter paper No. 1 and transferred into a microcentrifuge tube. The tube was kept in the absence of light and later centrifuged before transferring the solution into a 96-well microplate. The quantification of the contents of the chlorophylls and carotenoid was carried out based on spectrophotometric analysis using absorption measurements spanning the range 350–700 nm. The calculations were performed based on Equations 2–4:

$$\text{Chloroplyll a} = \frac{13.7A_{665} - 5.76A_{649}}{\text{mass} \times 200} \quad (2)$$

$$\text{Chloroplyll a} = \frac{13.7A_{665} - 5.76A_{649}}{\text{mass} \times 200} \quad (3)$$

$$\text{Carotenoids} = \frac{4.7A_{440} - 0.263c_{\text{chla+chlb}}}{\text{mass} \times 200} \quad (4)$$

where A_x is the absorption at the wavelength x, chla is the amount of chlorophyll a, chlb is the amount of chlorophyll b and the three parameters are all measured in milligrams per gram.

Nitrate determination

The nitrate content was determined based on a colorimetric method using salicylic acid, as described by Hachiya and Okamoto (2017). The sunflower microgreen samples were washed with tap water and subsequently rinsed twice with deionized water. Initially, 2 g of each microgreen samples were extracted with 5 mL of deionized water and then incubated in a boiling water bath for 20 min. After cooling to room temperature, the supernatant was collected via centrifugation at 20,100×*g* at 4°C for 10 min. A 10 µL portion of the resulting extract was mixed with 40 µL of a 0.05% (weight per volume; w/v) salicylic acid solution in sulfuric acid within a 1.5 mL microtube. This mixture was thoroughly vortexed. Next, the solution samples were left at room temperature for 20 min and then 1 mL of 8% (w/v) NaOH solution in deionized water was added to each sample. Subsequently, the absorbance was measured at 410 nm using a spectrophotometer. The nitrate content in each sample, was expressed in millimoles per unit of fresh weight and determined using Equation 5:

$$[\text{True nitrate concentration}] = [\text{Extracted volume}] / [\text{Fresh weight}] \quad (5)$$

where the concentration is measured in micromoles, the volume is measured in milliliters and the weight is measured in grams.

Nitrite determination

The nitrite content was determined using the Griess reaction method (Hachiya and Okamoto, 2017). Initially, each sunflower microgreen sample was washed with tap water and then rinsed twice with deionized water. Next, 2 g of the microgreen sample were initially extracted with 5 mL of deionized water. Subsequently, the supernatant was separated using centrifugation at 20,100×*g* and kept at 4°C for 10 min. Then, a mixture was prepared, combining 260 µL of the supernatant with 65 µL 1% (w/v) sulfanilamide in 1 mol/L hydrochloric acid, 65 µL 0.02% (w/v) N-1-naphthylethylenediamine dihydrochloride in deionized water and 910 µL deionized water. This mixture was transferred to a 1.5 mL microtube and incubated at room temperature for 15 min. The absorbance of the solution was measured at 540 nm using a spectrophotometer. The nitrite content of each sample, was expressed in micromoles per gram of fresh weight and was determined using Equation 6:

$$[\text{True nitrite concentration}] = [\text{Extracted volume}] / [\text{Fresh weight}] \quad (6)$$

where the concentration is measured in micromoles, the volume is measured in milliliters and the weight is measured in grams.

Ammonium determination

The ammonium content was determined using the ammonia-salicylate method (Hachiya and Okamoto, 2017). The salicylate/nitroprusside solution was made by dissolving 150 g sodium salicylate and 0.30 g sodium nitroprusside and then diluting to a final volume of 1 L. The hypochlorite solution was prepared by diluting 6 mL 5.25% sodium hypochlorite to 100 mL. Each sunflower microgreen sample underwent an initial wash with tap water, followed by two subsequent rinses with deionized water. In the first step of the extraction process, 2 g of the microgreen sample were treated with 5 mL 0.1 M potassium chloride. The resulting supernatant was collected using centrifugation at 20,100×*g* and kept at 4°C for 10 min. Subsequently, 40 µL of the supernatant was carefully pipetted into a 96-well plate. Following this, 80 µL salicylate/nitroprusside solution and 80 µL hypochlorite solution were added to each well. All aliquots were mixed thoroughly by pipetting up and down. Then, the mixtures were incubated at room temperature for 45 min. The absorbance of the solutions was measured at 650 nm using a spectrophotometer. The apparent ammonium concentration of the supernatant was determined using a standard curve. The ammonium content of the sample, was expressed in micromoles per gram of fresh weight and calculated using Equation 7:

$$[\text{True ammonium concentration}] = [\text{Extracted volume}] / [\text{Fresh weight}] \quad (7)$$

where the concentration is measured in micromoles, the volume is measured in milliliters and the weight is measured in grams.

