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Research article

Combined effect of an agro-industrial compost and light spectra composition on yield and phytochemical profile in mizuna and pak choi microgreens

Cinthia Nájera [a,b], Margarita Ros [b,*], Diego A. Moreno [c], Alicia Hernández-Lara [b], José Antonio Pascual [b]

[a] Department of Agronomy, University of Almeria, 04120 Almeria, Spain
[b] Department of Soil and Water Conservation and Organic Wastes Management, CEBAS-CSIC, Campus Universitario de Espinardo – 25, E-30100 Murcia, Spain
[c] Phytochemistry and Healthy Food Lab (LabFAS), Food Science and Technology Department, CEBAS-CSIC, Campus Universitario de Espinardo – 25, E-30100 Murcia, Spain

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A B S T R A C T

This work aimed to evaluate the growth of two species of microgreens (mizuna and pak choi), using agro-industrial compost as growing media in two different mixes versus one hundred percent peat, under two different LED illumination spectra (LED 1 and LED 2) in a 14 h photoperiod. The experiment was carried-out for two times. Biomass yield, glucosinolates, and phenolic compounds, and nitrate (NO_3^-) content were analysed in leaf tissues. In both species, the highest fresh and dry biomass production was in compost:peat (50:50%) and LED 2 (Blue/Red/Far Red). In general, compost had a greater influence on nitrate content than light, but in the microgreen pak choi, the anthocyanin content was inhibited by the compost treatment. In the other hand both LED illumination had a positive effect on mizuna for glucosinolates and anthocyanins, and LED 2 also showed a positive effect on pak choi for anthocyanin. Therefore, the use of agri-food compost: peat (50:50%) with LED 2 (blue/red) lighting treatment to obtain microgreens in indoor crops is a plausible technology that provides nutritionally and phytochemically rich crops.

1. Introduction

The global market for sprouts and microgreens has increased considerably, they were worth $1445 million in 2021, and the global economic value is estimated to reach nearly $4 billion by 2030, according to Straits Research (2022), with North America and Europe being the two largest regions in the market. In Spain, there are 14 companies established by Royal Decree 379/2014 in BOE, as sprout producers, which have registered more than 50 species to produce microgreens and sprouts. The species most produced in Spain, according to the Ministry of Agriculture, Fisheries and Food (MAPA) are soya, alfalfa, onion, cabbage, radish, broccoli, and lentils; and in the European Union they are those belonging to the families *Brassicaceae, Asteraceae, Chenopodiaceae, Lamiaceae, Apiaceae, Amarillydaceae* and *Cucurbitaceae*. Riggio et al. [1] defines microgreen as a fresh tiny green plant that is used, both, as a visual and

* Corresponding author.
E-mail addresses: cnajera4@ual.es (C. Nájera), margaros@cebas.csic.es (M. Ros), dmoreno@cebas.csic.es (D.A. Moreno), ahernandez@cebas.csic.es (A. Hernández-Lara), jpascual@cebas.csic.es (J.A. Pascual).

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flavour component or ingredient in fine dining. They are smaller than "baby greens" and different from "sprouts" by their shape, size, and production time. Microgreens have primary leaves of varied sizes, shapes, colours, and textures, with varying flavours. A decade ago, bioactive compounds in microgreens were not as well studied [2], however, mainly in *Brassicaceae* glucosinolates, anthocyanins, phenols, and carotenoids were the main compounds identified in large quantities, compounds studied for their ability to inhibit cancer cell growth. Production of plant species under innovative farming technologies that mean plants grown in indoor environments without sunlight and using new growing media (vertical farming) is gaining ground worldwide. The growing conditions (mineral nutrition, biofortification, light intensity, and spectrum composition) prior to harvest significantly influence the nutritional and functional quality of microgreens [3].

Regarding lighting conditions, microgreens production is conditioned to the quality and quantity of artificial lighting provided. Light-emitting diodes (LED), an artificial lighting system that is becoming today one of the most promising technological advances. LED spectral combinations have positively increased the content of nutritional compounds of broccoli (*Brassica oleracea* var. Italica) microgreens and glucosinolates when grown under blue spectrum LED lighting [4]. Likewise, carotenoids such as β-carotene, lutein, neoxanthin, violaxanthin and zeaxanthin were also increased in lettuce cultivation under of supplemental blue light [5]. In red pak choi species, grown under red and blue LED lighting, compared to those grown in open field greenhouses, showed an increase in total anthocyanins and a decrease in macro-elements [6]. On the other hand, the use of LED light has used to reduce the amount of nitrates considered as antinutritional compound with negative effect on human health. In spinach, Ohashi et al. [7] showed that nitrate concentration is reduced during the night when supplemental light was incorporated, and more recently, Brazaitytė et al. [6] obtained lower nitrate and nitrite concentrations when they grew mustard microgreens with a blue LED light treatment relative to different combinations of red and blue LEDs.

Regarding the substrate or medium where microgreens are grown, they are mainly produced by using soilless culture carried out on nutrient solution or an organic (peat) or inorganic (perlite and vermiculite) substrate or in a mixture of both. In fact, peat is the most used growing medium because it has the right characteristics for optimal plant growth and seed germination. However, peat is a non-renewable natural resource whose exploitation has generated concern in accordance with circular economy criteria [8]; in addition, peat is a substrate susceptible to the development of diseases such as damping off (*Pythium* spp.), which can lead to production losses and, therefore, to economic losses. Recently, alternatives have sought to totally or partially replace peat in horticultural crops, such as compost from agri-food industry organic waste [9]. This alternative has given increases of yield and nutritional quality on crops e.g. baby lettuces and even biopesticide effect against the pathogen *Pythium ultimum* [10], converting compost as a friendly alternative to be used. However, the use of agro-industrial compost as a growing medium in the production of microgreens is little studied, and the use of waste from the agri-food industry after composting (compost) could be a choice to produce microgreens.

Taking all these considerations, the objective was to find the best combination of LED lights and compost growing media to obtain phytochemically rich microgreens of mizuna and pak choi, with a remarkable reduction in the presence of nitrates as a secondary but also interesting outcome, in addition to good productivity.

2. Materials and methods

2.1. Plant material and growing media

Mizuna seeds (*Brassica rapa nipposinica* "Red Empire F1") and pak choi (*Brassica rapa* subsp. *chinensis* "Red Wizard F1") of the company CN Seeds® (Cambridgeshire, UK) were used.

