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Exploring the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown *Cichorium spinosum* L.

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Abstract: Nutrient-efficient plants and agricultural systems could tackle issues resulting from conventional agriculture. Spiny chicory (*Cichorium spinosum* L.), a very adaptive, wild edible vegetable, is gaining commercial interest as a functional food. Floating-raft hydroponics is a method commonly used for the commercial cultivation of leafy vegetables due to numerous advantages compared to soil cultivation. In this paper, the simultaneous effects of different potassium, calcium and magnesium ratios and different electrical conductivity (EC) levels on the growth and mineral composition of hydroponically grown C. spinosum were investigated. Four nutrient solutions (NS) were compared, two NS with low EC (L, 2.4 dS/m) and two with high EC (H, 3.6 dS/m) with K:Ca:Mg ratios of either 50:40:10 or 40:50:10. The results showed no interactions between the two factors. No significant effects were observed on the fresh and dry weight, leaf number and leaf area. High EC levels increased the K content and decreased the Mn and Zn content in the leaf tissues. The 40:50:10 ratio led to increased Ca content in plant tissues. The Nitrate-N was only affected by the EC level and was increased under H conditions, whereas the total-N was not affected.

Keywords: hydroponics; stamnagathi; floating; macrocation ratio; electrical conductivity

Citation: Voutsinos-Frantzis, O.; Ntatsi, G.; Karavidas, I.; Neofytou, I.; Deriziotis, K.; Ropokis, A.; Consentino, B.B.; Sabatino, L.; Savvas, D. Exploring the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown *Cichorium spinosum* L.. *Agronomy* **2022**, *12*, 2214. https://doi.org/10.3390/agronomy12092214

Academic Editor: Byoung Ryong Jeong

Received: 31 August 2022
Accepted: 13 September 2022
Published: 16 September 2022

1. Introduction

Efficient energy and resource use management are crucial for more sustainable agriculture [1]. There has been a growing interest in mineral efficiency in crops since at least 40% of the world's arable land suffers from mineral deficiencies [2] which usually demand the use of inorganic fertilizers in order to sustain high yields. In addition, it has been suggested from dietary surveys that an approximate 10% of the energetically well-nourished adult population in developed countries assumes a suboptimal intake of macronutrients such as K, Ca and Mg and is considered to be at risk of deficiency [3]. Meanwhile, the significant increase in the fertilizers' cost and growing concerns regarding the environmental impact of the recently excessive fertilizer inputs, coupled with the increasing demand for fresh and nutritious food on an overpopulated planet, further enhances the importance of both nutrient-efficient cultivars and agricultural systems [4,5]. Nevertheless, even though, in the past 30 years, many experiments have been conducted in order to identify nutrient-efficient species and cultivars, or even landraces and ecotypes [6–10], there has been little success in breeding and introducing those cultivars into mainstream agriculture.

Soilless culture, in either greenhouses or indoor growing modules, is becoming a more popular alternative to open-field production both due to energy use efficiency and

also due to the high risk of open-field systems induced by climate change [11–13]. Year-round production, regardless of the outdoor conditions or soil quality, can nowadays be achieved through protected, controlled environment agricultural systems, such as hydroponic greenhouses [14,15] or even vertical farms [16,17], covering the rising demand for high-value fresh leafy vegetables [18].

The Mediterranean basin is known for its abundance of wild, edible underutilized horticultural species [19]. These plants grow spontaneously in nature and were traditionally included in the locals' diets [20]. Nowadays, even though urbanization has alienated people from the lifestyle associated with the gathering of these crops, consumer demand for these species has increased [21]. The need to diversify a monotonous diet and consume foods with nutraceutical compounds played a critical role in this increase [22–25]. Spiny chicory (*Cichorium spinosum* L.), also known as "stamnagathi", is one of those wild edible plants that has been gaining attention in the Mediterranean basin for the past 20 years. In Greece, stamnagathi is mainly grown in Crete, where different ecotypes have been introduced to commercial cultivation, both in soil and soilless culture systems [26–31]. Moreover, stamnagathi commands high prices on the market thanks to its unique taste and richness in health-promoting compounds [32]. However, upscaling efforts of the cultivation of stamnagathi, with a potential for it to become a highly profitable crop, are limited. In addition, the information regarding its response to various environmental conditions is largely fragmented [33].

