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Effect of Magnesium Sulfate Application on Selected Microgreen Species

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Abstract: This study investigated the effect of applying five concentrations of magnesium sulfate on selected species of microgreens. Six plant species (broccoli, mustard, cress, basil, sunflower, and cucumber) were treated with $MgSO_4$ solutions containing magnesium at concentrations of 0, 10, 20, 30, 40, and 50 mg·L^{-1}. The water-soluble magnesium and calcium content of plants, fresh yield, dry matter content, vitamin C, total phenols, flavonoids, and antioxidant activity were monitored for all species. The highest content of water-soluble magnesium (after the application of 50 mg·L^{-1}) was found in cucumber (1,076 mg·kg^{-1} FW), while the lowest was in sunflower (369 mg·kg^{-1} FW). The application of 50 mg·L^{-1} resulted in an increase in magnesium content in the plants, ranging from 67% in mustard to 137% in broccoli, and up to 262% in basil. It was observed that the highest applied concentration positively influenced the total phenol content, flavonoids, vitamin C, and antioxidant activity in broccoli. Dry matter content (excluding mustard) and fresh weight were generally not significantly affected in any species. All species were successfully enriched with magnesium; however, it seems that each species responded differently to the application of magnesium sulfate. Although the highest Mg content in treated plants was found in cucumber, broccoli appears to be a more promising species in terms of high antioxidant activity, vitamin C content, total phenols, and flavonoids.

Keywords: magnesium; functional food; agronomic biofortification; $MgSO_4$; phytochemical analysis

1. Introduction

Microgreens, in their modern form, were first introduced in the 1980s in San Francisco, where chefs at select restaurants began preparing them [1]. They are young plants harvested between 1 and 3 weeks of growth, typically reaching heights of 3–10 cm, with fully developed cotyledons or the first true leaves.

A wide range of species and cultivars can be grown as microgreens, including vegetables, ornamental plants, and weeds. The choice of species depends on factors such as health safety, seed cost, availability, growth rate, sensory qualities, and potential nutritional value. *Brassicaceae* species, particularly broccoli, mustard, radish, kale, and cress, are most commonly chosen, with other families like *Amaranthaceae*, *Apiaceae*, *Cucurbitaceae*, and *Lamiaceae* also frequently used [2,3].

Microgreens are rich in minerals, phytonutrients, and secondary metabolites, including chlorophyll, beta-carotene, vitamins, antioxidants, and phenolic compounds. Many microgreens contain higher concentrations of these compounds than conventionally grown vegetables [4,5]. This has been confirmed by multiple studies [6–8].

The short cultivation period of microgreens also means they require minimal space, substrates, and water. Fertilisation is often unnecessary in standard production. Microgreens can be cultivated in densely populated areas, with options for soilless vertical farming, which optimises space and reduces costs [1,9].

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Over a quarter of the population suffers from micronutrient deficiencies, such as vitamin A, zinc, iron, magnesium, or iodine [10]. Biofortification offers a potential solution by enhancing nutrients in plants without compromising yield or quality [11]. Biofortification methods include breeding, genetic manipulation, nanotechnology, green technologies, and agronomic biofortification. Agronomic biofortification optimises fertilisation to increase nutrient accumulation in plant tissues [12]. Soilless cultivation systems using nutrient solutions with precise compositions minimise environmental risks, such as nutrient leaching or soil accumulation [13,14], while also improving crop quality and offering a sustainable solution for microgreens cultivation. Studies on biofortification have focused on vitamins C, iron, zinc, selenium, and iodine [15–18].

Magnesium, the fourth most abundant cation in the human body, is essential for activating over 300 enzymes, supporting muscle and nerve function, maintaining healthy bone and tooth structure, and boosting the immune system. The recommended daily intake (RDI) is 420 mg for men and 320 mg for women [19]. Magnesium deficiency is common in developed countries [20,21]. The depletion of magnesium in soils, due to poor agronomic practices, and food processing, which reduces magnesium content, are primary causes of dietary deficiency. For example, processing grains into flour can decrease magnesium content by 82–97% [22,23].

This study is among the first to focus on the agronomic biofortification of microgreens with magnesium. An experiment was conducted with six species (broccoli, mustard, cress, basil, sunflower, and cucumber) using magnesium sulfate. The aim was to assess whether $MgSO_4$ application effectively increases magnesium content in these species, making them suitable sources of magnesium. The impact of $MgSO_4$ on secondary metabolites and yield was also evaluated.

2. Materials and Methods

2.1. Experimental Design

Six plant species were used in the experiment. Three commonly used species in microgreens production from the *Brassicaceae* family were selected: cress (*Lepidium sativum* L. cv. 'Dánská'), mustard (*Sinapis alba* L.), and broccoli (*Brassica oleracea* L. var. *italica* cv. 'Limba'). Additionally, representatives from three other families were chosen: *Lamiaceae* (basil, *Ocimum basilicum* L.), *Asteraceae* (sunflower, *Helianthus annuus* L.), and *Cucurbitaceae* (cucumber, *Cucumis sativus* L. cv. 'Othello F1'). All seeds were purchased from MORAVOSEED CZ a.s. (Mikulov, CZ).

The plants were cultivated in a climate chamber at the Faculty of Horticulture, Mendel University in Brno. The phytotron FYTOSCOPE FS-SI-4600 was equipped with white LED lighting, providing a Photosynthetic Photon Flux Density (PPFD) of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a measured light intensity of 7,800 lux at plant height. The light period was set to 16/8 hours (day/night), with a temperature of 24/20°C and relative humidity (RH) ranging from 60 to 70%. The plants were grown in PP trays measuring 15 × 11 × 5 cm. Laboratory filter paper (120 $g \cdot m^{-2}$) (Papírna Perštejn Ltd.) was used as the substrate in two layers. Sowing density was species-specific (Table 1).

For the first few days, the plants were covered with an opaque lid. The duration of coverage depended on the species, and the plants were uncovered once germination was complete and the cotyledons began to develop. Subsequently, they were covered with transparent trays until the end of cultivation (Table 1).

Table 1. Cultivation parameters of the microgreens species.