Protein determination and amino acid estimation

The protein contents of the sunflower microgreens were determined using the following procedure and to estimate the amino acid compositions. Initially, the microgreens were dried and subsequently ground into a fine powder using a mortar and pestle. A sample of 1.25 g per treatment was accurately weighed and transferred into a 50 mL beaker. Subsequently, 10 mL of methanol was added to the sample and the mixture was gently heated on a hotplate while stirring. After a brief heating period, the sample was left standing for 15 min. Following this, the solution was passed through filter paper No. 1 into a 25 mL volumetric flask and then diluted with a methanol solution. The absorbance was measured across a wavelength range of 200–600 nm using an ultraviolet-visible wavelength spectrophotometer (Okoronkwo et al., 2017). Amino acids were identified at specific wavelengths, according to Okoronkwo et al. (2017): cysteine (204–220 nm), phenylalanine (240–265 nm), tyrosine (274–330 nm) and tryptophan (275–312 nm). A percent solution extinction coefficient (ε) was used to determine the protein concentration. While the extinction coefficients in most proteins typically are in the range 4.0–24.0, an average value of around 10 is often used for a mixture of various proteins (Thermo Scientific, 2002). In this study, the protein concentration was calculated using Equation 8:

$$\text{Concentration} = (\text{Absorbance} / {}^{\varepsilon}\text{percent}) \times 50 \quad (8)$$

where the concentration is measured in milligrams per milliliter.

Results

Fresh weight, dry weight, and water content

The HNS-U significantly induced the highest fresh weight of sunflower microgreens (0.27 g/seedling) but was not significantly different to that treated with HNS-MSG (0.26 g/seedling), as shown in Fig. 1A. The HNS-MSG treatment (0.0133 g/seedling) had the highest dry weight value, closely followed by the HNS-U, HNS-AS, HNS, and DW treatments at 0.0130 g/seedling, 0.0130 g/seedling, 0.0127 g/seedling and 0.0120 g/seedling, respectively (Fig. 1B).

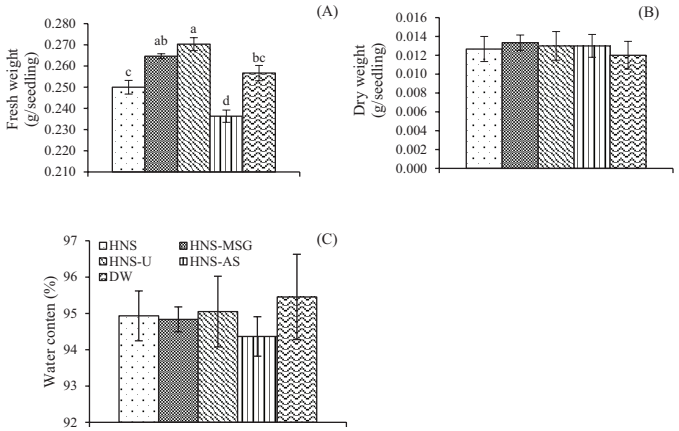

Fig. 1 Evaluation of sunflower microgreens under different foliar nutrient solutions: (A) fresh weight; (B) dry weight; (C) water content, where HNS = hydroponic nutrient solution; HNS-AS = HNS + ammonium sulfate; HNS-U = HNS + urea; HNS-MSG = HNS + monosodium glutamate; DW = distilled water, error bars indicate ± SD for three replications; Different lowercase letters above the bars indicate significant ($p < 0.05$) differences based on least significant difference test ($n = 15$).

Based on the findings, the water content in the sunflower microgreens was highest in the DW treatment (95.5%), followed by the HNS-U, HNS, HNS-MSG and HNS-AS treatments, with values of 95.1%, 94.9%, 94.8% and 94.4%, respectively (Fig. 1C). Nonetheless, there was no clear impact of the different nutrient solutions on either the dry weight (Fig. 1B) or water content (Fig. 1C) of the sunflower microgreens.

Chlorophyll a, chlorophyll b, total chlorophylls, carotenoid contents and pigment ratios

The HNS-AS foliar application significantly induced the highest chlorophyll a content (0.33 mg/g fresh weight; FW) which was not significantly different from that of DW (0.31 mg/g FW), whereas the HNS treatment produced the lowest value (0.27 mg/g FW), as shown in Table 1.

The HNS-MSG foliar application significantly induced the highest chlorophyll b content (0.31 mg/g FW) which was not significantly different from that of HNS-U (0.28 mg/g FW), whereas both the HNS and DW treatments produced the lowest value (0.23 mg/g FW), as shown in Table 1.

The HNS-MSG foliar application significantly induced the highest total chlorophyll content of 0.60 mg/g FW which was not significantly different from that of HNS-AS (0.57 mg/g FW) and HNS-U (0.56 mg/g FW), whereas the HNS treatment produced the lowest value (0.50 mg/g FW), as shown in Table 1.

The HNS-AS foliar application significantly induced the highest carotenoid content (0.21 mg/g FW) which was not significantly different from that of DW (0.20 mg/g FW), whereas the HNS treatment produced the lowest value (0.16 mg/g FW), as shown in Table 1.