The growing medium was prepared by mixing Pinstrup peat and agro-industrial compost (54% vineyard pruning residues, 46% tomato residues, and 20% coffee as an additive), according to Hernández-Lara et al. [9] to obtain compost with good effects on plant growth. Additional Compost and peat properties characteristics are in Table 1, Hernández-Lara et al. [9] and Giménez et al. [10].

Table 1
Main chemical characteristics of the composts and peat.

	Compost	Peat
TOC (g Kg^{-1})	393 ± 2.1	466 ± 0.2
Total N (g Kg^{-1})	36.1 ± 0.1	9.4 ± 0.3
Total K (g Kg^{-1})	19.7 ± 0.2	3.2 ± 0.3
Total P (g Kg^{-1})	7.5 ± 0.1	4.5 ± 0.2
Ca (g Kg^{-1})	3.2 ± 0.1	18.1 ± 3.0
Mg (g Kg^{-1})	0.57 ± 0.1	1.8 ± 0.8
B (mg Kg^{-1})	40.62 ± 0.2	0.3 ± 0.1
Cu (mg Kg^{-1})	19.97 ± 0.1	5.5 ± 1.1
Fe (mg Kg^{-1})	2315.15 ± 0.8	1.2 ± 0.1
Mn (mg Kg^{-1})	140.78 ± 0.9	70.8 ± 5.7
Mo (mg Kg^{-1})	0.98 ± 0.3	1.5 ± 0.1
Zn (mg kg^{-1})	41.7 ± 0.2	14.3 ± 1.1

TOC: total organic carbon; Total N: total nitrogen; Total K: total potassium; Total P: total phosphorus; Ca: calcium; Mg: magnesium; B: boron; Cu: copper; Fe: iron; Mn: manganese; Mo: molybdenum; Zn: zinc.

2.2. LED light treatments

Two types of LED lights were used as follows: LED 1: Xitanium LED (Phillips®, Koninklijke Philips N.V., Netherlands) and LED 2: Arize® Top Horticulture LED Grow Lights (GE Current®, China). Lighting spectra and spectrum composition (%) (Table 2) were measured with the AsenseTek® lighting passport spectrophotometer model ALP-01 (Taiwan) and the data were obtained through the application Spectrum Genius Agricultural Lighting®. Photosynthetic Photon Flux Density (PPFD) was fixed to 260 μmol m^{-2} d^{-1} within the lighting treatments. Therefore, a daily light integral (DLI) of 13.1 mol m^{-2} d^{-1} was established by providing a 14 h per day photoperiod.

2.3. Experimental design

The experiment was carried out in an indoor and controlled growth chamber located at CEBAS-CSIC (Department of Soil and Water Conservation and Organic Wastes Management, Murcia, Spain). Two independent experiments under the same conditions (experiment 1 and experiment 2) were carried out to support the experimental data [11]. In each experiment, five trays (replicates) were used per growing media treatment (3) and light conditions (2). Three grams of seeds (1300 and 1150 seeds of mizuna and pak choi, respectively), from the certified commercial bag of each species, were directly placed in 250 ml drainage holed food-grade polystyrene trays previously filled with 50 g of the different growing media: mix compost:peat (v:v) (0:100; 50:50; 75:25). Seeds were gently spread onto 40 g of the growing medium and covered with a thin, layer of 10 g of it. For germination, the trays were kept in the dark for 2 days and then subjected to the two treatment lights. During the experiments the temperature and relative humidity were 23 °C and 75% respectively, and 14/10 h light/darkness photoperiod.

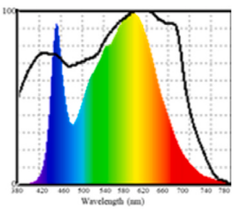
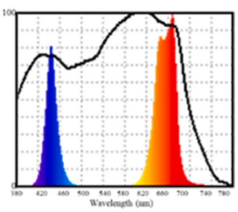
2.4. Harvest, fresh and dry weight

During germination and elongation of the microgreens, irrigation with water (without nutrient solution) was applied to prevent desiccation and maintain acceptable moisture. After 10 days since sowing in each of the carried out independent experiments, just when plants passed the sprouts stage, and with two of the true leaves emerging, they were harvested by cutting approximately 1 cm above the potting mix surface. Fresh weight and dry weight were measured on a RADWAG® precision balance, obtaining the results in kg and g square meter per treatment, respectively. To determine the dry weight, a subsample (20 g average for each repetition) was dried in a forced air oven at 60 °C to a constant dry weight. The rest of the fresh plant yield in each of the two carried out independent experiments was stored frozen at −80 °C until its subsequent lyophilized for analysis of glucosinolates, and phenolic compounds, including anthocyanins.

2.5. Nitrate (NO_3^-) content

The nitrate content was determined using a LAQUA Twin Nitrate (NO_3^-) rapid response digital meter (LAQUA Twin Nitrate Meter,

Table 2
Percentage of the specific spectral composition of each LED lamp used in the production of two microgreens species.

	Spectrum composition (%)				PPFD (μmol·m^{-2} d^{-1})	Spectrum figure (Relative intensity %)
Treatment*	Blue (B)	Green (G)	Red (R)	Far Red (FR)		
LED 1	18.00	44.82	34.36	2.83	265	
LED 2	24.40	0	74.45	1.43	260	

*LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. PPFD: Photosynthetic Photon Flux Density.

Spectrum Technologies, Inc.). The fresh microgreens from each of the two carried out independent experiments were crushed in a laboratory mortar to obtain the sap that was introduced directly into the microsensor. The measurement obtained was in $mg \cdot NO_3^{-} \cdot kg^{-1}$·fresh weight.

2.6. Extraction and determination of extracts analytical

2.6.1. Glucosinolates and phenolic compounds extraction

A modified version of the methodology described by Velasco et al. [12] for the simultaneous extraction and analysis of glucosinolates and phenolics was used for each of the two carried-out independent experiments. Consequently, once the fresh material has been freeze-dried and crushed, 100 mg of each sample were extracted in 1 mL 70% methanol at 70 °C for 20 min with vortex mixing every 5 min. Samples were centrifuged (15000 g, 15 min), and filtered through 0.22 μm∅ syringe PVDF filters (Análisis Vínicos, Tomelloso, Spain). Samples were stored at 4 °C until HPLC-DAD analysis.