Common name	Scientific name	Determined TSW (g)	Density of seeds per tray (g)	Density of seeds per cm²	Growing in the dark (day)	Light exposure (day)	Cultivation days (total)
Cress	*Lepidium sativum* L.	2.4	3	7.6	2	5	7
Mustard	*Sinapis alba* L.	5.8	7	7.3	3	3	6
Broccoli	*Brassica oleracea* L. var. *italica*	3.1	7	13.7	3	7	10
Basil	*Ocimum basilicum* L.	1.5	3	12.1	3	3	6
Sunflower	*Helianthus annuus* L.	60.9	18	1.8	4	5	9
Cucumber	*Cucumis sativus* L.	22.8	6	1.6	3	8	11

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Six treatments of $MgSO_4$ solution were used for biofortification at concentrations of 10, 20, 30, 40, and 50 mg·L^{-1}, along with a control using distilled water (Table 2). The treatments were labelled as C (control), Mg1, Mg2, Mg3, Mg4, and Mg5. Each treatment was replicated five times, resulting in a total of 180 units (6 species × 6 treatments × 5 repetitions). Three of the most representative repetitions were then selected for further analysis.

Table 2. Parameters of the $MgSO_4$ solution.

Treatment	Mg concentration (mg·L^{-1})	pH value	EC (μs·cm^{-1})
Mg1	10	8.04	700
Mg2	20	7.83	1210
Mg3	30	7.66	1670
Mg4	40	7.47	1990
Mg5	50	7.20	2500

2.2. Plant Material and Sample Preparation

Before sowing, 20 ml of the treatment solution was pipetted into each growing tray. After sowing, seeds were irrigated with mechanical sprayers, and the dose applied was based on the specific needs of each species. The total solution dose was then converted to the total magnesium supply to the plants (Table 3). For sunflower, seed coats were removed from the leaves two days before harvest. At harvest, all species had fully developed cotyledons and the first true leaves.

The above-ground plant parts were separated from the substrate at a height of a few millimetres using sharp blades. For cress, mustard, and basil, the entire plants, including roots, were harvested due to their delicate root systems. Harvested plants were weighed to determine fresh yield (g·m^{-2}). Samples from each repetition were used for gravimetric dry matter determination following Zbíral et al. [24]. These samples were dried for 4 hours at 105°C in a hot air steriliser (STERIMAT 574.2, BMT Medical Technology Ltd., Czech Republic). All determinations were performed in triplicate, and dry matter content was expressed as a percentage.

Table 3. Total $MgSO_4$ supply depending on the cultivated species and treatment (mg).

Cultivated species	Cultivation days	Used amount of Mg (mg·L^{-1})				
		10	20	30	40	50
Cress	7	1.01	2.03	3.04	4.05	5.07
Mustard	6	0.74	1.48	2.22	2.95	3.69
Broccoli	10	1.20	2.40	3.61	4.80	6.01
Basil	6	0.50	1.00	1.50	2.00	2.50
Sunflower	9	1.11	2.23	3.35	4.45	5.57
Cucumber	11	1.97	3.93	5.90	7.86	9.82

2.3. Ascorbic Acid

Ascorbic acid was determined by high-performance liquid chromatography (HPLC) following sample preparation [25]. A reversed-phase (RP) mode was used, with detection in the ultraviolet region. A fresh sample (5–10 g) was blended with 20–40 ml of oxalic acid solution, filtered, and transferred to a 100 ml volumetric flask. The sample was brought to volume with oxalic acid solution. From this, 20 ml was centrifuged at 4,000 rpm for 10 minutes, then filtered through a 0.45 μm PVDF microfilter. The analysis was performed with RP-HPLC (ECOM, Czech Republic) using a UV-VIS detector. All samples were analysed in triplicate and expressed in mg·kg^{-1}.

2.4. Total Phenols and Flavonoids

A methanol extract was prepared for measuring antioxidant activity, flavonoids, and total phenols [26]. Fresh plant material (5 g) was mixed with 20 ml of 75% methanol and extracted for 24

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hours. The sample was filtered, transferred to a volumetric flask, and diluted with 75% methanol. For phenol determination, 10 ml distilled water, 1 ml extract, and 1 ml Folin-Ciocalteu reagent were added. After 5 minutes, 10 ml sodium carbonate solution was added, and the flask was filled to volume with distilled water. Absorbance was measured at 765 nm after 90 minutes. For flavonoid determination, 0.5 ml extract, 1.5 ml water, 0.2 ml sodium nitrite, and 0.2 ml aluminium chloride were added, followed by 1.5 ml sodium hydroxide and 1 ml water. After 15 minutes, absorbance was measured at 510 nm.

2.5. Antioxidant Activity

Total antioxidant capacity (TAA) was measured using the DPPH free radical method. The same methanol extract was used for analysis. Absorbance was measured at 515 nm after 30 minutes using a UV-VIS spectrophotometer.

2.6. Magnesium and Calcium Content

Water-soluble magnesium and calcium were determined by isotachophoretic analysis [27]. One gram of dried plant material was shaken with 50 ml deionized water for 60 minutes, then filtered and brought to volume. The Mg and Ca content was measured using an IONOSEP 2003 analyzer (Recman Ltd., Czech Republic).

2.7. Statistical Analysis

Data processing was performed in Microsoft 365 Excel. Statistical analysis was carried out using TIBCO STATISTICA 14.0.0 (2020). One-way ANOVA was used, and significant differences were tested with the Tukey HSD test ($p < 0.05$). Pearson's correlation analysis and linear regression were used to evaluate parameter relationships. Data are presented as means ± standard error (SE).

3. Results and Discussion

3.1. Water-Soluble Magnesium Content

Significant differences in magnesium content were observed in all six species treated with higher Mg concentrations compared to the control ($p < 0.05$). Some treatments showed minor variations in Mg content, such as in sunflower, where the Mg4 treatment resulted in slightly lower Mg levels than the lower concentrations, excluding the control (Figure 1, Table 4). This lower Mg content in sunflower (Mg4) may be due to stress, indicated by the lowest average dry matter content (8% vs. 9.9% in other treatments) (Appendix A, Table A1). Stress, possibly from fungal infestation, could have impaired the plant's Mg accumulation.