The biomass and composition of hydroponically grown *C. spinosum* are modulated interactively by the chosen ecotype, the salinity level and type and EC level related to nitrogen amount and supply [28,32–34]. It has been demonstrated that different macronutrient compositions of nutrient solutions can have a significant impact on the yield and chemical compositions of certain crops, such as strawberries [35], melon [36], tomato [37] and lettuce [38–40]. When the nutrient solution is within the optimum range of the crop's nutrition, its effect can be exerted by the genotype and growing conditions, as observed by ref. [41] on lettuce. In this work, we assess the interaction of different EC levels and K:Ca:Mg ratios on the growth and nutritional value of *Cichorium spinosum* L. cultivated in a hydroponic floating-raft system.

2. Materials and Methods

2.1. Plant Material and Experimental Setting

The experiment took place during the spring growing season, from 5 April 2021 to 21 May 2021, under natural lighting conditions in a glass greenhouse of the Laboratory of Vegetable Production at the Agricultural University of Athens (Athens, Greece), located at latitude 37.98°, longitude 23.7 and altitude 38 m. The greenhouse chamber was equipped with a heating system with aerial hot water pipes, while the cooling of the greenhouse was achieved through the opening of the side windows and roof. The greenhouse was protected from insects by covering all windows and door openings with insect netting.

On 5 April 2021, intact achenes of stamnagathi (*Cichorium spinosum* L.) were sown on rockwool plugs (AO Plug, Grodan, Roermond, Netherlands) and covered with a thin layer of vermiculite. On 10 May 2021, the resultant seedlings that had reached 2–6 true leaves were separated and transplanted evenly on the floating rafts used for the experiment by placing the rockwool plugs in 55 mm plastic net pots and then placing the pots in the holes of each raft (Figure 1). Each tank used for this experiment was developed from stainless steel by Intelagro (Intelagro, Thessaloniki, Greece) and was 1.5 m × 0.6 m × 0.4 m (length × width × depth). Each replication was filled with 180 L of a particular starter nutrient solution for each treatment, and would accommodate up to 48 plants, having a plant density of 53 plants/m^2. Because the NS level was maintained by connecting the main tank to a 50-L replenishment solution tank through a float valve, four plants that would be placed in the final row were never used. Their place was covered with styrofoam to stop sunlight from reaching the NS and avoid the growth of algae. For the oxygenation and uniformity of the NSs, an air stone and an aquarium pump were placed inside each tank.

The average day/night temperatures and relative humidity values (±standard deviations) inside the glasshouse were 33 ± 9 °C and 32 ± 10% and +19 ± 3 °C and 61 ± 10%.

Figure 1. View of the chamber where the experiment took place, with the 16 tanks and floating rafts, after transplanting took place.

As soon as the seedlings reached a number and stage suitable for transferring to the floating rafts, 704 seedlings were chosen and separated into three groups based on their size and the number of true leaves developed (large, medium and small, see Figure 2). These groups were then distributed into 16 tanks so that each tank had the same number of small, medium and large seedlings.

Figure 2. Separating the seedling into 3 groups based on their size and leaf number before transplanting them to the tanks.

The experiment was carried out as a Randomized Complete Block Design. One factor was the total ionic content, having either high (H-) or low (L-) electrical conductivity (EC) and the other factor was the macrocation ratio of potassium, calcium and magnesium. Two K:Ca:Mg ratios were used for the experiment, 50:40:10 and 40:50:10. The four treatments were labeled based on their EC and K:Ca:Mg ratio as L-50:40:10, L-40:50:10, H-50:40:10 and H-40:50:10. Each treatment was replicated four times making a total of 16 tanks (see Supplementary Material Figure S1). The nutrient solution composition of each treatment, plus that of the common treatment replenishment solution, is shown in Table 1. The percentages of K:Ca:Mg have been chosen so that the starvation effect of either of the elements is avoided. The selection of the K:Ca:Mg ratio 50:40:10 was based on findings from a previous paper [42] since that ratio was found similar to the molar ratio of these nutrients in the leaf tissue. It is well known that the uptake of sufficient amounts of Ca by plants requires the maintenance of a substantially higher Ca concentration in the root zone than the uptake concentration of Ca (i.e., the Ca/water ratio in mmol/L) [43]. Therefore, in our research, we compared this standard K:Ca ratio (50% K, 40% Ca) with an increased Ca ratio compared to that of the other two micronutrient cations (40% K, 50% Ca). This was important in this research in order to fine-tune the nutrient solution for the cultivation of spiny chicory since bigger ratio differences could induce severe changes in the anatomy,

physiology and biochemistry by affecting the photosynthetic carbon assimilation, which was not desired in this study [44].

Table 1. Composition of the 4 nutrient solutions and the replenishment solution.