2.6.2. Anthocyanins extraction

Lyophilized samples (100 mg) for each of the two carried out independent experiments, were extracted with 1 mL of methanol/water/formic acid (25:24:1, v:v:v), according to Moreno et al. [13], with slight modifications. Immediately, samples were vortexed and extracted in an ultrasonic bath for 60 min at room temperature and were kept at 4 °C overnight. Samples were then again placed for another extra 60 min in the United States, and then centrifuged (15000 g, 15 min) to separate the supernatant from the solid residue. This supernatant was filtered through a 0.22 μm∅ syringe PVDF filters. Samples were stored at 4 °C until HPLC-DAD analysis.

2.6.3. HPLC-DAD-ESI/MSn identification and HPLC-DAD quantitation of compounds

It was carried out following the method described in Abellán et al. [14], using a Kinetex column (5 μm, C18, 100 A, 150 × 4.6 mm, Phenomenex, Macclesfield, UK), using mobile phases with (A) 1% formic acid and (B) acetonitrile, using a gradient to obtain 25% B at 25 min, and 60% B at 40 min, to then wash-out the column, and return to initial conditions. The flow rate was 0.8 ml/min and injection volume of 20 μL. Spectral peaks were detected between 200 and 600 nm, recording chromatograms of 227 nm for glucosinolates, 320 nm for phenolic acids, and 520 nm for anthocyanins. HPLC-PAD-ESI/MSn analyses were carried out on an Agilent 1200 series HPLC equipped with a photodiode array detector and a serial mass detector (Agilent Technologies, Waldbronn, Germany). The HPLC systems were controlled by ChemStation software (Agilent, version 08.03). Retention times, and fragmentation patterns, for the identification of glucosinolates, phenolic acids, and anthocyanins from two experiments were shown in Table 1S (experiment 1) and Table 2S (experiment 2).

2.7. Growing media properties

For each of the two carried-out independent experiments, dry plant biomass was used for total nutrients in the mixing of the culture medium after harvest were measured by ICP-MS (Agilent 7500CE); and total organic carbon (TOC) and total nitrogen (Total N) was also analysed by with an elemental CHNS–O analyser (EA-1108, Carlo Erba). P, K, Na, Ca, Mg, Fe, and heavy metals were determined by inductively coupled plasma-mass spectrophotometry (ICP-MS PQExCell, VG-Thermo Elemental, Winsford, Cheshire, UK), after

Table 3
Effect of LED light and growing media treatments on fresh weight and dry weight of the two microgreens species of experiment 1.

	Fresh weight (kg m^{-2})		Dry matter (g m^{-2})	
	Mizuna	Pak choi	Mizuna	Pak choi
LED (A)				
LED 1[†]	1.12 ± 0.19 b	1.01 ± 0.29 a	103.12 ± 7.70 b	94.06 ± 11.45 a
LED 2	1.58 ± 0.13 a	1.15 ± 0.22 a	121.68 ± 6.63 a	100.56 ± 10.31 a
Growing media (B)				
0:100[††]	1.05 ± 0.25 b	0.92 ± 0.17 b	108.28 ± 9.63 b	91.56 ± 6.29 b
50:50	2.61 ± 0.45 a	1.64 ± 0.27 a	129.19 ± 10.21 a	112.45 ± 9.94 a
75:25	1.21 ± 0.48 b	0.90 ± 0.37 b	105.17 ± 13.05 b	94.10 ± 7.06 b
A * B				
LED 1 0:100	1.03 ± 0.06 b	0.94 ± 0.10 bc	102.28 ± 4 86 c	78.86 ± 5.11 c
LED 1 50:50	2.60 ± 0.34 a	1.68 ± 0.18 a	120.08 ± 5.21 b	112.04 ± 8.89 a
LED 1 75:25	1.12 ± 0.22 b	0.72 ± 0.12 c	102.86 ± 4.89 c	94.06 ± 8.03 b
LED 2 0:100	1.27 ± 0.28 b	1.01 ± 0.14 b	113.43 ± 8.28 bc	97.26 ± 7.69 b
LED 2 50:50	3.08 ± 0.43 a	1.57 ± 0.26 a	140.45 ± 5.66 a	112.86 ± 5.74 a
LED 2 75:25	1.50 ± 0.45 b	1.08 ± 0.19 b	120.05 ± 6.72 b	94.14 ± 9.05 b
Significance				
A	*	ns	*	ns
B	***	***	*	*
A * B	***	***	*	**

[†]LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. [††]0:100 (0%compost:100% peat); 50:50 (50%compost:50% peat); 75:25 (75% compost:25% peat). $n = 5$. *, **, *** and ns indicate significant differences for columns at $P \le 0.05$, $P \le 0.01$, and $P \le 0.001$, and non-significant, respectively.

HNO3/HClO4 high-pressure digestion.

2.8. Statistical analysis

Each experiment was conducted independently in the same chamber and under the same conditions using for each once a randomised complete block design, with five replicates per treatment. The data were subjected to analysis of variance and their means were compared by Tukey's test using Statgraphics Centurion® (version 19.04.02; Warrenton, VA).

3. Results and discussion

3.1. Effect of compost on growing media and different LED illumination on microgreens growth

Mizuna production shows that the light spectrum LED 2 significantly increases the fresh weight and dry matter, and no significant differences were observed for pak choi according to LED light treatment, although LED 2 also showed a higher trend than LED 1 (Table 3). In the case of experiment 2, no significant statistical differences were seen concerning the LED treatments in the fresh weight of both species, but there were significant differences in the dry weights, where LED 2 also showed higher dry matter in both species (Table 4). According to these results, Kong et al. [15], showed that a higher proportion of blue light enhanced different microgreen growth such as arugula, cabbage, and kale. This behaviour may be similar to that of red light and phytochrome [15], where shade-avoiding plants exert a mechanism to reduce phytochrome activity in blue monochromatic light. Lower values of fresh and dry weights of mizuna and pak choi under LED 1, are also agreed to Ma et al. [16], which mentioned that blue light in its activation of the photoreceptor cryptochrome 1 could physically interact and inhibit with the phytochrome interaction factor 4 that it would be related to hypocotyl elongation that it would be reduced by having a lower proportion of blue spectrum.