Table 1 Pigments and pigment ratios of sunflower microgreens under foliar application of various nutrient solutions

Pigment	HNS	HNS-MSG	HNS-U	HNS-AS	DW
Chlorophyll a	0.27 ± 0.01^{d}	0.29 ± 0.02^{bc}	0.29 ± 0.00^{cd}	0.33 ± 0.01^{a}	0.31 ± 0.01^{ab}
Chlorophyll b	0.23 ± 0.01^{b}	0.31 ± 0.07^{a}	0.28 ± 0.01^{ab}	0.25 ± 0.02^{b}	0.23 ± 0.02^{b}
Total chlorophylls	0.50 ± 0.01^{c}	0.60 ± 0.05^{a}	0.56 ± 0.01^{ab}	0.57 ± 0.02^{ab}	0.54 ± 0.03^{bc}
Carotenoids	0.16 ± 0.01^{c}	0.19 ± 0.02^{b}	0.19 ± 0.09^{b}	0.21 ± 0.00^{a}	0.20 ± 0.01^{ab}
Pigment ratio					
Chlorophyll a-to-b ratio	1.19 ± 0.08^{ab}	0.97 ± 0.19^{b}	1.04 ± 0.03^{b}	1.33 ± 0.08^{a}	1.36 ± 0.03^{a}
Chlorophyll a-to-total chlorophyll ratio	0.54 ± 0.02^{ab}	0.49 ± 0.05^{b}	0.51 ± 0.01^{b}	0.57 ± 0.02^{a}	0.58 ± 0.01^{a}
Chlorophyll a-to-carotenoid ratio	1.73 ± 0.08^{a}	1.53 ± 0.08^{b}	1.52 ± 0.01^{b}	1.55 ± 0.03^{b}	1.57 ± 0.01^{b}
Chlorophyll b-to-total chlorophyll ratio	0.46 ± 0.02^{ab}	0.51 ± 0.05^{a}	0.49 ± 0.01^{a}	0.43 ± 0.02^{b}	0.42 ± 0.01^{b}
Chlorophyll b-to-carotenoid ratio	1.47 ± 0.04^{ab}	1.65 ± 0.35^{a}	1.46 ± 0.04^{ab}	1.17 ± 0.08^{b}	1.15 ± 0.03^{b}
Total chlorophylls-to-carotenoid ratio	3.20 ± 0.05^{a}	3.18 ± 0.36^{a}	2.98 ± 0.05^{ab}	2.72 ± 0.09^{b}	2.72 ± 0.03^{b}

HNS = hydroponic nutrient solution; HNS-AS = HNS + ammonium sulfate; HNS-U = HNS + urea; HNS-MSG = HNS + monosodium glutamate; DW = distilled water.

Mean ± SD in each row with different lowercase superscripts are significantly ($p < 0.05$) different based on least significant difference test ($n = 15$).

The DW treatment produced the significantly highest chlorophyll a-to-chlorophyll b ratio (1.36) which was not significantly different from that of HNS-AS (1.33) and HNS (1.19), whereas the HNS-MSG treatment had the lowest ratio (0.97), as shown in Table 1.

Similarly, the DW treatment had the significantly highest chlorophyll a-to-total chlorophyll ratio (0.58) which was not significantly different from that of HNS-AS (0.57) and HNS (0.54), whereas the HNS-MSG treatment had the lowest ratio (0.49), as shown in Table 1.

The HNS treatment had the significantly highest chlorophyll a-to-carotenoid ratio (1.73), whereas the HNS-U treatment had the lowest ratio (1.52), as shown in Table 1.

The HNS-MSG treatment had the significantly highest chlorophyll b-to-total chlorophyll ratio (0.51) which was not significantly different from that of HNS-U (0.49) and HNS (0.46), whereas the DW treatment had the lowest ratio (0.42), as shown in Table 1.

The HNS-MSG treatment had the significantly highest chlorophyll b-to-carotenoid ratio (1.65) which was not significantly different from that of HNS (1.47) and HNS-U (1.46), whereas the DW treatment had the lowest ratio (1.15), as shown in Table 1.

The HNS treatment had the significantly highest total chlorophyll-to-carotenoid ratio (3.20) which was not significantly different from that of HNS-MSG (3.18) and HNS-U (2.98), whereas the DW and HNS-AS treatments both had the lowest ratio (2.72), as shown in Table 1.

Nitrate, nitrite, ammonium and protein contents

The foliar application of HNS significantly induced the highest content of nitrate in the sunflower microgreens (0.60 mmol/g FW), while treating these tiny greens with HNS-AS and DW resulted in the equally lowest nitrate contents (0.21 mmol/g FW), as shown in Fig. 2A.

The foliar application of HNS had highest content of nitrite in the microgreens (5.31 µmol/g FW), whereas the HNS-AS treatment had the lowest nitrite content (2.39 µmol/g FW). However, there were no significant differences in the nitrite content of sunflower microgreens among the treatments (Fig. 2B).

The foliar application of HNS-MSG induced the significantly highest content of ammonium in the sunflower microgreens (235.61 µmol/g FW); however, this was no significantly different from that of HNS (220.94 µmol/g FW) and HNS-U (214.94 µmol/g FW), whereas the DW treatment had the lowest ammonium contents (91.61 µmol/g FW), as shown in Fig. 2C.

