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Domestication of Wild Edible Species: The Response of *Scolymus hispanicus* Plants to Different Fertigation Regimes

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Abstract: *Scolymus hispanicus* L. is a wild edible species with wide distribution in the Mediterranean area. Recent research has focused on the domestication of wild edible greens, which is essential for the preservation of agroecosystems and the increase in biodiversity, especially under the adversely changing climate conditions. In the present work, the aim was to evaluate the response of *S. hispanicus* plants to different fertilization regimes that varied in the amounts of nitrogen, phosphorus and potassium in regard to plant growth and chemical composition of leaves. For this purpose, plants were grown in pots within an unheated greenhouse. Seven experimental treatments were used, including six fertigation regimes (SH1-SH6) and the control treatment (SHC), where no fertilizers were added. Fresh yield was beneficially affected by the treatments that included a high content of P and K (e.g., SH3 and SH5), while lesser amounts of these macronutrients (e.g., SH1 and SH4) resulted in higher chlorophyll content (SPAD index) and leaf area. In terms of mineral profile, high amounts of P and K improved dietary fiber and carbohydrates content, whereas the untreated plants had the highest content of ash, fat and crude protein. Oxalic and quinic acid were the major organic acids detected, with fertigation regimes significantly reducing their content compared to the control treatment. α-tocopherol was the only isoform of vitamin E detected in all the samples, while glucose and fructose were the most abundant sugars, with their highest content detected in control and SH4 treatments, respectively. *Scolymus hispanicus* leaves were rich in macro and micro minerals, while their contents varied depending on the fertigation regime. Finally, α-linolenic, palmitic, and linoleic acid were the major fatty acids detected, while their contents were beneficially affected by low nutrient inputs (e.g., untreated plants and SH1 and SH2 treatments). In conclusion, the regulation of nutrient solution seems to be an effective practice to increase fresh yield in *S. hispanicus* without compromising the nutritional profile of the edible product, while low inputs of macronutrients such as P and K may improve the chemical composition of the species, especially in terms of n-fatty acids.

Keywords: common golden thistle; nutritional value; mineral profile; chemical composition; wild edible greens; Mediterranean diet; Spanish oyster thistle; organic acids; tocopherols

1. Introduction

Modern farming systems aim to obtain maximum yields to ensure the alarmingly increasing food demands [1,2], while at the same time, the anthropogenic activities tend to gradually reduce the available arable land [3]. Therefore, a new approach is needed focusing not only on intensification of cropping systems, but also on maximizing the efficient use of the available arable land, including degraded soils that cannot be grown with conventional crops. In this context, the valorization of underutilized and neglected species seems to be a promising alternative to the existing crops, especially when considering the ongoing climate crisis that severely affects conventional crop production [4], which may provide new sources of food with improved nutritional properties [5]. Moreover, the integration of such species in farming systems ensures the preservation of agrobiodiversity heritage and reduces the risk of genetic erosion in agroecosystems due to intensified cropping systems [1,6–8].

Scolymus hispanicus L. (also known as common golden thistle or Spanish oyster thistle) is a wild annual or perennial herb belonging to the Asteraceae family with wide distribution in the Mediterranean basin [9–11]. It is usually found in agricultural ecosystems and is considered a difficult-to-control and noxious weed [12,13]. However, although it is undesirable in commercial farms, it is highly appreciated as a wild edible green due to its high nutritional value and beneficial health effects, especially in the countries of southern Europe [9,14]. It is a common ingredient in various gourmet and local dishes of the so-called Mediterranean diet where it is consumed in raw, boiled or fried form [9,11,15,16]. The most commonly consumed plant parts are the flowers, midribs and petioles of leaves, as well as the cortex of the roots, which after post-harvest processing can be used in various dishes [10,17]. According to Disciglio et al. [18], wild *S. hispanicus* plants are rich sources of Mg and Ca and contain low amounts of nitrates, while Rubio et al. [19,20] identified various flavonoids and phenolic acids. Petropoulos et al. [21] suggested luteolin and kaempferol glucuronides as the major phenolic compounds, while Vardavas et al. [22] detected moderate amounts of vitamins (K1 and C), carotenoids (lutein and carotene), Tbatou et al. [23] detected high dietary fiber content, and Morales et al. [24] detected low amounts of α- and total tocopherol. Moreover, Vardavas et al. [25] reported a balanced content of n-6 and n-3 fatty acids (a ratio of 1.06) and high amounts of palmitic, linoleic and α-linolenic acids.