Comparing the growing media in the two carried out experiments, the compost:peat 50:50 mix shows the significant highest fresh weight and dry matter, compared to the other two growing media treatments (0:100 and 75:25) (tables 3 and 4). Compost:peat (75:25) treatment shows lower yield than 50:50 treatment, that it can be caused by compost compounds, they could inhibit microgreen seedlings at this higher dose [17]. The interaction between the two factors (growing media and LED treatments) for both experiments were significant for both microgreens species. Both LEDs light and 50:50 growing media showed the highest values of fresh weight and dry matter, indicating that the substitution of 50% of peat by compost made from agro-food industry waste increases mizuna and pak choi production, as it was observed for red baby leaf lettuce using this mixture of compost:peat by Hernández-Lara et al. [9]. Similar results were also observed by Saleh et al. [18] on pak choi obtaining higher yields when sown in a mixture of 30% vermicompost, 30% mushroom compost, 20% sawdust, and 20% perlite compared to potting medium alone. Contrary, D'Imperio et al. [19] obtained higher yields on 100% peat or a lower proportion of *Posidonia* (*Posidonia oceanica* L.) residues mix than if it was increased to 50% as a growing medium in the cultivation of *Brassica* microgreens.

3.2. Nitrate content in microgreens grown on different compost growing media under different LED illumination

Nitrate content is of special interest in leafy vegetables that are preferably consumed fresh, since the consumption of vegetables

Table 4
Effect of LED light and growing media treatments on fresh weight and dry weight of the two microgreens species of experiment 2.

	Fresh weight (kg m^{-2})		Dry matter (g m^{-2})	
	Mizuna	Pak choi	Mizuna	Pak choi
LED (A)				
LED 1[†]	1.31 ± 0.23 a	1.01 ± 0.19 a	115.45 ± 9.65 b	107.57 ± 7.15 b
LED 2	1.20 ± 0.14 a	1.14 ± 0.23 a	137.24 ± 12.84 a	123.26 ± 5.61 a
Growing media (B)				
0:100[††]	0.98 ± 0.11 b	1.15 ± 0.11 b	133.52 ± 4.56 a	120.74 ± 5.42 b
50:50	1.59 ± 0.21 a	1.44 ± 0.18 a	128.55 ± 3.63 b	142.45 ± 6.39 a
75:25	1.19 ± 0.17 b	0.63 ± 0.16 c	116.97 ± 5.41 b	83.06 ± 8.16 c
A * B				
LED 1 0:100	1.28 ± 0.09 b	1.22 ± 0.11 b	119.60 ± 11.12 b	46.46 ± 8.69 d
LED 1 50:50	1.56 ± 0.16 a	1.52 ± 0.10 a	137.50 ± 8.46 a	143.49 ± 7.12 a
LED 1 75:25	1.20 ± 0.12 b	0.30 ± 0.16 c	123.63 ± 7.93 b	132.76 ± 4.55 b
LED 2 0:100	1.10 ± 0.11 b	1.20 ± 0.13 b	116.44 ± 13.24 b	108.71 ± 9.10 c
LED 2 50:50	1.50 ± 0.15 a	1.41 ± 0.08 a	140.06 ± 9.87 a	141.41 ± 4.96 a
LED 2 75:25	1.03 ± 0.20 b	1.21 ± 0.14 b	110.31 ± 14.84 b	119.65 ± 5.60 c
Significance				
A	ns	ns	*	*
B	***	**	*	*
A * B	***	***	*	**

[†]LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. [††]0:100 (0%compost:100% peat); 50:50 (50%compost:50% peat); 75:25 (75% compost:25% peat). $n = 5$. *, **, *** and ns indicate significant differences for columns at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, and non-significant, respectively.

with a nitrate concentration above the EU thresholds is associated with negative effects on human health, especially in infants and children under 6 years of age who are susceptible to methemoglobinemia caused by excessive nitrate consumption according to European Food Safety Authority.

Nitrate accumulation content in the two microgreens species and two experiments showed no significant statistical differences between the two-lighting treatment [Fig. 1A (experiment 1) and Fig. 1B (experiment 2)]. Instead, the results show that the growing medium plays a significant effect on nitrate accumulation in microgreens. The ones grown in only peat (0:100) maintains nitrate levels on average 60 and 10% below the maximum consumption limits reported by the Regulatory Commission European Union in *Brassica* and lettuce, which are among the most studied nitrate-accumulator species. In the same way, using compost:peat 50:50 shows a significant reduction in nitrate accumulation between 35 and 15% below the maximum consumption limits. Giménez et al. [10] obtained a decrease of nitrate in spinach plants when fertilised with compost extract (mix: 45.6% vine pruning, 20.8% leek waste and 33.7% olive mill waste) in open field. However, the mixture with the highest proportion of compost (compost:peat 75:25 treatment), the nitrate values are on average of 30% in the two species studied above the EU recommended values, probably due to the content of nitrogen to the mixture (Table 1). Also, Weber [20] studied the nutrient content of *Brassica* (*Brassica oleracea* var. capitata) and lettuce (*Lactuca sativa*) microgreens grown with vermicompost *vs* hydroponic pads as growing media and found that microgreens accumulate higher nutrient content when grown on vermicompost, because nutrients in the substrate had been made bioavailable through the decomposition of nutrient-rich food waste and this is likely responsible for the higher levels of nutrients taken up by microgreen plants. Not only, but the nitrogen content of organic substrate is also responsible to NO_3^- accumulation in plants, there are other nutritional (e.g. nitrogen fertilization, treatment with physiologically active substances, and sorbents and amount and kind of nutrients in the soil) environmental (e.g. light intensity, temperature), and physiological factors (e.g. genotypic variability, nitrate distribution within the plant, and diurnal effects).

3.3. Functional compounds in microgreens grown on different compost in growing media under different LED illumination

3.3.1. Glucosinolates

In our assays, eight glucosinolates have been identified in mizuna and pak choi microgreens (tables 1S and 2S) in both experiments