The foliar application of HNS-MSG induced the significantly highest protein contents in the sunflower microgreens (9.75 mg/mL) which was not significantly different from that of HNS (9.71 mg/mL), whereas the DW treatment had lowest nitrate contents (7.10 mg/mL), as shown in Fig. 2D.

Estimation of amino acid compositions

The foliar application of HNS-MSG stimulated the formation or production of all four amino acids, while the formation or production of both cysteine and phenylalanine was not detected following foliar fertilization with HNS (Table 2). However, tyrosine and tryptophan were obtained in all treatments.

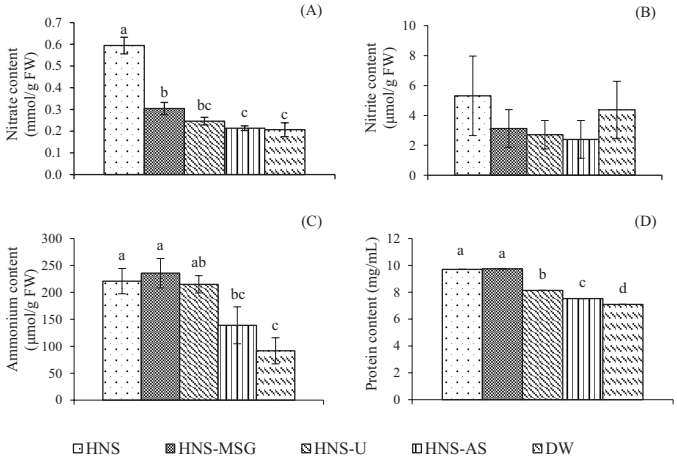

Fig. 2 Evaluation of sunflower microgreens under different foliar nutrient solutions: (A) nitrate content; (B) nitrite content; (C) ammonium content; (D) protein content, where HNS = hydroponic nutrient solution; HNS-AS = HNS + ammonium sulfate; HNS-U = HNS + urea; HNS-MSG = HNS + monosodium glutamate; DW = distilled water, error bars indicate ± SD for three replications; Different lowercase letters above the bars indicate significant ($p < 0.05$) differences based on least significant difference test ($n = 15$).

Table 2 Estimation of amino acids (cysteine, phenylalanine, tyrosine and tryptophan) in sunflower microgreens under various foliar nutrient solutions

Treatment	Cysteine	Phenylalanine	Tyrosine	Tryptophan
HNS	-	-	+	+
HNS-MSG	+	+	+	+
HNS-U	+	-	+	+
HNS-AS	+	-	+	+
DW	+	-	+	+

HNS = hydroponic nutrient solution; HNS-AS = HNS + ammonium sulfate; HNS-U = HNS + urea; HNS-MSG = HNS + monosodium glutamate; DW = distilled water.
+ = presence of amino acid; - = absence of amino acid.

Discussion

The current research investigated the impact of the foliar application of various foliar nutrient solutions on the growth, pigments, minerals, protein contents and estimated amino acid composition in sunflower microgreens.

Fresh weight, dry weight and water content

The fresh and dry weights of microgreens are key factors in determining microgreen yield, with particular emphasis on fresh weight. For example, Petropoulos et al. (2021) observed that the fresh yields of microgreens were influenced by the nutrient solution. They investigated the effects of different durations of feeding nutrient solution before harvesting spinach microgreens and found that an extended nutrient solution feeding period (20 d) resulted in the highest fresh weight. Based on the current investigation, HNS-U produced the highest fresh weight of sunflower microgreens which was not different from that obtained in the HNS-MSG treatment; however, the HNS-MSG foliar application did not significantly increase this fresh yield compared to the DW treatment. Furthermore, DW did not result in any different fresh yield from that following HNS. Although the HNS-AS treatment had the lowest fresh yield.

Fayezizadeh et al. (2023) investigated the impact of different concentrations of Hoagland's nutrient solution on the yield of basil microgreens. They found that the maximum fresh weight was achieved at a 50% concentration of the nutrient solution, with no significant differences observed at 75% and 100% concentrations of the nutrient solution. In contrast, Hassama et al. (2022) investigated the influence of different nitrogen fertilizer sources, including monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulfate, ammonium nitrate, urea and monosodium glutamate, on the growth of sunflower microgreens. Their results suggested that there were no significant variations in the fresh weight of sunflower microgreens among the various nitrogen fertilizer sources. Therefore, the enhanced fresh weight of microgreens resulting from the foliar application of the modified Hoagland nutrient solution could be attributed to its comprehensive nutrient composition, meeting the essential requirements for plant growth.

Dry weight is a crucial parameter indicative of yield quality. The current investigation measured the dry weight of sunflower microgreens and observed no notable differences among the treatments. This finding aligned with the results reported by Hassama et al. (2022), who similarly concluded that there were no significant differences in the dry weight of sunflower microgreens treated with a foliar application of various nitrogen sources.