Considering consumers' awareness regarding the origin of food and the production practices implemented, especially regarding the inputs of agrochemicals, the use of sustainable means for crop production is essential for fulfilling the market demands [26]. The introduction of alternative crops such as the various wild and underutilized species falls within this context due to their low requirements in agrochemicals and natural resources (e.g., fertilizers and water) and their efficient adaption strategies to various abiotic and biotic stressors [27,28]. Moreover, the commercialization of these species is pivotal for the reduction of genetic erosion risks related to irrational harvest and anthropogenic activities [29]. During the last few years, several studies have focused on the chemical characterization and the bioactivities of various wild edible plants [30–32], while numerous ethnopharmacological studies have highlighted their contemporary uses in modern diets and their positive health effects [13,22,33–38]. However, in order to domesticate these species, useful information regarding the best practice guides that farmers should follow in order to achieve high yields and high quality of the final product should be also provided [9,13]. Thus far, various species have been suggested for commercial exploitation in small-scale farming systems of the Mediterranean, including *Cichorium spinosum* [39–41], *Portulaca oleracea* [42–44], *Sanguisorba minor* [45,46] *Crithmum maritimum* [47–49] and several others [21,31,50,51]. Among the cultivation practices, the fertilization regime has a significant impact on the yield, the chemical composition and bioactive properties, and the optimum fertilization has to be considered for the commercial production of final products with similar quality as the wild counterparts [3,52–55]. Moreover, the existing genotypic diversity among the numerous ecotypes of these species suggests a wide variation in chemical composition, which along with the effect of growing condition, may result in significant differences in the chemical profile of wild edible greens [56,57].

Despite the prolific studies regarding the chemical properties and the cultivation practices of various wild edible species, there is scarce literature for *Scolymus hispanicus* since most of the studies focus on the chemical characterization and bioactivities of plants collected from the wild [18,36,58]. On the other hand, Papadimitriou et al. [59] suggested that *S. hispanicus* is moderately tolerant to salinity species that could be utilized in saline agriculture, while they also suggested its introduction in soilless cropping systems with reduced macronutrients requirements [60]. Considering the limited information about *S. hispanicus* cultivation, the aim of the present study was to evaluate the impact of different fertilization regimes that varied in the amounts of the main macronutrients (e.g., N, P, K) on the growth, nutritional and mineral profile and chemical composition of *S. hispanicus* plants. The presented results will be helpful for the integration of the species as an innovative crop in the existing farming systems, especially in the small-scale farms of the Mediterranean, while they provide a best-practice guide for the fertilization of the species, focusing on high yields without compromising the quality of the final product.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The trial was conducted at the experimental farm of the University of Thessaly in Velestino (Greece; 39°37′18.6″ N, 22°22′55.1″ E) during the growing period of October 2020–April 2021. The experiment was conducted in the unheated glasshouse of the experimental field. Seeds of *Scolymus hispanicus* were sown in seed trays on October 2021, and young seedlings were transplanted in 6 L plastic pots with peat (Klassman–Deilmann KTS2, Geeste, Germany) and perlite (1:1, *v*/*v*) in January 2021, when the plants had formed 3–4 true leaves. Physicochemical properties of peat were as follows: bulk density 0.12 g/cm3, water holding capacity 218.5%, pH 6.0, electrical conductivity (EC) 0.35 dS/m, organic matter 47.5%, carbon 27.5%, nitrogen 0.14%, C/N 196.8, P 160 mg/L, and K (cmol/kg) 46.03 [51]. Mean air temperature throughout the experimental period was as follows: October, 16.4 °C; November, 10.7 °C; December, 7.8 °C, January, 6.5 °C, February, 8.6 °C; March 12.4 °C; and April 16.7 °C. Data for temperatures inside the greenhouse were obtained from Onset HOBO RH/Temp data logger (Onset Computer Corporation, MA, USA) [61]. The tested fertigation treatments have already been described in detail in a similar study by our team regarding the commercial cultivation of *Cichorium spinosum* [39,52]. In brief, the applied treatments differed in the amounts of N:P:K, e.g., 100:100:100 (SH1), 200:100:100 (SH2), 200:200:200 (SH3), 300:100:100 (SH4), 300:200:200 (SH5) and 300:300:300 ppm (SH6) of N:P:K, as well as the control treatment without the addition of fertilizers (SHC). Stock solutions were prepared with Atlas 20 -20–20 (4.8% ammonium N, 5.0% nitric N, 10.2% ureic N; 20% P2O5; 20% K2O)+ TE (trace elements) fertilizer (Gavriel; S.A., Volos, Greece) for the preparation of 100:100:100 ppm (SH1), 200:200:200 ppm (SH3), and 300:300:300 ppm (SH6), while for the rest of the solutions (200:100:100 ppm (SH2), 300:100:100 ppm (SH4), 300:200:200 ppm (SH5)), the extra amount of nitrogen was achieved with the addition of ammonium nitrate fertilizer (34.5% of N; Gavriel; S.A., Volos, Greece). The control treatment included tap water with no fertilizers added (SHC) [39,52]. The application of treatments was performed manually once or twice per week via the abovementioned solutions, while each treatment included fifteen plants (n = 15) with one plant per pot and 105 plants in total. The total amount of nutrient solution for all the treatments was 1.8 L. Therefore, the abovementioned treatments refer to the following amounts of N:P:K per hectare (assuming a soil depth of 0,15 m as the pot height): SH1, 135–135-135 kg of N:P:K per hectare; SH2, 270-135-135 kg of N:P:K per hectare; SH3, 270-270-270 kg of N:P:K per hectare; SH4, 405-135-135 kg of N:P:K per hectare; SH5, 405-270-135 kg of N:P:K per hectare; SH6, 405-405-405 kg of N:P:K per hectare. The experiment was laid out according to completely randomized design (CRD).