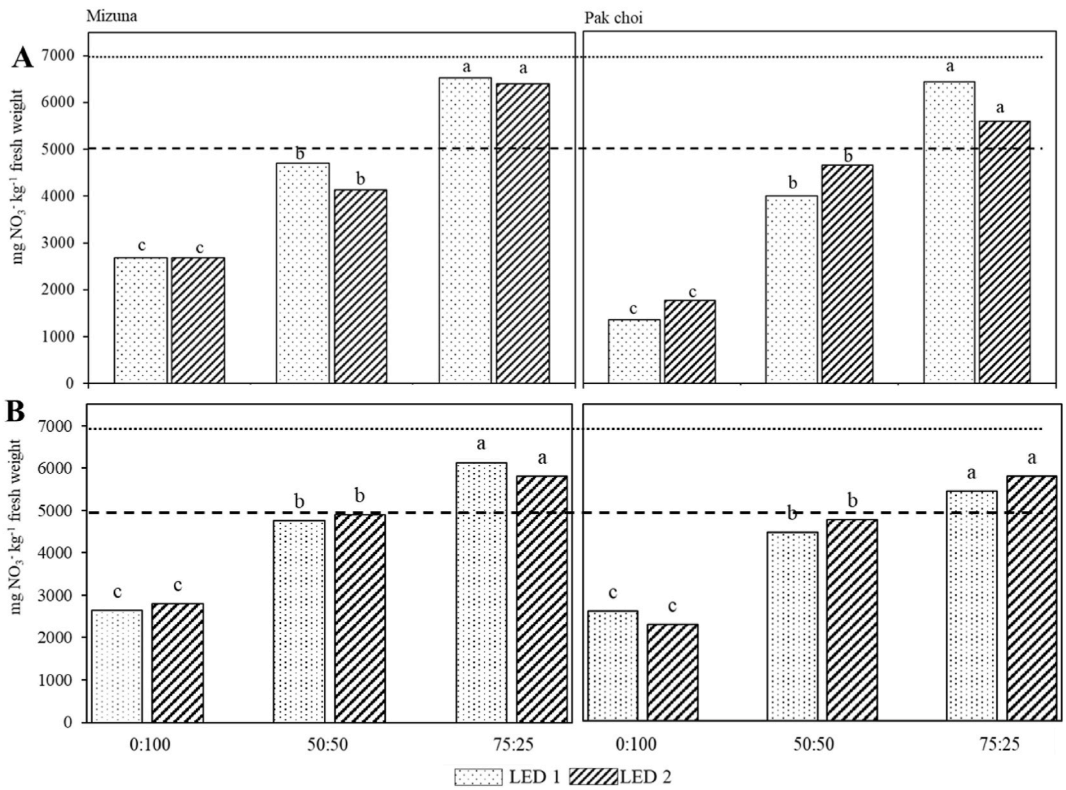


Fig. 1. Nitrate content of two species of microgreens in the two carried-out experiments [1 (**A**) and 2 (**B**)].
LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. 0:100 (0%compost:100% peat); 50:50 (50%compost:50% peat); 75:25 (75% compost:25% peat). Different letters indicate significant differences between growing medium and LED at $P \leq 0.05$ using the Tukey's test. $n = 5$. *, **, *** and ns indicate significant differences for columns at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, and non-significant, respectively.
·······Maximum nitrate levels in *Brassica tenuifolia* grown in winter regulated by the Regulatory Commission, Official Journal of the European Union.
———Maximum nitrate levels in lettuce grown in greenhouses regulated by the Regulatory Commission, Official Journal of the European Union.

and the total glucosinolate content is shown in Fig. 2. In experiment 1 (Fig. 2A) indole-GLS have been separated from aliphatic-GLS, reflected a similar proportion in all cases (20–30% indole-GLS to total glucosinolates), with indole-GLS content being lower in all treatments, as was also observed by Li et al. [21] in mustard sprouts. This could be due to the increasing blue spectrum that influences the accumulation of aliphatic-GLS [22]. In experiment 2 (Fig. 2B) the glucosinolates were also divided into indole-GLS and aliphatic-GLS, and additionally, the aromatic-GLS was identified. In the two experiments, the results and their trend were particularly similar. Indole-GLS in pak choi was not detected with the addition of compost in experiment 2, while in mizuna it was even higher than in experiment 1. It has been studied that the appearance of a volatile compound such as aromatic may be due to environmental storage conditions [23].

For mizuna microgreens, the accumulation of total glucosinolates is higher in the compost:peat 0:100 and 50:50 treatment than compost:peat 75:25, and significantly higher in LED 1 than LED 2 in both experiments (1 and 2). For pak choi microgreens, no significant differences were observed neither due to the type of light nor to the mixture of growing media. Neugart et al. [24] used a treatment with 5% coffee residue + control (35 % volcanic clay, 50 % turf and 15 % bark humus) and their glucosinolate values in *Brassica rap*a were lower compared to the treatment without additional coffee. Glucosinolates have been found to be nitrogen-containing compounds, and therefore extra nitrogen input strongly affects their concentration [24]. Regarding the effect of light, Demir et al. [25] observed lower glucosinolate content in cabbage grown with a light treatment including a far red (FR) portion similar to our LED 2. While to the spectrum of LED 1 which contains a higher proportion of green light (44.82%), influences the expression of genes related to glucosinolate biosynthesis (MAM1 and CYP79F1) in pak choi [26], and even the green spectrum can positively activate other enzymes related to the glucosinolate pathway (CYP83A1, CYP83B1, SUR1, UGT74B1.MAM1, CYP79F1 y CYP83A1) in broccoli sprouts [27].

The importance of glucosinolates for human health is well known and studied, due to the effect of inhibiting the growth of cancer cells, and their wide range of more than 200 naturally occurring secondary metabolites [28], as well as other studies have linked glucosinolate content to the enhancement of the pungent taste of some microgreen species [29], so using different spectra and culture media like ours may be an option to achieve flavour differences in microgreens.

3.3.2. Phenolics acid

Phenolic acids in both experiments showed similar behaviour (Fig. 2A and B). Although, the interaction (0:100) growing media and LED 1 treatment showed significant higher phenolic acids probably due to this interaction induce plant stress, that it would be showed by increasing them [30]. In general, the growing media 0:100 (compost:peat) mixture treatment in the two microgreens species shows higher phenolic acids similar to Saleh et al. [18] who reported higher phenols in a commercial media compared to a vermicompost, sawdust, perlite and peat mix. In the other hand, Gonani et al. [31] reported higher phenolic acid content with a mushroom-based agro-industrial compost compared to a sandy-loam soil medium.