The water content stands out as one of the prevalent physiological parameters that can constrain the efficiency of photosynthesis and biomass productivity in plants (Jin et al., 2017). There were no significant differences among treatments in the water content of the sunflower microgreens in the current treatments using various foliar nutrient solutions. This result suggested that foliar nutrient solutions did not have an impact on the water content of the sunflower microgreens. Furthermore, this result, with the water content exceeding 90%, was consistent with findings by Xiao et al. (2013) and Bulgari et al. (2017).

Plant pigments serve vital physiological functions, contributing to processes, such as photosynthesis, and defending against light stress (Petibon and Wiesenberg, 2022). Additionally, they offer health benefits to humans due to their antioxidant action and anticarcinogenic properties (Neto et al., 2017). Chlorophylls, the green pigments situated within plant cell chloroplasts, play a key role in the photosynthesis process. Their noteworthy antioxidant activity implies a variety of potential health advantages, including anti-inflammatory, anti-cancer, and anti-obesity properties (Ebrahimi et al., 2023). Many factors contribute to the diversity in chlorophyll content among plants, including light intensity (Wu, 2021), temperature (Zhao et al., 2020), genetic factors (Li et al., 2018), water availability (Enneb et al., 2021), pH levels (Huh and Lee, 2022), plant stress (Swoczyna et al., 2022), plant age (Kamble et al., 2015) and nutrient availability (Amujoyegbe et al., 2007). Chlorophyll exists in various forms, including chlorophyll a, chlorophyll b, chlorophyll c, chlorophyll d and chlorophyll e (Björn et al., 2009). The predominant forms in plants are chlorophyll a and b. Chlorophyll a efficiently absorbs light in the red and blue regions of the spectrum, with peak absorption occurring around 430 and 662 nanometers, respectively (Martins et al., 2023). *In vitro* investigations conducted by Ferruzzi et al. (2002) revealed that standard chlorophyll a derivatives had a higher antioxidant capacity than chlorophyll b derivatives. Wojdyło et al. (2020) examined the chlorophyll a content in the microgreens of kale, radish, beetroot, green peas and amaranth, with values in the range 88.1–336.2 μg/g FW. In the current study, the impact of foliar nutrient solution on sunflower microgreens was reflected in the chlorophyll a content range of 0.27–0.33 mg/g FW,

indicating that the chlorophyll a content of sunflower microgreens treated with HNS-AS and DW was significantly higher than in those treated with HNS, HNS-MSG and HNS-U. Notably, the foliar nutrient solutions (HNS, HNS-MSG and HNS-U) had a decreasing effect on chlorophyll a content compared with the DW treatment, except for the HNS-AS treatment, which had the highest chlorophyll a content. However, the decreased chlorophyll contents in HNS, HNS-MSG and HNS-U could be attributed to nitrogen assimilation for protein synthesis and subsequent transfer to other plant parts, as evidenced by the elevated protein contents observed in those treatments (Figure 2D). Hassama et al. (2022) investigated the impact of a foliar application of various nitrogen nutrient solutions (monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulfate, ammonium nitrate, urea, monosodium glutamate and deionized water) on the chlorophyll a content of sunflower microgreens. Their results revealed that compared to the deionized water treatment (the control), the chlorophyll a content of the other treatments were not significantly different.

Chlorophyll b represents an alternate form of chlorophyll present in plants, algae and certain bacteria. While its chemical structure closely resembles that of chlorophyll a, it contains a slightly different porphyrin ring (Martins et al., 2023), enabling chlorophyll b to absorb light in the blue-green region of the spectrum, peaking at approximately 453 nm. While chlorophyll b contributes to photosynthesis, its primary function is to shield chlorophyll a from excessive light (Martins et al., 2023). In the realm of microgreens, Wojdyło et al. (2020) examined the chlorophyll b content in varieties such as kale, radish, beetroot, green peas and amaranth, reporting values in the range 57.0–186.3 µg/g FW. In the current investigation, the chlorophyll b content was in the range 0.23–0.31 mg/g FW and exhibited sensitivity to various foliar nutrient solutions. These findings indicated that the highest chlorophyll b content was observed in sunflower microgreens treated with the HNS-MSG solution. Chlorophyll a is synthesized from glutamate within approximately 1 hr (Gomez-Silva et al., 1985), potentially followed by the immediate synthesis of chlorophyll b. Thus, the HNS-MSG treatment produced a higher chlorophyll b content than the other treatments. In contrast, Hassama et al. (2022) reported that the chlorophyll b content in sunflower microgreens treated with MSG solution was notably lower than in the ammonium nitrate treatment. However, there were no significant differences compared to the treatments involving monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulfate, ammonium nitrate, urea and deionized water.