In control treatments, the highest Mg content was found in cucumber, followed by mustard, broccoli, cress, sunflower, and basil (Table 4). Xiao et al. [7] reported an average Mg content of 510 $mg \cdot kg^{-1}$ FW in broccoli, while mustard's values were similar to those found here (350 $mg \cdot kg^{-1}$ FW). Di Gioia et al. [28] noted higher Mg levels in sunflower microgreens (390 $mg \cdot kg^{-1}$ FW). After applying 50 $mg \cdot L^{-1}$ Mg, cucumber had the highest Mg content, followed by broccoli, mustard, cress, and basil, with sunflower having the lowest (Table 4, Figure 1).

Table 4. Magnesium content in the studied species ($mg \cdot kg^{-1}$ FW).

Treatment	Cress	Mustard	Broccoli	Basil	Sunflower	Cucumber
C	216.8 ±22.4d	369.8 ±14.6d	312.3 ±2.3d	150.7 ±17.5e	203.7 ±5.5c	376.8 ±19.8e
Mg1	377.8 ±51.2cd	389.8 ±21.7d	404.4 ±10.6cd	214.9 ±19.6d	292.5 ±36.1ab	491.4 ±6.9d
Mg2	366.5 ±7.0cd	427.4 ±15.6cd	476.5 ±24.5bc	307.7 ±16.8c	294.0 ±25.9ab	683.4 ±18.5c
Mg3	451.8 ±38.9c	493.9 ±15.1bc	516.5 ±25.9bc	393.8 ±65.8b	307.5 ±7.3ab	823.7 ±55.8ab
Mg4	533.1 ±39.3ab	561.9 ±26.7ab	581.0 ±19.5b	379.6 ±17.3b	276.9 ±10.8b	850.9 ±122.9ab
Mg5	589.7 ±66.2ab	616.8 ±13.4a	724.2 ±38.3a	545.8 ±57.0a	368.5 ±18.1a	1076.1 ±30.1a

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Values are expressed as mean ± standard error (n = 3). Statistical differences between the values were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Different letters indicate statistically significant differences between the groups.

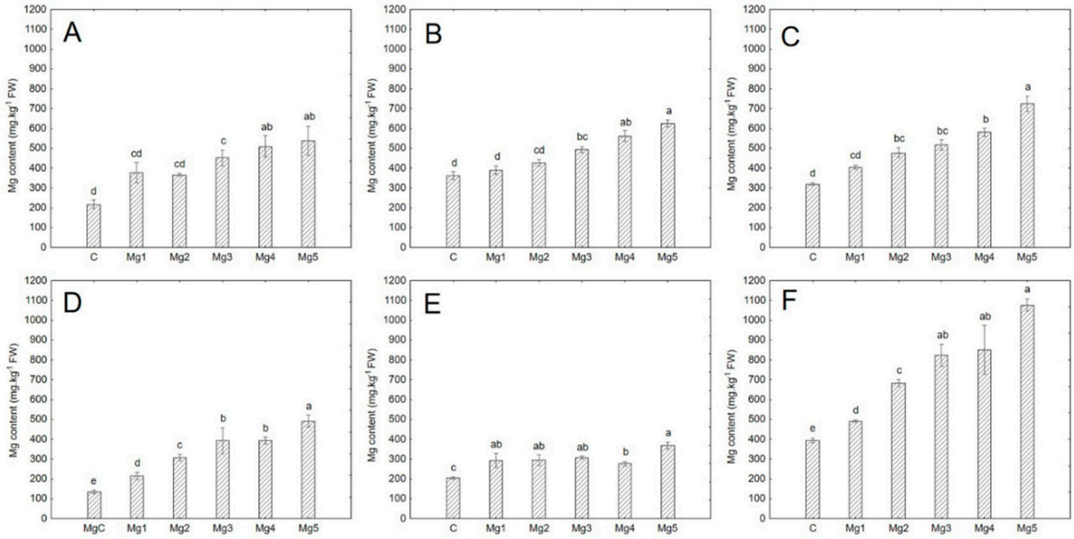

Figure 1. Magnesium content in the studied species. Data are expressed as mean ± standard error (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$). Bars marked with different letters indicate statistically significant differences between treatments. A = cress; B = mustard; C = broccoli; D = basil; E = sunflower; F = cucumber.

The increase in Mg content at the highest concentration (Mg5) compared to the control ranged from 67% (mustard) to 262% (basil) (Table 5). In basil, Mg increased by 262%, while sunflower showed only an 81% increase. However, the total Mg supplied through irrigation during cultivation was 5.6 mg for sunflower and 2.5 mg for basil (Table 3). This suggests that sunflower is not suitable for Mg biofortification, likely due to higher irrigation needs and greater stress susceptibility. Broccoli showed a 137% increase in Mg content. Przybysz et al. [29] reported that $MgSO_4$ concentrations of 50–300 $mg \cdot L^{-1}$ increased Mg in broccoli sprouts by 8–83%, although this study involved sprouts rather than microgreens.

Table 5. Percentage increase in magnesium content by treatment.

Treatment	Cress	Mustard	Broccoli	Basil	Sunflower	Cucumber
C	0%	0%	0%	0%	0%	0%
Mg1	74.23%	5.40%	26.75%	42.61%	43.62%	30.42%
Mg2	69.01%	15.57%	49.29%	104.21%	44.34%	81.37%
Mg3	108.36%	33.56%	61.83%	161.28%	50.97%	118.62%
Mg4	145.82%	61.49%	81.14%	151.85%	35.97%	125.85%
Mg5	171.96%	66.78%	136.93%	262.18%	80.92%	185.59%

For adults, the recommended daily intake (RDI) of magnesium is 420 mg for men and 320 mg for women [19]. To meet the RDI, men would need to consume 390 g of cucumber microgreens, while women would require 300 g. Since most people likely consume only 50% of the RDI [30], magnesium-enriched microgreens could provide an easily accessible dietary supplement. Even 100 g of such microgreens could meet daily magnesium needs. Broccoli, which had the third-highest Mg content in the control treatment and second-highest after 50 $mg \cdot L^{-1}$ Mg application (Table 4), also contains relatively high levels of vitamin C, flavonoids, total phenols, and antioxidant activity (Table 8). These could potentially be further enhanced with higher Mg concentrations (Figure 4), making broccoli an

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attractive option for biofortification. Future studies should explore the effects of magnesium biofortification on different broccoli cultivars.