Considering that LED 1 shows green light compared to the absence of this in LED 2, these data are not agreeing with the ones observed by Wong et al. [32] who observed that green light has been shown to reduce the accumulation of phenolic compounds. Appolloni et al. [33] mention that light exerts a positive effect on the increase of phenolic acids that is produced by the P450

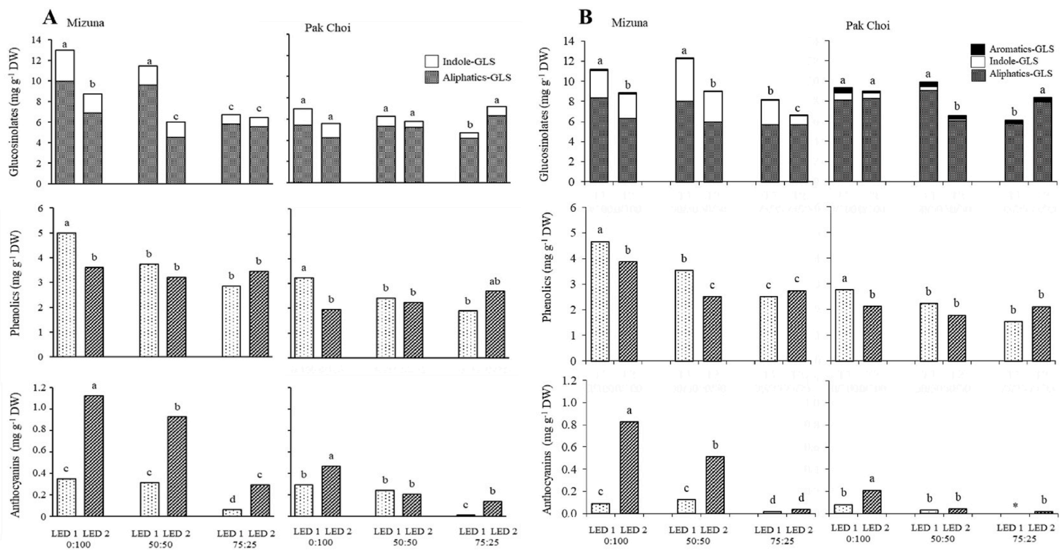


Fig. 2. Glucosinolates (Aromatic, Indole-GLS, and Aliphatics-GLS), and phenolics acid (including anthocyanins) content in the two microgreens species in the two carried-out experiments [1 (**A**) and 2 (**B**)].
LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. 0:100 (0%compost:100% peat); 50:50 (50%compost:50% peat); 75:25 (75% compost:25% peat). Different letters indicate significant differences between the growing medium and LED at $P \leq 0.05$ using Tukey's test. $n = 5$.
*Below the limit of quantification.

cytochrome, this enzyme complex obtains its maximum absorption at 450 nm (blue). Also, Routray et al. [34] also found that red light contributes less than blue light in promoting the antioxidant properties of blueberry (*Vaccinium corymbosum* L.) plants.

3.3.3. Anthocyanins

Anthocyanins are possibly the most versatile pigments for plant stress derived from the physicochemical property of light absorption, naturally occurring in plants in the form of glycosides, which are coupled with sugars with beneficial properties on cardiovascular diseases [35].

Anthocyanins content showed greater effect in both microgreens species in LED 2 compared to LED 1 in both experiments (Fig. 2). Higher values were observed in compost:peat 0:100 followed by 50:50 respect to the higher compost dose 75:25 (Fig. 2) in both species, particularly significant in mizuna. This indicates that increasing the percentage of compost leads to a decrease in anthocyanins. In this respect, the application of compost tea on hydroponic mustard leaves [36], and vermicompost on fenugreek plants [37], reduced anthocyanin content, compared to control treatments; fact that it could be reported through anthocyanin synthesis was inhibited by nitrogen increase [38], which corresponds to the results we have obtained with higher proportion of compost in the growing media combination, that showed higher amount of nitrogen (Table 1; Table 5; Table 6).

It has been studied that when plants are exposed to light, light stimulates anthocyanin production, especially when the light is a combination of red and blue [39], which coincides with the spectrum of our LED 2 treatment.

In mizuna, anthocyanins increased with LED 2 treatment by an average of 170%, regardless of the growing medium in both carried out experiments; and in pak choi, in both experiments the highest increase (500%) was seen with peat (0:100) combined with the LED 2 light treatment (Fig. 2). Therefore, in mizuna the most favourable correlation was due to the growing medium, and in pak choi the lights performed better (Fig. 2). Mizuno et al. [40] studied that the green LED spectrum decrease of anthocyanins in *Brassica oleracea* L.,

Table 5
Nutrient content of the culture substrates of two microgreen species at the end of experiment 1.