	Nutrient Solutions				
Treatment Name	**L-50:40:10**	**L-40:50:10**	**H-50:40:10**	**H-40:50:10**	**Replenishment**
EC (dS/m)	2.4		3.6		2.2
K:Ca:Mg	50:40:10	40:50:10	50:40:10	40:50:10	60:32:8
pH	5.6				6.1
K^+ (mmol/L)	6.93	5.14	10.69	7.9	7.54
Ca^{2+} (mmol/L)	5.54	6.52	8.55	10.07	4.02
Mg^{2+} (mmol/L)	1.39	1.30	2.14	2.01	1.01
SO_4^{2-} (mmol/L)	3.08		5.18		1.98
NH_4^+ (mmol/L)	1.15		1.87		1.68
NO_3^- (mmol/L)	14.17		21.97		11.4
$H_2PO_4^-$ (mmol/L)	1.4				1.12
Fe (µmol/L)	20				14.88
Mn^{++} (µmol/L)	9				8.37
Zn^{++} (µmol/L)	5				3.72
Cu^{++} (µmol/L)	0.8				0.65
B (µmol/L)	30				23.25
Mo (µmol/L)	0.5				0.47
Cl^- (µmol/L)	0.4				
Na^{++} (µmol/L)	0.6				
HCO_3^- (µmol/L)	0.4				

For the determination of the composition of each nutrient solution and the quantities of the fertilizers required, NUTRISENSE (accessed on 1 April 2021, https://nutrisense.online/), an online Decision Support System (DSS) program, was used [45] (Laboratory of Vegetable Production, Agricultural University of Athens, Athens, Greece). The final solution was prepared by dilution of the dense A and B nutrient solutions 100 times until the chosen EC levels (2.4 dS/m or 3.6 dS/m) were reached. Then, nitric acid was used to reach the pH values (5.5–6.5). The dilution took place in a 300 L barrel that was connected with a pump that either recirculated the solution or pumped it via rubber tubing into the experiment chamber's tanks.

2.2. EC and pH Values Monitoring and Water Consumption Determination

During preparation and after pumping the NSs from the dilution barrel to the chamber's tanks, the EC and pH values were monitored on a daily basis with pH and EC pens (Bluelab, Tauranga, New Zealand), and the readings were recorded manually. The consumption of the replenishment solution was carried out by having measured the weight of each replenishment tank while empty and then adding 15 L of replenishment solution, weighing again before connecting them with the main tank at the start of the experiment, and then throughout the growing period, the replenishment tanks were disconnected and weighed by placing them again on the scale, writing down the current weight and adding replenishment solution if needed. The sum of the replenishment solution removed from the replenishment tank was calculated at the end of the experiment.

2.3. Growth and Yield Parameters

The experiment ended when the average plant had reached the salable size. At that time, all the plants from every tank were harvested, and their fresh weight was measured to determine the yield of the crop. Apart from the fresh weight, five plants of each tank

were used to measure the leaf number, leaf area and dry weight. For the plant weight, a Mettler PE 3600 (Mettler-Toledo, Columbus, OH, USA) balancer was used, while the leaf area measurement was conducted by separating leaves by hand and placing them on the transparent belt of LI-3100C (LI-COR, Inc. Lincoln, NE, USA). The separated leaves were placed in a paper bag along with the stem and then placed in a drying oven (STF-N 400, FALC Instruments S.L.R, Treviglio, Italia) at 65 °C for 7 days in order to dry the plant tissue until their weight became stable. After the 7-day period, the dry plant tissues' weight was measured, followed by grating the samples by passing them through the MF 10 Microfine grinder (IKA Werke, Staufen, Germany) at the highest speed option (6000–6500 rpm) and collecting the grated tissues in sealable plastic bags to avoid humidity degrading the sample.

2.4. Nutrient Concentration in Plant Tissues

In order to proceed with the analysis of the nutrient content of the plants, the dry ashing method was followed. In total, 0.5 g of dry plant tissue were placed in porcelain cups which were then placed in chamber furnace LM-112 (Linn High Therm, Hirschbach, Germany) for 8 h at 550 °C until they became ash. The ash was then used to produce plant tissue solutions by adding 10 mL of HCl solution (0.25 N) in the porcelain pots and then infiltrating the content through 125 mm, Macherey-Nagel filter paper and filling the volumetric flasks with distilled water until the solution level reached 100 mL. Finally, the aqueous tissue extracts of each sample were poured inside a 100 mL plastic bottle and stored in a refrigerator until the chemical analysis was carried out.