3.2. Water-Soluble Calcium Content

Among the species observed, cucumber and cress appear to have the highest water-soluble calcium content, while sunflower exhibited the lowest concentration of this element (Table 6). No significant effect of $MgSO_4$ application on calcium content was recorded for basil and sunflower. In contrast, an increase ($p < 0.05$) in calcium content was evident for mustard, broccoli, and cucumber with increasing concentrations of magnesium. In cress, the water-soluble calcium content increased significantly across all treatments, except for Mg2, which showed the lowest calcium content among the evaluated treatments. Compared to the control treatment, the Mg5 treatment resulted in an increase in calcium content of 49% (mustard), 27% (cucumber), 24% (broccoli), and 38% (cress) (Table 6).

Table 6. Water-soluble calcium content in the studied species (mg·kg^{-1} FW).

Treatment	Cress	Mustard	Broccoli	Basil	Sunflower	Cucumber
C	195.3 ±0.6ab	162.6 ±6.9c	125.1 ±6.8b	158.8 ±24.5a	118.2 ±2.7a	199.5 ±16.2ab
Mg1	218.3 ±22.5ab	161.7 ±5.2c	132.6 ±5.1ab	148.2 ±9.6a	135.9 ±16.7a	182.0 ±6.6b
Mg2	177.3 ±7.5b	160.4 ±5.7c	132.4 ±8.9ab	158.2 ±10.8a	116.3 ±10.5a	225.9 ±3.2ab
Mg3	233.0 ±21.8ab	204.2 ±4.1b	130.8 ±4.0b	166.3 ±21.6a	125.0 ±2.7a	241.6 ±12.8a
Mg4	272.0 ±25.6a	227.8 ±9.4ab	149.9 ±3.4ab	136.6 ±8.4a	107.0 ±7.5a	232.9 ±20.1ab
Mg5	270.4 ±19.9a	241.8 ±2.7a	164.5 ±6.9a	152.3 ±13.0a	117.5 ±10.1a	252.8 ±10.4a

Values are expressed as mean ± standard error (n = 3). Statistical differences between the values were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Different letters indicate statistically significant differences between the groups.

3.2.1. Ca/Mg Ratio

Based on the results (Table 6), each species of microgreens appears to respond differently to increasing levels of magnesium in plants (Figure 2). Mustard, cucumber, broccoli, and cress showed a slight increase in calcium content, while the other species either exhibited negligible changes or no significant effect.

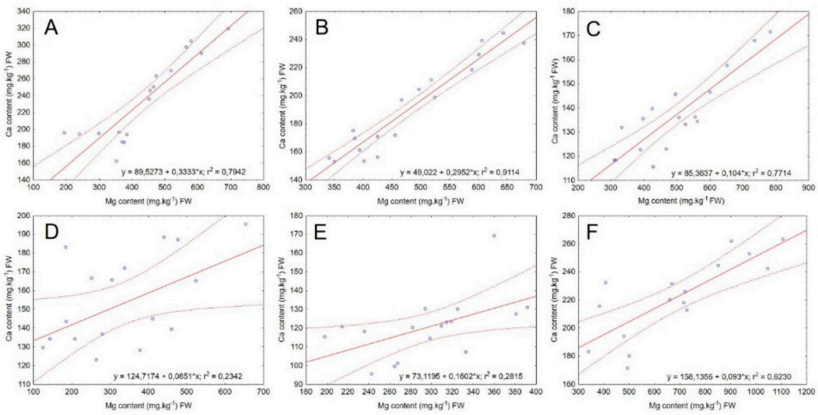

Figure 2. Correlation analysis of Ca and Mg content across different microgreens species (A–F). The linear regression equations and coefficients of determination (R^2) are shown. Dashed lines represent the 95% confidence intervals of the regression lines. A = cress; B = mustard; C = broccoli; D = basil; E = sunflower; F = cucumber.

The limited impact of Mg on Ca content in the plants may be related to the short cultivation period of the microgreens. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) are the two most abundant divalent cations in plants, and they can interact antagonistically. Specifically, they compete for the same binding sites on enzymatic and transport proteins [31,32]. However, a proposed hypothesis suggests that high levels of external Mg^{2+} may cause a transient increase in cytosolic Ca^{2+} within plant cells [33]. This mechanism could explain the slight increase in calcium content observed in some species, though further research is needed to confirm this.

3.3. Dry Matter

Regardless of the treatment, basil had the highest average dry matter content, while cress had the lowest. The remaining species (cucumber, broccoli, sunflower, and mustard) had similar dry matter content (Table 8). In mustard, the Mg3, Mg4, and Mg5 treatments resulted in significantly higher dry matter content ($p < 0.05$) compared to the lower concentrations and control (Table A1). Broccoli's dry matter content averaged 9.2% (excluding the Mg5 treatment), which is higher than the 7.9% reported by Xiao et al. [7]. The higher dry matter content in this study may be attributed to differences in growing conditions or cultivar, as well as a one-day longer cultivation period. The average dry matter content in basil (12.3%) aligns with other studies, where green basil's dry matter content ranged from 10.1 to 11.9% [16]. A recent study on the biofortification of broccoli microgreens with ascorbic acid showed a correlation between higher ascorbic acid doses and dry matter content, with the control having an average of 7.5% dry matter, which increased to 8.9% with 0.25% ascorbic acid [34]. While no significant effect of Mg on dry matter content in broccoli was observed in this study, the average dry matter content was still higher, and the Mg5 treatment showed slightly higher dry matter content compared to the lower concentrations and control (Table A1), though these results were not statistically significant ($p > 0.05$).

3.4. Yield

In this study, magnesium concentrations did not significantly affect the yield of any species ($p > 0.05$) (Table A2, Figure 3). The average fresh yield (including roots) per gram of seeds, regardless of the treatment, was highest for cucumber and lowest for sunflower (Table 7). When considering yield per area, sunflower had the highest average yield, while basil had the lowest (Table 7). For broccoli, cucumber, and sunflower, the edible parts and roots were harvested and weighed separately.

Differences in yield among species are attributed to their physiological characteristics, sowing density, and cultivation duration. Magnesium biofortification did not show any significant differences in biomass yield compared to the control treatments. It seems that the application of $MgSO_4$, along with the high electrical conductivity, did not induce toxicity symptoms in the plants. These findings align with a study on the effect of magnesium enrichment in plant sprouts, which also reported negligible impacts on yield [29].