The total chlorophyll content is the combined amount of chlorophyll a and b. Typically microgreens have a higher total chlorophyll content than mature vegetables such as broccoli, celery, lettuce and artichoke (Bohn et al., 2004). Furthermore, the total chlorophyll contents have been identified to be more abundant in microgreens than sprouts (Fuente et al., 2019). Ghoora et al. (2020) indicated a total chlorophyll content range of 12.35–112.62 mg/100 g FW for microgreens. They reported the lowest concentration in green peas, while the highest was observed in lentil microgreens. Within the microgreen context, Wojdyło et al. (2020) investigated the total chlorophyll content in microgreens of kale, radish, beetroot, green peas and amaranth, with values in the range 195.6–638.5 µg/g FW. In the current study, the total chlorophyll content varied in the range 0.50–0.60 mg/g FW, with the HNS-MSG treatment producing the highest value which was not significantly different from the HNS-U and HNS-AS treatments. In addition, the HNS-MSG, HNS-U and HNS-AS treatments included urea, ammonium and glutamate, which can be converted into chlorophyll in plants (Gomez-Silva et al., 1985).

Carotenoids offer health advantages by lowering the risk of disease in humans, especially certain cancers and eye conditions (Johnson, 2002). Typically, microgreens have substantially higher levels of carotenoids compared to their fully mature plants (Xiao et al., 2013). Plant carotenoid concentrations can be influenced by nitrogen levels (Liu et al., 2022). Becker et al. (2015) reported that the carotenoid concentration in red and green lettuce typically diminished with a reduction in nitrogen concentration. Wojdyło et al. (2020) investigated the carotenoid content in microgreens of kale, radish, beetroot, green peas and amaranth, reporting a range of 1510.1–4073.5 µg/g FW. Throughout the current research, the carotenoid contents were in the range 0.16–0.21 mg/g FW. The sunflower microgreens treated with HNS-AS had the highest content, although there was no significant difference compared to the DW treatment. The carotenoid levels in the sunflower microgreens treated with HNS-U, HNS-MSG, and HNS were lower than those treated with DW, which could be attributed to nitrogen assimilation for protein synthesis and its subsequent translocation to other plant parts, supported by the elevated protein content observed in these treatments (Fig. 2D). In addition, stresses from external environmental factors can induce the biosynthesis of carotenoids in plants (Saini and Keum, 2018). The current experiment showed that

the HNS-AS and DW treatments resulted in higher carotenoid content than the other treatments, possibly due to nutritional stress.

Pigment ratios vary across different developmental stages, as well as in response to factors such as fertilizers, chemicals, moisture and various environmental conditions (Katayama and Shida, 1970). In the current experiment, the chlorophyll a-to-b ratio was in the range 0.97–1.36, reaching its highest point in the DW treatment, with no significant difference compared to the HNS-AS treatment. Nevertheless, the remaining treatments resulted in ratios significantly lower than for the DW treatment. Following the theory of optimal nitrogen distribution within a leaf, the expected result should be an increase in the chlorophyll a-to-b ratio when there is a reduction in leaf nitrogen content (Kitajima and Hogan, 2003). The chlorophyll a-to-total chlorophyll ratio was in the range 0.49–0.58. The highest ratio was observed in the DW treatment; however, there was no significant difference compared to the HNS and HNS-AS treatments. Other studies have reported that biotic and abiotic stresses decrease the chlorophyll content in plants (Turan and Tripathy, 2015; Rafique et al., 2020). However, sunflower microgreens treated with DW and HNS-AS in the current study had significantly higher contents of chlorophyll a and b compared to other treatments, suggesting that the foliar fertilizers (HNS, HNS-MSG and HNS-U) likely induced stress, leading to lower chlorophyll a and b contents in the sunflower microgreens. Consequently, the highest chlorophyll a-total chlorophyll ratio was observed in the DW and HNS-AS treatments. However, the HNS treatment also had a high ratio of chlorophyll a-to-total chlorophyll, which was not significantly different from the DW and HNS-AS treatments because HNS induced high chlorophyll a contents but lower chlorophyll b contents, resulting in a high chlorophyll a-to-total chlorophyll ratio.

The chlorophyll b-to-total chlorophyll ratio was in the range 0.42–0.51, with the highest ratio observed in the HNS-MSG treatment, with no significant difference between the HNS and HNS-U treatments. These results indicated that the HNS-MSG, HNS and HNS-U treatments induced high chlorophyll b compared to the total chlorophylls.

There is an alteration in the chlorophyll-to-carotenoids ratio, during periods of stress or leaf senescence in plants, accompanied by a change in the contents of individual pigments. Generally, chlorophylls exhibit a more rapid decline than carotenoids under these conditions (Zhou et al., 2019). Indeed, understanding the chlorophyll-to-carotenoids ratio can offer more valuable information on the physiological status of plants than by focusing solely on the absolute amounts of individual pigments. This approach allows differences among plant species and varieties to be disregarded, providing a more accurate indication of plant senescence (Zhou et al., 2019). Based on the results from the current study, the ratios of chlorophyll a-to-carotenoids and of total chlorophyll-to-carotenoids were highest in sunflower microgreens treated with HNS.

The chlorophyll b-to-carotenoids ratio was highest in the HNS-MSG treatment, which could be is attributed to the elevated levels of chlorophyll b and the reduced carotenoid contents in the sunflower microgreens treated with HNS-MSG (Table 1). However, no significant differences were noted among the ratios of chlorophyll b-to-carotenoids and total chlorophyll-to-carotenoids among the HNS, HNS-MSG and HNS-U treatments.