The nutrient concentration of the aqueous tissue extracts was determined with various methods depending on the element. For the determination of phosphorus, the molybdenum blue reaction for the determination of orthophosphate [46] was used, and photometry was carried out in the Anthos Zenyth 200 (Biochrom Ltd., Cambridge, UK). Calcium, magnesium, iron, manganese, zinc and copper were all measured by placing the aqueous tissue extracts, diluted or undiluted, in the Atomic Absorption Spectrophotometer Shimadzu AA-7000 (Shimadzu, Kyoto, Japan). Potassium and sodium were measured by placing the plant tissue solution, diluted or undiluted, in the Sherwood Flame Photometer 410 (Sherwood, Cambridge, UK). Total nitrogen was determined by the Kjeldahl method.Digestion was carried out on Labtec DT 220 with the simultaneous use of Scrubber Labtec SR 210, while distillation was carried out using Tecator Kjeltec 8200 (FOSS A/S, Hillerød, Denmark). The determination of the total nitrogen content was completed through manual titration for each distilled sample by measuring the ml of HCl solution (0.05 N) needed to turn the solution's color from green to pink. The determination of nitrate content in dry tissues [47] was conducted colorimetrically by nitration of salicylic acid and photometering using Anthos Zenyth 200 (Biochrom Ltd., Cambridge, UK). The results were expressed in ug g^{-1} of fresh weight. Finally, boron was determined spectrophotometrically with an azomethine H derivative [48], and photometering was carried out using Anthos Zenyth 200.

2.5. Statistical Analyses

Experimental data were analyzed by applying a two-way ANOVA to assess the main effects of electrical conductivity (EC) and potassium-calcium-magnesium ratio (K:Ca:Mg ratio) on first-order interaction (EC × K:Ca:Mg ratio). Multiple comparison of means was performed by applying Duncan's Multiple Range Test at a confidence level of 0.05 after performing a two-way ANOVA. All statistical analyses were carried out using the version 9.0 STATISTICA software package for windows (StatSoft Inc., Tulsa, OK, USA). Normality was respected for both parameters, and no data transformation was required for either parameter.

3. Results

3.1. EC and pH Levels

EC and pH levels (see Figures 3 and 4) were monitored throughout the cultivation period. The levels of EC increased as time passed, while pH values decreased with the passage of time.

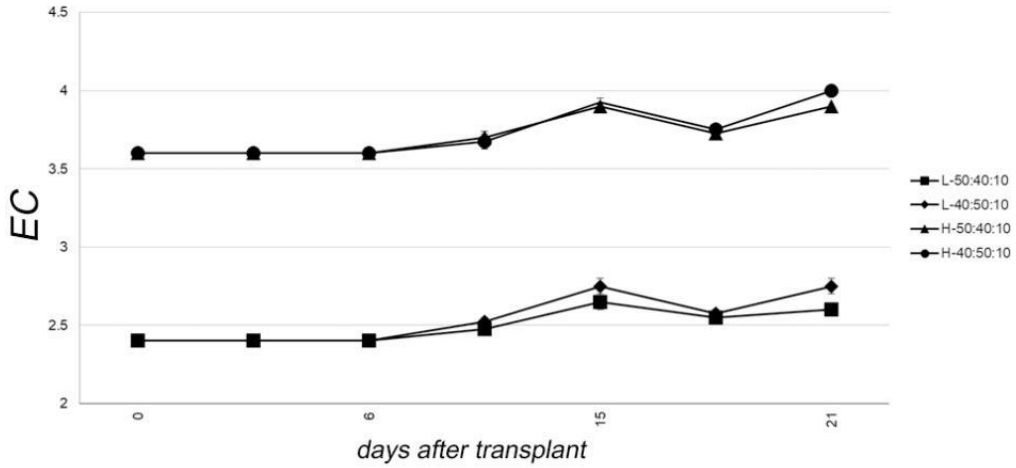

Figure 3. Mean weakly EC levels during the cultivation period. Vertical bars denote ± standard errors of means (n = 4).