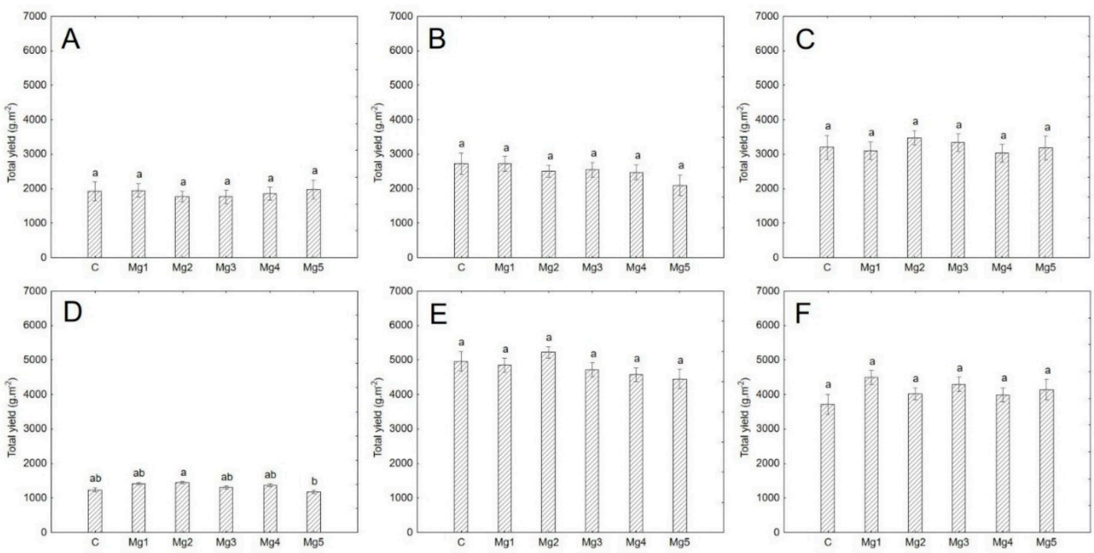

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Figure 3. Yield by treatment. Data are expressed as mean ± standard error (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$). Bars marked with different letters indicate statistically significant differences between treatments. A = cress; B = mustard; C = broccoli; D = basil; E = sunflower; F = cucumber.

Di Gioia et al. [28] examined the yield of 17 microgreen species, including broccoli, basil, cress, and sunflower, which were also part of this study. These plants were cultivated in a soilless system using a natural fiber mat as the growing substrate. In their study, the reported yield of broccoli was 1461 $g \cdot m^{-2}$, whereas our study achieved a higher yield of 1925 $g \cdot m^{-2}$, excluding root mass (Table 7). Notably, our experimental conditions included a sowing density of 13.7 $seeds \cdot cm^{-2}$, compared to their lower density of 2.7 $seeds \cdot cm^{-2}$. Additionally, our cultivation period was 10 days, while theirs extended to 11 days (Table 1; Table 7). Sunflower yields of 1656 $g \cdot m^{-2}$ have been documented at a sowing density of 1 seed/cm^2 over 10 days. In our study, we achieved a higher yield of 2342 $g \cdot m^{-2}$ (excluding roots) with a density of 1.8 seeds/cm^2 over 9 days (Table 1; Table 7). Vrkić et al. [35] reported a yield range of 1219 to 1590 $g \cdot m^{-2}$ for mustard, depending on the LED lighting intensity, with a sowing density of 5 $seeds \cdot cm^{-2}$ and an 8-day cultivation cycle. In contrast, our study achieved a significantly higher yield of 2547 $g \cdot m^{-2}$ at a sowing density of 7.3 $seeds \cdot cm^{-2}$ over 6 days (Table 1; Table 7). The high sowing density in this study may not always correlate with increased yield. Variations in results may also arise from differences in harvesting methods (e.g., harvesting the entire plant with roots, precision of above-ground part harvesting, or time since the last watering, affecting turgor). Future studies should focus on optimizing the sowing density-to-yield ratio. Additionally, seed quality, cultivar choice, and cultivation conditions should be considered.

Table 7. Interspecies comparison of yield.

Interspecies comparison of yield					
Species	**FWY·gs⁻¹ [g]***	**Yield [g·m⁻²]**	**Edible part**	**FWY·gs⁻¹ [g]**	**Yield [g·m⁻²]**
Cress	9.9 ±0.3b	1805 ±53e		The entire part is edible	
Mustard	6.0 ±0.1c	2547 ±49d		The entire part is edible	
Broccoli	7.7 ±0.1d	3259 ±46c		4.5 ±0.1b	1925 ±22c
Basil	7.3 ±0.2d	1318 ±27f		The entire part is edible	
Sunflower	4.5 ±0.1e	4861 ±77a		2.2 ±0.1c	2341 ±72a
Cucumber	11.3 ±0.2a	4114 ±73b		5.8 ±0.1a	2107 ±36b

*$FWY \cdot gs^{-1}$ [g] - Fresh biomass yield per gram of seed. Values are expressed as mean ± standard error (n = 3). Statistical differences between the values were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Different letters indicate statistically significant differences between the groups.

3.5. Ascorbic Acid

Vitamin C content varied significantly across species. Significant differences within the treatments were observed only in broccoli, where the Mg5 treatment resulted in significantly higher values ($p < 0.05$) compared to the other treatments (Table A1, Figure 4). Regardless of the concentration used, basil had the lowest ascorbic acid content, while broccoli had the highest (excluding the Mg5 treatment) (Table 8). The average ascorbic acid content in broccoli, excluding the Mg5 treatment, was 748.4 $mg \cdot kg^{-1}$ FW (Table 8), while at the highest magnesium concentration (50 $mg \cdot L^{-1}$), it increased to 883 $mg \cdot kg^{-1}$ FW (Table A1). Koh et al. [36] analysed 80 mature commercial samples of broccoli and found an average vitamin C content of 872 $mg \cdot kg^{-1}$ FW. For broccoli microgreens, values range from 791 $mg \cdot kg^{-1}$ FW [8] to 893 $mg \cdot kg^{-1}$ FW [37].

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Table 8. Comparison of analytical parameters within species.