Nitrate, ammonium, urea and amino acids represent nitrogen forms accessible to plants (Muratore et al., 2021). Following the uptake of nitrate into the roots, nitrate undergoes stimulation to produce nitrite, ammonium, glutamine acid and glutamate acid through the actions of nitrate reductase, nitrite reductase, glutamine synthetase and glutamate synthase (Masclaux-Daubresse et al., 2010; Htwe and Ruangrak, 2021). While nitrates are generally stable, dietary nitrate undergoes conversion to nitrite through a non-enzymatic process (Ma et al., 2018).

Nitrate was once considered potentially harmful due to the possible generation of nitrosamines, particularly under conditions such as an acidic stomach, with nitrosamines having been linked to various cancers, including esophageal cancer, gastric cancer, colon cancer and other tumors (Park et al., 2015; Bedale et al., 2016). Consequently, the World Health Organization suggested an upper limit for the daily intake of nitrate of 3.7 mg/kg and for nitrite of 0.06–0.07 mg/kg (Weitzberg and Lundberg, 2013). Based on the results of the current research, the nitrate content in sunflower microgreens was in the range 0.21–0.60 mmol/g FW, with the content tripling in the NHS treatment compared to the DW treatment, perhaps due to nitrogen assimilation for ammonium (Fig. 2C) and protein synthesis (Fig. 2D). However, there was no subsequent translocation to other plant parts, as indicated by the low fresh weight (Fig. 1A). According to Pintoa et al. (2015), mature lettuces supply four times the amount of nitrate compared to microgreens. Consequently, heightened nitrate concentrations can impact infants and children, who are particularly prone to methaemoglobinemia, following the consumption of vegetables (Sadeq et al., 2008; Martinez et al., 2013). Microgreens have emerged as a beneficial

alternative for lowering nitrate intake while simultaneously enhancing essential mineral consumption (Pintoa et al., 2015). Based on the results of the current study, using the HNS foliar fertilizer induced the accumulation of nitrate content in sunflower microgreens more than the other treatments.

Nitrite is a naturally occurring compound in both nature and biology and has been applied in the preservation of food, especially meat, by effectively slowing down the generation of botulinum toxin and playing a role in creating the unique flavor and color commonly associated with cured meats (Bryan, 2006). Nitrite plays a vital role as a biomarker signifying endogenous nitric oxide synthase activity, as emphasized in research conducted by Kleinbongard et al. (2003) and Dejam et al. (2005). Furthermore, Lundberg and Govoni (2004) demonstrated that nitrite played a fundamental role in cell signaling and pathology, with and disturbances in stable nitrite concentrations possibly having serious health consequences. Nitrites are commonly found in plants, especially in green vegetables, where they exist in the nitrate form (Gassara et al., 2016). Nitrate presents a potential risk to human health as it undergoes conversion by nitrate reductase in saliva and gastric fluid, leading to the formation of nitrite (Santamaria, 2006). Nitrite, upon reacting with amino acids, amides and amines, can generate N-nitroso compounds, which are known to be associated with various types of cancers (Santamaria, 2006). The current results indicated the nitrite content was in the range 2.39–5.31 µmol/g FW. However, there were no significant difference among the treatments in inducing the accumulation of nitrite content in sunflower microgreens.

Ammonium serves as a crucial nitrogen source for plants, being absorbed by plant cells through ammonium transporters located in the plasma membrane (Howitt and Udvardi, 2000). Subsequently, it is distributed to various intracellular compartments, including chloroplasts, mitochondria, and vacuoles, likely through distinct transporters in each instance typically being followed by local assimilation, facilitated by glutamine synthetases in both the cytoplasm and plastids (Howitt and Udvardi, 2000). The current research revealed the ammonium content in the sunflower microgreens was in the range 91.61–235.61 µmol/g FW, with the highest ammonium accumulation in the HNS-MSG treatment. However, there were no significant differences between the HNS and HNS-MSG treatments (Fig. 2C), with these treatments contained a significant nitrogen source, with plants storing it in the form of ammonium, awaiting assimilation for protein synthesis to be followed by the transfer of synthesized proteins to other parts of the plant. However, the HNS-AS treatment produced a significantly lower ammonium content than the HNS and NSH-MSG treatments, possibly because HNS-AS provided excess ammonium that became toxic for the sunflower microgreens resulted in a reduced fresh weights (Fig. 1A).

There is a growing positive trend among consumers for protein, with an increasing demand for both plant and animal sources of protein (Henchion et al., 2017). Plant proteins confer long-term health benefits and contribute to the prevention of chronic diseases, including cardiovascular health, metabolic syndrome, diabetes, cancer, renal function and lean body mass, strength, as well as overall morbidity and mortality (Hertzler et al., 2020). In tropical spinach, both microgreens and baby greens had a higher protein content (32.5%) than the marketable foliage from the field-grown crop (25%). Similarly, in roselle, the microgreens had a higher protein content (30%), in contrast to the mature plant (22%), according to Ayeni (2021). In the current study, the protein content was in the range 7.10–9.75 mg/mL, with the HNS and HNS-MSG treatments having significantly higher levels than the HNS-U, HNS-AS and DW treatments. This result demonstrated that sunflower microgreens treated with HNS and HNS-MSG promote protein synthesis more effectively than the other treatments.