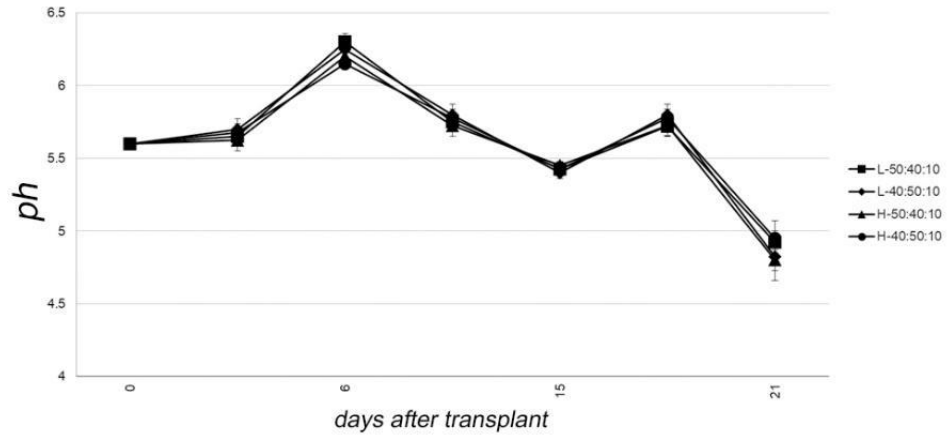

Figure 4. Mean weakly pH levels during the cultivation period. Vertical bars denote ± standard errors of means (n = 4).

3.2. Effects on Growth

To assess the effects on the yield of spiny chicory, we measured fresh and dry weight, as well as leaf number and leaf area at the harvest stage. The outcome of the statistical analysis of each factor separately (EC and K:Ca:Mg ratio), as well as their interactions, showed that none of the agronomical characteristics of *Cichorium spinosum* L. were significantly affected by the different NSs (Table 2).

Table 2. Impact of different EC levels (low and high, as L and H, respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the fresh weight (FW), dry weight (DW), leaf number (LN) and leaf area (LA) of spiny chicory cultivated in a hydroponic floating raft system.

Main Effects					
Factor	**Treatment**	**FW (g)**	**DW (g)**	**LN**	**LA (cm^2)**
EC level	L	9.179	0.815	20.98	137.75
	H	7.8625	0.823	17.56	123.43
K:Ca:Mg ratio	40:50:10	7.763	0.766	18.19	123.67
	50:40:10	9.279	0.873	20.34	137.50
Statistical Significance					
EC		NS	NS	NS	NS
K:Ca:Mg ratio		NS	NS	NS	NS
EC × K:Ca:Mg ratio		NS	NS	NS	NS

The values are means (n = 4). For each factor, or all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to Duncan's multiple comparison test ($p < 0.05$): NS indicates "not significant" at $p < 0.05$.

3.3. Effect on the Nutrient Composition of Leaf Tissue

The effects of the studied factors on the macronutrient content of the leaf tissues are shown in Table 3. The high EC level had a positive effect on the potassium content of the leaf tissues compared to the low EC level. The 40:50:10 K:Ca:Mg ratio had a positive effect on the calcium content of the leaf tissues. There appears to be no interaction of the two factors (EC and K:Ca:Mg ratio) on the concentration of macronutrients in the leaf tissues of hydroponically grown spiny chicory.

Table 3. Impact of different EC levels (low and high, as L and H, respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of macronutrients (K, Ca, Mg and P) in leaf tissues of hydroponically grown spiny chicory.

Main Effects					
Factor	**Treatment**	**K (mg/g)**	**Ca (mg/g)**	**Mg (mg/g)**	**P (mg/g)**
EC level	L	51.25	3.68	2.27	7.66
	H	56.75	3.72	2.074	8.62
K:Ca:Mg ratio	40:50:10	54.00	4.31	2.17	8.24
	50:40:10	54.00	3.10	2.17	8.04
Statistical Significance					
EC		*	NS	NS	NS
K:Ca:Mg ratio		NS	*	NS	NS
EC × K:Ca:Mg ratio		NS	NS	NS	NS

The values are means (n = 4). For each factor, or all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to Duncan's multiple comparison test ($p < 0.05$): * indicate significance at $p < 0.05$; NS = not significant.

The effects of the studied factors on the micronutrient content of the leaf tissues are shown in Table 4. The EC levels appear to affect the concentration of Mn and Zn in the leaf tissues since concentrations of those elements appeared significantly higher in the L treatments. When looking at the K:Ca:Mg ratio factor, no significant differences occurred between the treatments. Finally, no interactions between EC and the K:Ca:Mg ratio appeared through statistical analysis.

Table 4. Impact of different EC levels (low and high, as L and H, respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of micronutrients (Fe, Mn, B, Zn, Cu, Na) in leaf tissues of hydroponically grown spiny chicory.