Mizuna	N total† (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (g kg^{-1})	S (g kg^{-1})
LED (A)							
LED 1††	24.1 ± 3.1 a	5.0 ± 0.8 a	5.5 ± 1.2 a	41.0 ± 2.3 a	5.0 ± 1.3 a	265.43 ± 12.6 a	3.3 ± 0.8 a
LED 2	23.3 ± 3.4 a	5.5 ± 0.9 a	5.4 ± 0.8 a	38.7 ± 2.1 a	4.9 ± 0.7 a	279.07 ± 10.8 a	3.4 ± 0.6 a
Compost (B)							
0:100†††	12.1 ± 4.2 c	0.4 ± 0.2 c	0.5 ± 0.2 c	25.5 ± 1.5 b	1.9 ± 0.6 b	216.11 ± 22.1 b	1.6 ± 1.1 c
50:50	25.6 ± 2.2 b	6.6 ± 1.3 b	6.3 ± 1.6 b	43.9 ± 2.6 a	5.9 ± 1.2 a	255.23 ± 33.9 ab	3.9 ± 0.3 b
75:25	30.4 ± 3.4 a	8.8 ± 0.7 a	9.6 ± 1.3 a	50.0 ± 1.7 a	7.1 ± 0.9 a	345.42 ± 54.6 a	4.7 ± 0.6 a
A × B							
LED 1 0:100	12.0 ± 6.5 b	0.4 ± 0.3 c	0.6 ± 0.3 b	25.4 ± 2.3 c	1.9 ± 0.8 b	253.51 ± 56.6 bc	1.5 ± 0.8 b
LED 1 50:50	24.5 ± 6.6 ab	5.7 ± 1.2 b	6.1 ± 2.6 a	40.1 ± 5.6 b	5.7 ± 2.3 a	239.97 ± 44.1 bc	3.7 ± 1.2 a
LED 1 75:25	29.6 ± 3.9 a	8.7 ± 1.1 a	9.8 ± 2.7 a	57.3 ± 6.7 a	7.4 ± 1.9 a	302.83 ± 46.5 b	4.7 ± 1.6 a
LED 2 0:100	12.2 ± 7.1 b	0.4 ± 0.2 c	0.3 ± 0.1 b	25.6 ± 1.9 c	1.8 ± 0.7 b	178.71 ± 54.9 c	1.6 ± 0.7 b
LED 2 50:50	26.5 ± 4.2 a	7.4 ± 0.7 ab	6.4 ± 1.9 a	47.7 ± 3.6 ab	6.1 ± 1.8 a	270.48 ± 32.5 b	4.0 ± 1.0 a
LED 2 75:25	31.0 ± 3.7 a	8.7 ± 0.7 a	9.4 ± 2.9 a	42.7 ± 4.9 ab	6.8 ± 2.1 a	388.01 ± 36.8 a	4.6 ± 0.8 a
Significance							
A	ns	ns	ns	ns	ns	ns	ns
B	***	***	***	**	***	*	***
A × B	***	***	**	*	***	**	***
Pak choi	N total (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (g kg^{-1})	S (g kg^{-1})
LED (A)							
LED 1	22.3 ± 0.3 a	5.3 ± 1.3 a	5.1 ± 0.9 a	39.5 ± 8.3 a	4.7 ± 1.6 a	515.35 ± 79.8 a	3.3 ± 0.6 a
LED 2	22.5 ± 0.4 a	5.4 ± 0.9 a	5.2 ± 1.1 a	43.1 ± 10.9 a	4.8 ± 1.7 a	264.04 ± 84.7 b	3.4 ± 0.9 a
Compost (B)							
0:100	11.9 ± 2.6 c	0.4 ± 0.1 c	0.5 ± 0.3 c	26.6 ± 5.6 b	1.7 ± 0.8 c	181.27 ± 55.7 b	1.6 ± 0.6 c
50:50	25.2 ± 1.8 b	6.5 ± 1.2 b	6.4 ± 0.8 b	41.5 ± 7.8 ab	5.5 ± 0.7 b	280.11 ± 66.2 b	3.9 ± 0.3 b
75:25	30.0 ± 2.1 a	9.3 ± 1.5 a	8.7 ± 1.1 a	56.0 ± 6.1 a	7.1 ± 1.1 a	707.70 110.2 a	4.7 ± 0.5 a
A × B							
LED 1 0:100	11.4 ± 1.5 b	0.4 ± 0.1 c	0.4 ± 0.2 b	28.9 ± 16.5 a	1.6 ± 0.9 c	163.66 ± 79.6 a	1.0 ± 0.3 c
LED 1 50:50	24.7 ± 3.6 a	6.3 ± 1.6 b	6.2 ± 1.1 a	39.7 ± 17.6 a	5.2 ± 0.7 b	310.36 ± 82.7 a	3.8 ± 0.2 b
LED 1 75:25	30.7 ± 4.1 a	9.3 ± 1.4 a	9.0 ± 1.8 a	50.1 ± 12.6 a	7.3 ± 1.2 a	172.02 ± 93.6 a	4.6 ± 0.3 a
LED 2 0:100	12.3 ± 2.6 b	0.5 ± 0.2 c	0.6 ± 0.2 b	24.4 ± 17.8 a	1.8 ± 1.1 c	198.88 ± 88.0 a	1.6 ± 0.4 c
LED 2 50:50	25.8 ± 4.3 a	6.7 ± 1.3 b	6.5 ± 1.3 a	43.4 ± 19.5 a	5.7 ± 1.2 ab	249.86 ± 65.4 a	4.0 ± 0.3 b
LED 2 75:25	29.2 ± 4.6 a	9.2 ± 1.2 a	8.5 ± 1.5 a	61.8 ± 21.7 a	6.8 ± 1.4 ab	343.38 ± 103.7 a	4.8 ± 0.3 a
Significance							
A	ns	ns	ns	ns	ns	***	ns
B	***	***	***	**	***	***	***
A × B	***	***	***	ns	***	ns	***

†N total: total nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; Fe: iron; S: sulphur. ††LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. †††0:100 (0%compost:100% peat); 50:50 (50%compost:50% peat); 75:25 (75%compost:25% peat). $n = 5$. *, **, *** and ns indicate significant differences for columns at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, and non-significant, respectively.

Table 6
Nutrient content of the culture substrates of two microgreen species at the end of experiment 2.