Species	Vitamin C [mg·kg⁻¹ FW]	TAC DPPH [mg·kg⁻¹ FW]	Flavonoids [mg·kg⁻¹ FW]	Total phenols [mg·kg⁻¹ FW]	Dry matter [%]
Cress	167.7 ±7.4c	886.9 ±24.6b	305.2 ±7.1d	1909.7 ±68.5b	7.9 ±0.2e
Mustard	312.2 ±7.8b	917.8 ±21.7b	341.0 ±10.5c	3445.5 ±102.1a	9.7 ±0.2bc
Broccoli*	748.4 ±6.5a	997.9 ±8.2a	353.9 ±5.4c	1726.9 ±22.3c	9.2 ±0.2cd
Basil	43.3 ±2.0f	571.6 ±23.2c	528.9 ±30.0a	1022.7 ±37.5d	12.3 ±0.5a
Sunflower	50.7 ±2.1e	382.3 ±32.5d	408.9 ±22.8b	923.3 ±46.3e	9.4 ±0.3bc
Cucumber	75.8 ±2.5d	152.8 ±6.1e	147.7 ±4.2e	661.8 ±14.8f	9.2 ±0.2cd

* The Mg 5 treatment was excluded from the comparison because it exhibited higher values for most parameters than the other treatments. Values are expressed as mean ± standard error (n = 3). Statistical differences between the values were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Different letters indicate statistically significant differences between the groups.

While lower magnesium sulfate concentrations (up to 40 $mg \cdot L^{-1}$) did not significantly affect ascorbic acid content compared to the control, the highest concentration caused a noticeable increase in ascorbic acid levels in broccoli. Further studies are needed to confirm these findings, and it would be beneficial to test even higher concentrations than those used in this study to explore why the ascorbic acid content was not significantly affected at the second-highest magnesium sulfate concentration. The application of magnesium sulfate at certain concentrations might influence ascorbate oxidase activity, which could be related to the increase in ascorbic acid content in plants [38,39]. The effect of magnesium on ascorbic acid content appears species-dependent and may also depend on the form of magnesium used. Borowski et al. [40] found that foliar application of magnesium salts negatively affected ascorbic acid content in spinach. Yadav et al. [41] reported higher ascorbic acid content in cucumber microgreens compared to mature cucumbers. The literature shows varying values for ascorbic acid concentrations in cucumbers, indicating cultivar dependency. However, according to the USDA [42], the average ascorbic acid content in mature cucumber is 28 $mg \cdot kg^{-1}$ FW. In this study, cucumber had relatively low ascorbic acid content compared to other species, yet the average value of 75.78 $mg \cdot kg^{-1}$ FW (Table 8) was still higher than that of mature cucumber fruits. The recommended daily intake of vitamin C is 90 mg for men and 75 mg for women [43]. Given the highest average vitamin C values found in this study (883 $mg \cdot kg^{-1}$ for broccoli), daily consumption of 102 grams of broccoli microgreens for men and 85 grams for women would meet the recommended daily intake. Future studies should also consider the impact of different broccoli cultivars on vitamin C content.

3.6. Total Phenolic Content

No significant differences in total phenolic content were observed among the treatments, except in broccoli, where the Mg5 treatment led to significantly higher total phenol content ($p < 0.05$) (Table A1, Figure 4). Regardless of treatment, cucumber had the lowest average total phenolic content, while mustard had the highest (Table 8). The results suggest that mustard is the richest source of total phenols, with species from the *Brassicaceae* family generally being good sources of phenolic compounds. Mustard seeds are well known for their high phenolic content [44], and this study supports the idea that young mustard plants have a similar phenolic profile. In broccoli, the Mg5 treatment resulted in a total phenol content of 1884 $mg \cdot kg^{-1}$ FW (Table A1), which was significantly higher than in the other treatments (Table 8). Xuan et al. [45] reported higher concentrations of total phenols in rice seedlings when magnesium sulfate was applied compared to the control.

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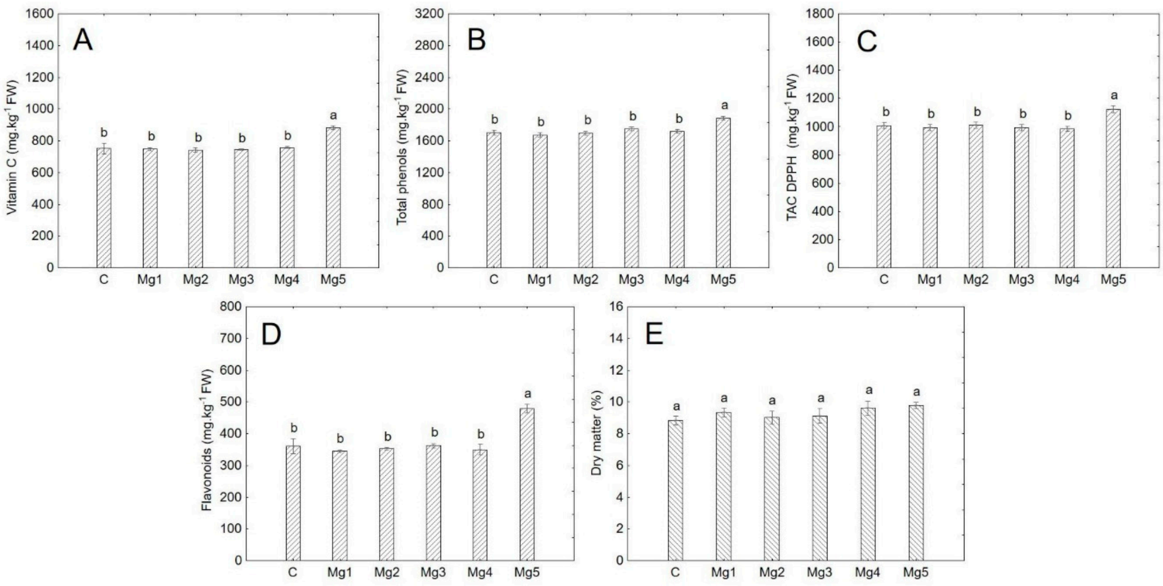

Figure 4. Analysed parameters in broccoli. Data are expressed as mean ± standard error (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$). Bars marked with different letters indicate statistically significant differences between treatments. A = vitamin C, B = total phenols, C = antioxidant activity, D = flavonoids, E = dry matter.