Main Effects							
Factor	**Treatment**	**Fe (μg/g)**	**Mn (μg/g)**	**B (μg/g)**	**Zn (μg/g)**	**Cu (μg/g)**	**Na (mg/g)**
EC level	L	114.69	131.7 a	63.21	112.59 a	22.5	0.68
	H	110.75	112.79 b	62.87	99.28 b	16.88	0.66
K:Ca:Mg ratio	40:50:10	116.11	122.68	64.53	105.84	21.12	0.69
	50:40:10	109.33	121.82	61.55	106.03	18.26	0.66
Statistical Significance							
EC		NS	*	NS	*	NS	NS
K:Ca:Mg ratio		NS	NS	NS	NS	NS	NS
EC × K:Ca:Mg ratio		NS	NS	NS	NS	NS	NS

The values are means (n = 4). For each factor, or all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to Duncan's multiple comparison test ($p < 0.05$): * indicate significance at $p < 0.05$; ns = not significant.

The nitrate-N was expressed in mg kg^{-1} of fresh weight. From Table 5, it is clear that only the EC level affected the nitrate-N levels, which were higher under the high EC levels. On the other hand, the total N content, expressed in mg g^{-1} of dry weight, was not affected by either the EC level or the K:Ca:Mg ratio.

Table 5. Impact of different EC levels (low and high, as L and H, respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of nitrates in fresh tissues and total nitrogen in dry tissues of hydroponically grown spiny chicory.

Main Effects			
Factor	**Treatment**	**NO_3-N (mg kg^{-1} fw)**	**Total N (mg g^{-1} dw)**
EC level	L	721.21 b	48.99
	H	933.28 a	47.98
K:Ca:Mg ratio	40:50:10	822.53	49.05
	50:40:10	833.53	47.92
Statistical Significance			
EC		*	NS
K:Ca:Mg ratio		NS	NS
EC × K:Ca:Mg ratio		NS	NS

The values are means (n = 4). For each factor, or all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to Duncan's multiple comparison test ($p < 0.05$): * indicate significance at $p < 0.05$; ns = not significant.

4. Discussion

In recent years, few studies have been conducted on stamnagathi. Despite this, there has been a focus on its nutritional value, chemical composition and bioactive compounds content under salinity stress induced by NaCl [28] or other salts such as KCl, Na_2SO_4 and $CaCl_2$ [34]. Other studies have focused on the effects of different ecotypes, salinity and nitrogen supply on the biomass production of hydroponic spiny chicory [32] and on successive harvestings [49]. In every study, *C. spinosum* is identified as a highly adaptable wild edible vegetable, always rich in nutrients, especially when it comes to its potassium, calcium, iron, magnesium and zinc content [50]. In this study, the simultaneous effect of different EC levels (high, H and low,

L) and K:Ca:Mg ratios (50:40:10 and 40:50:10) on the growth and nutrient concentration in dry leaf tissues of *C. spinosum* cultivated on a floating raft hydroponic system was evaluated. The two K:Ca:Mg ratios were chosen based on a previous study where the 50:40:10 ratio of those elements was also observed in the leaf tissues [42]. The uptake of sufficient amounts of Ca by plants requires the maintenance of a substantially higher Ca concentration in the root zone than the uptake concentration of Ca [43]. In order to avoid the starvation effects of either of the elements that would further induce changes in the anatomy, physiology and biochemistry of the plants, the 40:50:10 ratio was chosen. By studying the effect of this ratio on the growth of *C. spinosum*, we aimed to fine-tune the hydroponic solution supplied in hydroponics [44,51]. The findings of the present work also showcase *C. spinosum* as a highly adaptable edible green.

It has been suggested that increased K levels in the nutrient solution can increase the leaf area and number of leaves due to the effect that K ions have on cell division and in the control of cell expansion and turgor [52]. Moreover, it has been suggested that for lettuce and mustard, increased calcium in the nutrient solution could as also increase leaf number, with or without the simultaneous increase in fresh weight [53]. Nevertheless, this was not observed in the current work, as our results indicate that none of the agronomical characteristics (fresh weight, dry weight, leaf number and leaf area) were affected by either the examined factors or their interaction (Table 2). These results indicate that stamnagathi has a wide range of adequacy for K and Ca, and the K and Ca levels applied through the NS in both tested ratios were within this range.

Throughout the experiment, the changes in EC and pH levels in the nutrient solution of each tank were monitored (Figures 3 and 4). The EC levels increased slightly by the end of the growing period. This is ascribed to the accumulation of certain nutrients that were present in the starter solution and later supplied by the replenishment solution at a pace that was greater than the plants could absorb. The EC levels during a particular cropping period might also increase when the nutrient uptake rates remain stable, if the rates of water uptake increase due to changes in climatic conditions, since these two processes are physiologically independent [54]. This also underlines the importance of more balanced management of the nutrient solution, which should take into consideration the greenhouse microclimate.