Mizuna	N total[†] (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (g kg^{-1})	S (g kg^{-1})
LED (A)							
LED 1[††]	21.4 ± 7.3 a	2.5 ± 1.7 a	7.7 ± 5.3 a	43.6 ± 12.7 a	3.8 ± 1.1 b	383.52 ± 151.0 a	2.9 ± 1.1 a
LED 2	20.8 ± 8.1 a	2.6 ± 1.8 a	7.2 ± 5.1 a	48.9 ± 22.7 a	6.1 ± 1.3 a	199.31 ± 47.7 b	2.9 ± 1.2 a
Compost (B)							
0:100[†††]	11.0 ± 0.2 c	0.3 ± 0.0 c	0.4 ± 0.0 c	26.8 ± 4.4 b	1.7 ± 0.1 b	145.32 ± 8.6 c	1.5 ± 0.1 c
50:50	24.4 ± 1.9 b	3.3 ± 0.3 b	8.1 ± 1.3 b	53.0 ± 7.9 a	5.9 ± 1.0 a	501.18 ± 233.0 a	3.3 ± 0.2 b
75:25	27.9 ± 0.4 a	4.0 ± 0.3 a	13.9 ± 1.8 a	58.9 ± 17.3 a	7.2 ± 1.9 a	227.74 ± 15.2 b	3.8 ± 0.2 a
A × B							
LED 1 0:100	10.9 ± 0.3 c	0.3 ± 0.0 c	0.4 ± 0.0 c	29.3 ± 5.7 b	1.8 ± 0.1 c	150.93 ± 9.7 c	1.5 ± 0.1 c
LED 1 50:50	25.6 ± 0.3 ab	3.3 ± 0.1 b	8.3 ± 1.0 b	52.3 ± 10.7 a	4.8 ± 0.4 b	784.38 ± 330.0 a	3.4 ± 0.1 b
LED 1 75:25	27.6 ± 0.1 a	3.9 ± 0.1 a	14.3 ± 1.8 a	49.3 ± 6.1 a	4.8 ± 0.1 b	215.24 ± 5.0 c	3.7 ± 0.2 a
LED 2 0:100	11.1 ± 0.1 c	0.3 ± 0.0 c	0.3 ± 0.8 c	24.4 ± 1.3 c	1.7 ± 0.2 c	139.71 ± 1.3 d	1.4 ± 0.1 c
LED 2 50:50	23.2 ± 1.6 b	3.2 ± 0.4 b	7.9 ± 2.0 b	53.7 ± 8.4 a	7.0 ± 1.8 a	217.98 ± 12.9 c	3.3 ± 0.4 b
LED 2 75:25	28.1 ± 0.4 a	4.2 ± 0.4 a	13.5 ± 2.4 a	68.5 ± 22.2 a	9.5 ± 2.5 a	240.25 ± 6.7 b	3.9 ± 0.2 a
Significance							
A	ns	ns	ns	ns	**	*	ns
B	***	**	***	**	***	***	**
A × B	***	***	***	**	***	***	***
Pak choi	N total (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (g kg^{-1})	S (g kg^{-1})
LED (A)							
LED 1	20.6 ± 7.7 a	2.6 ± 1.8 a	8.6 ± 6.6 a	40.9 ± 8.3 a	3.9 ± 1.4 a	207.8 ± 38.6 a	2.9 ± 1.1 a
LED 2	19.9 ± 7.1 a	2.5 ± 1.7 a	8.1 ± 5.4 a	42.5 ± 13.6 a	3.8 ± 1.7 a	216.1 ± 55.9 a	2.8 ± 1.1 a
Compost (B)							
0:100	11.0 ± 0.2 c	0.3 ± 0.0 c	0.4 ± 0.1 c	28.9 ± 3.1 b	1.9 ± 0.3 c	155.3 ± 15.1 b	1.5 ± 0.1 c
50:50	22.8 ± 0.4 b	3.3 ± 0.1 b	8.9 ± 1.1 b	45.0 ± 2.0 a	4.4 ± 0.1 b	251.9 ± 23.3 a	3.1 ± 0.1 b
75:25	26.7 ± 1.4 a	4.0 ± 0.3 a	15.6 ± 2.9 a	51.5 ± 6.2 a	5.2 ± 0.3 a	228.6 ± 14.5 a	3.9 ± 0.3 a
A × B							
LED 1 0:100	11.1 ± 0.3 c	0.3 ± 0.0 c	0.4 ± 0.0 d	30.5 ± 2.9 c	2.2 ± 0.1 c	163.5 ± 18.8 b	1.6 ± 0.0 d
LED 1 50:50	22.9 ± 0.1 b	3.3 ± 0.1 b	8.1 ± 0.1 c	44.8 ± 0.7 b	4.4 ± 0.0 b	240.3 ± 21.0 a	3.0 ± 0.0 c
LED 1 75:25	27.7 ± 1.3 a	4.1 ± 0.5 a	17.2 ± 2.5 a	47.4 ± 0.9 b	5.2 ± 0.5 a	219.7 ± 17.8 a	4.0 ± 0.4 a
LED 2 0:100	10.9 ± 0.1 c	0.4 ± 0.0 c	0.4 ± 0.1 d	26.7 ± 2.2 c	1.7 ± 0.0 d	147.2 ± 8.2 b	1.5 ± 0.0 e
LED 2 50:50	22.7 ± 0.6 b	3.2 ± 0.2 b	9.8 ± 0.7 b	45.3 ± 3.3 b	4.5 ± 0.2 b	263.5 ± 25.6 a	3.2 ± 0.1 b
LED 2 75:25	25.8 ± 1.1 a	3.9 ± 0.1 a	14.0 ± 3.1 a	55.6 ± 6.8 a	5.3 ± 0.1 a	237.4 ± 0.7 a	3.7 ± 0.2 a
Significance							
A	ns	ns	ns	ns	ns	ns	ns
B	***	***	***	**	***	**	***
A × B	***	**	***	**	***	*	**

[†]N total: total nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; Fe: iron; S: sulphur. [††]LED 1: Xitanium LED and LED 2: Arize® Top Horticulture LED. [†††]0:100 (0%compost:100% peat); 50:50 (50%compost:50% peat); 75:25 (75%compost:25% peat). $n = 5$. *, **, *** and ns indicate significant differences for columns at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, and non-significant, respectively.

data according to ours, where LED 1 incorporate green spectrum but not LED 2.

Although few studies are mentioning the positive effect of red light on anthocyanins, our study pointed out in this sense where the LED light with higher red proportion [74% (LED 2) vs 34% (LED 1)] stimulated anthocyanin production, similar to the results obtained by Miura & Iwata [41] in *Polygonum hydropiper*. Thus, the red-light receptor phytochrome may play a role in anthocyanin biosynthesis in mizuna and pak choi microgreens, as it was previously reported in 'Red Rookie' cabbage [42], and *Polygonum hydropiper* [41].

3.4. Nutritional content of growing media after microgreens harvested

tables 5 and 6 show the fertility levels of the growing media used at the end of the crop in the two carried-out experiments, respectively. No significant effect of different macro-micro-nutrients (total N, P, K, Ca, Mg, Fe, and S) is shown for mizuna and pak choi microgreens according to LED lighting treatments, except in pak choi for Fe where it significantly shows higher values in LED 1 treatment. In general, compost:peat 75:25 followed by the 50:50 treatment showed the highest values of macro-micro-nutrients for both microgreens species probably due to the high amount of nutrients in composts compare to peat [43]. This could indicate the possible use of this growing media for more than one production cycle of microgreens, due to the nutrient content microgreens production is higher than the ones observed before starting a crop. These findings are aligned to the circular economy, taking advantage of agri-food waste in microgreens crops. However, this will require further studies to analyse their behaviour and effect.

4. Conclusions

This study showed that the combination of agri-food compost and environmentally friendly lighting, such as LEDs, is an alternative for higher production of mizuna and pak choi microgreens within sustainable agriculture and circular economy. The use of compost:

peat (50:50%) with LED blue/red lighting treatment to obtain microgreens in indoor crops is a plausible technology that provides nutritionally and phytochemically (glucosinolates and anthocyanin content) rich food. Further investigation of the effects of these conditions on microgreens for further assess the suitability of these combinations of techniques for obtaining high-value fresh food and produce in indoor systems. Also, in view of the need for modern agriculture to counteract damage to the environment and human health.

Data availability statement

Has data associated with your study been deposited into a publicly available repository?
No.
Please select why.
Data included in article/supp. Material/referenced in article.

CRediT authorship contribution statement

Cinthia Nájera: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Margarita Ros:** Supervision, Methodology, Conceptualization. **Diego A. Moreno:** Supervision, Formal analysis. **Alicia Hernandez-Lara:** Formal analysis. **José Antonio Pascual:** Supervision, Methodology, Conceptualization.

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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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26390.

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