Amino acids serve as the primary building blocks for protein synthesis and other nitrogenous compounds in the body (Blanco and Blanco, 2022). Additionally, amino acids are essential compounds in plants, functioning as the fundamental elements for protein construction, the primary carriers of nitrogen, and signaling molecules (Guo et al., 2021). Plant-derived amino acids originate from processes such as root acquisition, nitrate reduction, and ammonium assimilation (Guo et al., 2021). Numerous amino acids were identified in sprouts and microgreens in the research conducted by Wojdylo et al. (2020), while the current study identified several amino acids (cysteine, phenylalanine, tyrosine and tryptophan).

Cysteine, classified as a non-essential amino acid, serves as a fundamental component necessary for protein synthesis (Devlin, 2010). The assimilation of cysteine can occur through various pathways, producing sulfur compounds tailored to the specific requirements of the cells (Stipanuk et al., 1992). Cysteine has gained importance in food supplements, not only for cancer prevention but also for promoting overall health (Yin et al., 2016; Aghajanpour et al., 2017). Wojdyło et al. (2020) investigated and observed cysteine content in kale, radish, beetroot, green peas and amaranth microgreens, reporting a range of 0.7–17.4 mg/100 g FW. In the current experiment, cysteine was observed in sunflower microgreens treated with HNS-MSG, HNS-U, HNS-AS and DW; however, it was

not detected in the HNS treatment. The use of HNS foliar fertilizer resulted in high contents of nitrate, ammonium and protein in the sunflower microgreens, causing an imbalance between nitrogen and sulfate due to insufficient sulfate. This suggested that a lack of sulfate initially reduced cysteine synthesis (Hesse et al., 2004).

Phenylalanine, an essential amino acid, serves as a fundamental constituent of proteins that is present in various food sources and can also be obtained through supplementation (Akram et al., 2020). Phenylalanine is obtained from either dietary sources or supplementation, encompassing items such as wheat germ, oats, dairy products and meat (Akram et al., 2020). It serves as a fundamental amino acid and can be converted into tyrosine (Gupta, 2008). Phenylalanine is present in various types of vegetables. For example, a study by Wojdyło et al. (2020) examined the phenylalanine content in microgreens (kale, radish, beetroot, green peas and amaranth) and found levels in the range 3.8–23.2 mg/100 g FW. In the current experiment, phenylalanine was detected in sunflower microgreens grown under the HNS-MSG treatment that contained glutamate, which aids in converting phenylpyruvate and prephenate to phenylalanine through the actions of phenylpyruvate aminotransferases and aminotransferase, respectively, in the biosynthesis of phenylalanine (Pascual et al., 2016).

Microgreens were investigated as a category of plants capable of producing tyrosine (Wojdyło et al., 2020), specifically investigating kale, radish, beetroot, green peas and amaranth, which had levels in the range 1.6–4.6 mg/100 g FW. The current study detected tyrosine in sunflower microgreens treated with HNS, HNS-MSG, HNS-U, HNS-AS, and DW, indicating that all the treatments may be conducive to promoting tyrosine synthesis in sunflower microgreens.

Wojdyło et al. (2020) recorded the ability of microgreens to produce tryptophan, with kale, radish, beetroot, green peas and amaranth, having amounts in the range 2.0–10.3 mg/100 g FW. Similar with these findings, the current research detected tryptophan in the sunflower microgreens treated with HNS, HNS-MSG, HNS-U, HNS-AS and DW, suggesting that all these treatments may facilitate tryptophan synthesis in sunflower microgreens.

In summary, the HNS-U treatment resulted in the highest increase in the fresh weight of sunflower microgreens. However, this increase was not significant different to the HNS-MSG treatment. Furthermore, there were no significant differences among the treatments with regard to both the dry weight and water content of the sunflower microgreens. The HNS-AS treatment produced the highest contents of chlorophyll a and carotenoids, whereas the HNS-MSG treatment had the highest values for chlorophyll b and total chlorophylls. The pigment ratios of chlorophyll a-to-chlorophyll b and chlorophyll a-to-total chlorophyll were significantly elevated in the DW treatment. Additionally, the HNS treatment showed notable increases in the ratios of chlorophyll a-to-carotenoids and total chlorophyll-to-carotenoids, while the HMS-MSG treatment had heightened ratios of chlorophyll b-to-total chlorophyll and chlorophyll b-to-carotenoids. Notably, the HNS-MSG treatment produced to a considerable increase in both the ammonium and protein contents in the sunflower microgreens. In contrast, the HNS treatment had the highest nitrate content among the treatments. However, there were no significant differences in the nitrite content among the various treatments. Additionally, amino acid analysis revealed that only the HNS-MSG treatment produced all four amino acids (cysteine, phenylalanine, tyrosine and tryptophan) in the sunflower microgreens. Overall, the results suggested that sunflower microgreens from the HNS-MSG treatment in the current study had enhanced yield and quality.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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