3.7. Flavonoids

Flavonoid content showed significant variability between species (Table 8). Similar to ascorbic acid, broccoli in the Mg5 treatment exhibited significantly higher flavonoid content ($p < 0.05$) compared to the control and lower concentration treatments (Table A1, Figure 4). While the other treatments and control averaged 354 mg·kg^{-1} FW (Table 8), the Mg5 treatment had an average flavonoid content of 479 mg·kg^{-1} FW (Table A1). The other species did not show significant changes in flavonoid content with varying magnesium concentrations. Ciscomani-Larios et al. [46] reported a positive effect of $MgSO_4$ application on flavonoid content in green beans, and similarly, magnesium sulfate increased flavonoid content in rice seedlings [45]. However, these studies focused on more advanced developmental stages of plants, and it's possible that a longer cultivation period would influence the flavonoid and total phenol content in the species examined in this study.

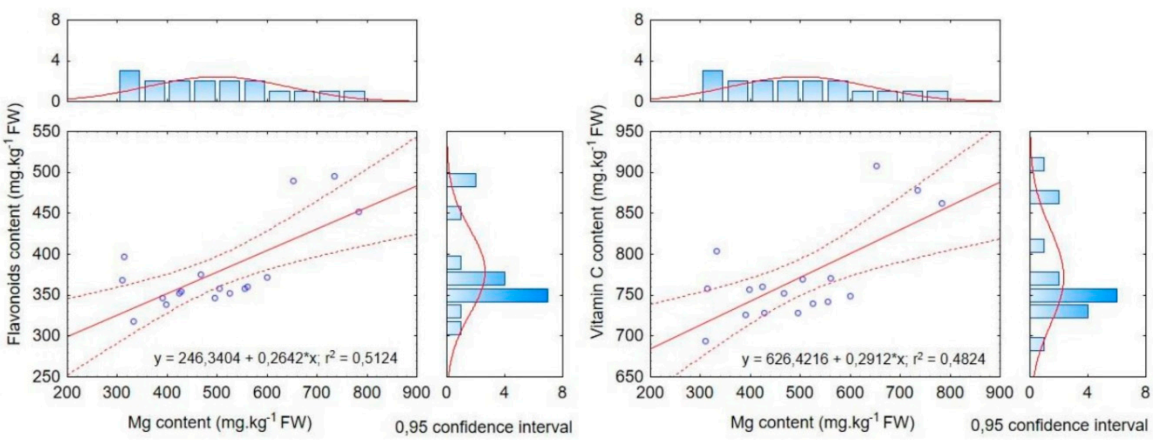

Figure 5. Relationship between magnesium content and selected parameters. Values represent individual data points with the fitted regression line and 95% confidence intervals. Linear regression analysis was performed to assess the relationship between magnesium content and the selected parameters. The coefficient of determination (R^2) indicates the proportion of variance explained by the model.

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Cucumber showed the lowest average flavonoid content across all treatments, while basil had the highest, indicating that basil is a good source of flavonoids, as confirmed by other studies [47,48]. In this study, basil stood out as the richest source of flavonoids among the species investigated, although its total phenol content was lower compared to other species. In contrast, mustard had an average flavonoid content, but its total phenol content was more than three times higher than that of basil. This could be due to genotypic variability or differences in the cultivation period.

3.8. Antioxidant Activity

While antioxidant activity was not significantly influenced by the treatments overall, significant differences were observed in broccoli, where the Mg5 treatment had significantly higher antioxidant activity ($p < 0.05$) compared to the other treatments (Table A1, Figure 4). Broccoli exhibited the highest average antioxidant activity, even when excluding the Mg5 treatment, while cucumber had the lowest values (Table 8). In fact, antioxidant activity in broccoli was 553% higher compared to cucumber. Przybysz et al. [29] reported that $MgSO_4$ concentrations between 50 and 300 mg·L^{-1} positively affected antioxidant activity in broccoli sprouts, though in this study, these findings were only partially confirmed (for the Mg5 treatment). This study found broccoli to have the highest antioxidant activity, consistent with other research showing that broccoli microgreens are considered an excellent source of antioxidants [49,50]. Additionally, no toxic effects of magnesium on antioxidant activity or other substances in the plants were observed.

4. Conclusions

This study demonstrated that $MgSO_4$ application effectively increased magnesium (Mg) content in the plants, with cucumber and broccoli showing the highest potential for Mg biofortification. Notably, no adverse effects were observed on yield or the levels of secondary metabolites such as antioxidants, phenols, flavonoids, and vitamin C. In broccoli, the highest Mg concentration (50 mg·L^{-1}) significantly boosted total phenols, flavonoids, ascorbic acid, and antioxidant activity. The study revealed substantial interspecies variability in both Mg uptake and secondary metabolite content, emphasizing the need for a species-specific approach to biofortification. This research, one of the first to focus on magnesium biofortification in microgreens, provides valuable insights into their potential as functional foods. Future studies should explore the impact of Mg biofortification on bioactive compounds, like glucosinolates in *Brassicaceae* species, and examine interactions between Mg and other minerals, such as sulphur. These efforts could help optimize microgreen biofortification, enhancing their role as healthy dietary supplements.

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Data Availability Statement: Data sharing not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Analysed parameters in other species.