Apart from the agronomical parameters, the nutrient concentrations in the leaf tissues were determined. The main finding is that there was no interaction between the two factors, but when looking only at the main effects, the EC factor affected the K concentration in the leaf tissues proportionally while the K:Ca:Mg ratio factor showed increased Ca concentration in the high Ca treatment (40:50:10). As seen from Tables 3–5, the different compositions of the NSs had a slight impact on the nutrient content of the plant tissues. From Table 3, it can be seen that the concentration of K in the leaf tissues increased from 51.25 mg/g in the L treatments to 56.75 mg/g in the H treatments, but the effect of the K:Ca:Mg ratio was insignificant. On the contrary, the concentration of Ca in the leaf tissues increased under a lower K to Ca ratio from 3.10 mg/g under 50:40:10 conditions to 4.31 mg/g under 40:50:10 conditions and was not affected by the EC level. Towards the end, statistical analysis did not show any interaction between the EC and the K:Ca:Mg ratio for the macronutrient concentrations in the leaf tissues.

The effect of the composition of the NSs was even slighter on the plants for the micronutrient concentration in the leaf tissues. As seen in Table 4, only the EC factor had a significant effect and only on the concentration of Mn. Manganese concentrations were greater under low EC conditions. Mn concentration was 131.7 μg/g in the L treatment and 112.79 μg/g in the H treatment. It is clear that the K:Ca:Mg ratio did not affect any micronutrient. Furthermore, no interaction appeared between the two factors for the micronutrient concentration of the leaf tissues.

Finally, no interaction between the EC and the K:Ca:Mg ratio occurred for leaf nitrate-N concentration (Table 5). Nitrate appeared higher in the H treatment, 933.28 mg per kg of fresh weight, whereas it was 721.21 mg per kg of fresh weight in the L treatment. Furthermore, it was not affected by the K:Ca:Mg ratio. Nitrate concentration is very important

to be kept under a certain threshold due to concerns that high amounts of NO_3^- in leafy vegetables could potentially result in life-threatening diseases such as methemoglobinemia or gastric cancer [55,56]. Since the high EC treatments had no significant impact on the yield, leaf number or leaf area, we consider the L treatments safer for consumption, even though both H and L maintained leaf nitrate levels dangerously close to the threshold for similar vegetables to *C. spinosum*, such as *C. intybus* [57]. The overall low nitrate level can also be ascribed to the design of the nutrient recipe and the use of a hydroponic floating raft system that can manipulate the root environment in a manner that contributes to the avoidance of increased nitrate levels in plant tissues [58,59]. Interestingly, the total nitrogen concentration of *C. spinosum* L. was not significantly affected by any of the studied factors or their interactions. The insignificant differences in the total nitrogen imply that the higher EC, which was imposed by increasing the concentration of N (and P, K, Ca, and Mg), did not further benefit the plants but only increased the nitrate concentration in the leaf tissues. Increased accumulation of nitrates in leafy vegetables occurs when the rate of absorbed nitrate is higher than the rate of its assimilation. Nitrate, being the main N source in hydroponic solutions and the main form that nitrogen is absorbed by plants, enters the plant cells and is reduced to nitrite via the nitrate reductase in the cytosol, and then nitrite, which is toxic to plant cells, is rapidly reduced to ammonium in the plastids via the nitrite reductase. Ammonium then is assimilated into organic nitrogen via the actions of glutamate synthase, the glutamine synthetase cycle and the glutamate dehydrogenase pathway [60]. In the current experiment, the plants were grown under the same natural light conditions in all treatments. Hence, the increased amounts of unassimilated nitrogen in the leaves of plants treated with the higher EC is ascribed to excess nitrate in the nutrient solution and not to differences in lighting regimes or any other environmental factor [61–63].

As a wild plant, *C. spinosum* L. has high physiological efficiency in terms of produced biomass compared to domesticated commercial vegetables such as lettuce. Therefore, the unaffected macrocation concentration in the leaf tissues can be ascribed to its ability to easily acclimate to different conditions [32]. For instance, in an experiment carried out on two butterhead lettuce cultivars by Corrado et al. [18], the effect of three NSs differing in the K:Ca:Mg ratios were examined as follows, 68:16:16, 16:68:16 and 16:16:68, and the authors found that the nutrient concentration in the leaf tissue was significantly affected by the treatments. The difference between that study and our results is ascribed to both the plant material used, lettuce being a much more domesticated plant than spiny chicory, and the more extreme differences in the K:Ca:Mg ratios.