Variant	Vitamin C [mg·kg⁻¹ FW]	TAC DPPH [mg·kg⁻¹ FW]	Flavonoids [mg·kg⁻¹ FW]	Total phenols [mg·kg⁻¹ FW]	Dry matter [%]
			Cress		
C	197.3 ±33.8ab	845.3 ±62.9a	289.9 ±2.1bc	1624.0 ±57.2cd	7.8 ±0.2bc
Mg1	193.6 ±19.3bc	886.2 ±81.7a	313.2 ±21.6ab	1829.3 ±260.8abc	8.4 ±0.7ab
Mg2	160.0 ±6.1cd	856.8 ±47.2a	278.9 ±8.7bc	1885.1 ±82.4abc	6.9 ±0.2d
Mg3	169.4 ±13.4bc	843.8 ±38.2a	303.6 ±8.8ab	1893.4 ±97.0abc	7.3 ±0.5cd
Mg4	159.7 ±16.1cd	952.9 ±83.9a	329.6 ±25.1ab	2128.2 ±230.6abc	8.2 ±0.5ab
Mg5	136.0 ±5.8e	922.5 ±62.2a	310.7 ±16.1ab	2003.0 ±123.7abc	8.8 ±0.7ab
			Mustard		
C	297.4 ±5.0bc	889.5 ±77.3bc	394.3 ±14.0ab	3431.8 ±224.7c	8.7 ±0.4d
Mg1	285.8 ±16.7bc	885.8 ±84.3bc	355.9 ±28.9ab	3919.3 ±148.4a	8.8 ±0.4d
Mg2	304.6 ±23.5ab	827.4 ±45.9c	309.6 ±6.6cd	2974.2 ±362.4c	8.9 ±0.2d
Mg3	321.6 ±5.9ab	936.7 ±71.4bc	308.3 ±19.3cd	3275.4 ±167.7c	9.8 ±0.1c
Mg4	309.7 ±9.9ab	962.5 ±28.9ab	318.1 ±8.2cd	3499.8 ±100.7c	10.8 ±0.6ab
Mg5	345.1 ±31.3a	938.6 ±18.2ab	356.8 ±24.3ab	3730.3 ±47.1b	10.5 ±0.1ab
			Broccoli		
C	751.3 ±31.9b	1008.5 ±19.5b	360.4 ±23.2b	1699.9 ±3.5b	8.8 ±0.3a
Mg1	747.3 ±10.8b	991.8 ±21.9b	345.5 ±3.9b	1673.9 ±7.0b	9.3 ±0.3a
Mg2	741.3 ±13.8b	1010.5 ±21.5b	352.9 ±3.6b	1694.0 ±42.2b	9.0 ±0.4a
Mg3	744.8 ±3.8b	993.2 ±22.1b	361.8 ±6.9b	1752.4 ±24.6b	9.1 ±0.5a
Mg4	757.5 ±6.4b	985.7 ±17.1b	349.3 ±17.0b	1717.2 ±26.7b	9.6 ±0.5a
Mg5	882.5 ±13.3a	1123.3 ±23.5a	478.5 ±13.5a	1884.0 ±16.5a	9.8 ±0.2a
			Basil		
C	44.9 ±2.7ab	551.2 ±86.4bc	474.8 ±107.9bcd	1041.9 ±120.0ab	11.2 ±1.2a
Mg1	43.1 ±5.8ab	618.4 ±26.7bc	575.1 ±50.3bc	1114.6 ±30.7ab	11.0 ±0.7a
Mg2	37.2 ±5.2bc	478.0 ±5.7cd	457.7 ±15.4cd	1016.5 ±121.4ab	12.3 ±0.6a
Mg3	39.1 ±1.3bc	669.7 ±12.2a	662.4 ±10.7a	1135.6 ±7.3ab	12.7 ±1.6a
Mg4	44.4 ±3.2ab	524.2 ±38.5bc	535.1 ±21.8bc	942.2 ±114.4bc	10.7 ±0.7a
Mg5	51.3 ±4.2a	478.0 ±53.5cd	378.3 ±31.1de	785.9 ±45.6d	13.7 ±1.3a
			Sunflower		
C	56.4 ±3.4ab	430.3 ±9.7ab	481.5 ±24.1a	1251.10 ±129.2ab	9.8 ±0.3a
Mg1	47.2 ±5.7cd	375.5 ±81.9ab	415.4 ±50.5a	872.8 ±70.0bc	10.4 ±1.2a
Mg2	41.6 ±2.3e	328.8 ±54.0cd	402.6 ±45.3a	849.3 ±77.9bc	9.2 ±0.8a
Mg3	50.5 ±2.1cd	349.1 ±26.5cd	382.4 ±46.9a	906.0 ±61.3bc	9.9 ±0.4a
Mg4	51.6 ±4.5ab	471.1 ±132.0ab	390.6 ±74.1a	1092.3 ±191.0ab	8.0 ±0.3b
Mg5	62.0 ±6.4a	564.5 ±146.5ab	478.9 ±84.3a	1054.7 ±180.7ab	10.4 ±0.6a
			Cucumber		
C	78.8 ±5.4ab	161.1 ±14.6ab	135.7 ±8.8cd	703.5 ±56.1ab	9.9 ±0.5a
Mg1	66.1 ±1.6c	154.5 ±7.7bc	144.3 ±11.5cd	650.5 ±14.6ab	8.2 ±0.1b
Mg2	73.5 ±6.6bc	180.9 ±8.4ab	145.4 ±6.7cd	694.5 ±26.9ab	9.2 ±0.1a
Mg3	70.5 ±5.4bc	127.1 ±9.9de	137.5 ±11.7ab	657.0 ±34.3ab	9.0 ±0.4a
Mg4	81.2 ±7.1ab	144.9 ±4.9bc	152.2 ±12.3bc	612.9 ±28.3bc	8.9 ±0.6a
Mg5	83.7 ±10.7ab	123.2 ±0.9de	169.3 ±9.9cd	620.6 ±17.1bc	9.7 ±0.4a

Values are expressed as mean ± standard error (n = 3). Statistical differences between the values were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Different letters indicate statistically significant differences between the groups.

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Table A2. Yield by treatment.

Treatment	Cress	Mustard	Broccoli	Basil	Sunflower	Cucumber
C	1932 ±5a	2400 ±202a	3183 ±86a	1265 ±54ab	5041 ±84a	3975 ±259a
Mg1	1949 ±111a	2561 ±131a	3275 ±85a	1395 ±46ab	5021 ±168a	4285 ±213a
Mg2	1775 ±104a	2624 ±89a	3446 ±141a	1324 ±112a	5224 ±203a	4017 ±254a
Mg3	1691 ±82a	2535 ±46a	3399 ±99a	1369 ±43ab	4674 ±113a	4241 ±104a
Mg4	1714 ±254a	2581 ±148a	3180 ±123a	1317 ±58ab	4657 ±141a	3987 ±45a
Mg5	1858 ±57a	2515 ±140a	3069 ±70a	1237 ±58b	4610 ±82a	4078 ±69a

Values are expressed as mean ± standard error (n = 3). Statistical differences between the values were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Different letters indicate statistically significant differences between the groups.

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