The fresh weight and dry weight of the current research appear lower compared to the outcome of Chatzigiani et al. [32], who explored the impact of different NaCl levels on the cultivation of montane and coastal ecotypes of spiny chicory grown in perlite bags. In addition, the leaf K, Mg and P concentrations in the current study appear close to those reported for the montane ecotype by Chatziginani et al. [32], while that for Ca was substantially lower in the current study. In another study by Petropoulos et al. [28], the leaf K and Mg concentrations were similar to those found in the current experiment, whereas that of Ca was higher compared to our study and closer to that reported by Chatzigiani et al. [32]. The differences in leaf Ca may be ascribed to the different growing systems and the differences in the nutrient solutions used in each experiment. Moreover, research carried out by Zamaniyan et al. [64] on *Cichorium intybus* L. demonstrated an increase in fresh weight when the K:Ca ratio reached 6:3, whereas further increase had a negative effect on fresh weight. Huett's [39] results from examining two lettuce cultivars under different NSs, with K:Ca ratios ranging from 1:3.5 to 3.5:1, suggested that the level of the effect is tied to the cultivar. In agreement with Huett's findings, a positive effect of elevated K levels was reported by Barickman et al. [40], who observed an increase in fresh weight and dry weight of lettuce plants. These findings demonstrate the heterogeneity in the available literature in terms of the effect of macrocation ratios on plant growth. As it has been previously noted by El-Nakhel et al. [65], this may be due to the difference

in the proportions of the accompanying ions, as well as their final concentration in the nutrient solution.

5. Conclusions

In this experiment, we tested four different nutrient solution recipes on the growth of *Cichorium spinosum* L., particularly two of high total ionic content (H-40:50:10 and H-50:40:10) and two of low total ionic content (L-40:50:10 and L-50:40:10), one of each had a K:Ca:Mg ratio 40:50:10 and the other 50:40:10. It was speculated that as a highly adaptable wild edible green, spiny chicory's growth would not be affected by the different nutrient solutions. Apart from a few significant differences in the nutrient content of the plant tissues of the cultivated plants, such as increased K content under high EC levels and increased Ca content under the 40:50:10 K:Ca:Mg ratio, the overall fresh and dry weight, leaf number and leaf area were not affected by either factor or their interactions. The fresh weight ranged from 7.8 to 9.3 g per plant and the dry weight from 0.77 to 0.87 g per plant. The leaf number ranged from 17 to 21 leaves per plant, and the leaf area from 123 to 137 cm^2. Nitrate content appeared lower in the L treatments (L-40:50:10 and -50:40:10) even though both EC levels produced a plant with safe for consumption nitrate levels. The different ratios of K:Ca:Mg might have had a different effect on the crop if the differences were more extreme since 50:40:10 and 40:50:10 were relatively subtle ratios and were chosen for this indicative experiment. When compared to other studies, the total yield of the plants was relatively low. Therefore, we believe that there are still parameters that need to be considered to optimize the growth process of *C. spinosum* L. and help integrate it into highly productive commercial systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092214/s1. Figure S1: Positioning of the different hydroponic rafts for the current experimental design. The conditions tested were the combined effect of different EC levels and different K:Ca:Mg ratios. There were 16 tanks in the greenhouse chamber. There were four treatments, and they were replicated four times. The treatments were L-50:40:10, L-40:50:10, H-50:40:10 and H-40:50:10 (L-, Low; H-, High; 50:40:10 and 40:50:10 refer to K:Ca:Mg ratio).

Author Contributions: Conceptualization, G.N. and D.S.; Data curation, O.V.-F., G.N., I.K., I.N., B.B.C., L.S. and D.S.; Formal analysis, O.V.-F., G.N., I.K., I.N., K.D., A.R., B.B.C., L.S. and D.S.; Funding acquisition, G.N.; Investigation, O.V.-F., G.N., I.K., I.N., K.D., A.R., B.B.C., L.S. and D.S.; Methodology, O.V.-F., G.N., I.K., I.N., K.D., A.R., B.B.C., L.S. and D.S.; Project administration, G.N.; Resources, G.N. and D.S.; Software, O.V.-F., G.N., I.K., I.N., K.D., A.R., L.S. and D.S.; Supervision, G.N., L.S. and D.S.; Validation, O.V.-F., G.N., I.K., I.N., K.D., L.S. and D.S.; Visualization, O.V.-F., G.N., I.K., A.R., L.S. and D.S.; Writing—original draft, O.V.-F., G.N., I.K. and D.S.; Writing—review and editing, O.V.-F., G.N., I.K., I.N., A.R., B.B.C., L.S. and D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the European Commission within the project 'RADIANT: Realising Dynamic Value Chains for Underutilised Crops', which has received funding from the European Union's Horizon 2020 research and innovation program under the grant agreement No. 101000622.

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

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