Before harvest, chlorophyll content of leaves (SPAD values) was recorded from 10 plants from each treatment. Harvest took place on April 9, 2021, and morphological traits such as the weight of plant (g), the number of leaves/plant, the weight of leaves/plant (g), the dry matter of leaves (%), the total leaf area (cm^2) and specific leaf area (m^2/kg) were also determined. Dry matter content of leaves, leaf area as well as specific leaf area were calculated from five plants from each treatment. Dry matter content was measured after drying fresh samples of leaves in a forced-air oven at 72 °C to constant weight. Leaf area was measured with a leaf area meter (LI-3100C, LICOR Biosciences; Hellamco S.A., Greece). Specific leaf area was calculated by dividing the dry weight of leaves of each plant by the corresponding leaf area.

2.2. Chemical Analysis

2.2.1. Nutritional Profile

The proximate composition of the edible leaves was performed according to the protocols of the Association of Official Analytical Chemists (crude protein: AOAC, 991.02; total fat: AOAC, 989.05; total dietary fiber: AOAC, 991.43 and 992.16; ash: AOAC, 935.42; and carbohydrates (by difference)) [62]. The results were expressed in g/100 g fw (fresh weight). The energy value was calculated using the formula: Energy = 4 × (protein + carbohydrate) + 2 × (total dietary fiber) + 9 × (total fat), and the results were expressed in kcal/100 g dw.

2.2.2. Organic Acids

Organic acids were analyzed in the dry powder of *S. hispanicus* by ultrafast liquid chromatography coupled to a photodiode array detector programmed to record at 215 nm as the preferred wavelength (UFLC-PDA; Shimadzu Corporation, Kyoto, Japan), and a C_{18} reverse phase column (250 x 4.6 mm, 5 µm, Phenomenex; Torrance, CA, USA) was used for compound separation at 35 °C [63]. The results were expressed in mg per 100 g of dry weight.

2.2.3. Tocopherols

The analysis of tocopherols was carried out based on the protocols described by Barros et al. [63], using a high performance liquid chromatography coupled to a fluorescence detector with a Polyamide II normal phase column (250 mm x 4.6 mm, 5 µm) at 35 °C (HPLC-FP, Knauer, Smartline system 1000, Berlin, Germany). The results were expressed in mg per 100 g of dry weight.

2.2.4. Free Sugars

Free sugars were determined with high-performance liquid chromatography coupled to a refractive index detector and a 100-5 NH_2 Eurospher column (HPLC-RI, Knauer, Smart-line system 1000, Berlin, Germany) as described by Barros et al. [63]. The results were expressed in g per 100 g of dry weight of the plant.

2.2.5. Mineral Content

The mineral composition was determined according to the respective AOAC protocol [62]. In brief, the dry powder *of S. hispanicus* was digested with 10 mL of nitric acid in a microwave extraction system at 200 °C and 1600 watts for 30 min and then made up to a final volume of 50 mL with distilled water. The mineral content in terms of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Co) was determined through atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA, USA).

2.2.6. Fatty Acids

Fatty acid methyl esters (FAME) were determined after trans-esterification of the lipid fraction obtained from the dry powder samples of *S. hispanicus* through Soxhlet extraction, following the protocol described by Barros et al. [63]. The results were expressed as relative percentage (%).

2.3. Statistical Analysis

Plant growth measurements were performed in 15 plants (n = 15) per treatment, except for SPAD index (n = 10), and dry matter content, leaf area and specific leaf area (n = 5). Chemical analyses were performed in triplicate in three batch samples of each treatment. All data were checked for normal distribution according to the Shapiro–Wilk test, while the mean values were compared by Duncan's multiple range test at $p = 0.05$. Statistical analysis was processed with JMP v. 16.1 (SAS Institute Inc.). The results are presented as mean values and standard deviations (SD).

3. Results and Discussion

The results of growth parameters are presented in Table 1. Significant statistical differences regarding the morphological traits in relation to the different fertilization regimes were recorded. The highest number of leaves/plant was achieved for the SH4 (300:100:100; 19.46) fertilization regime, being significantly different from the rest of the treatments, whereas SH1 (100:100:100) and SH6 (300:300:300) treatments recorded the lowest mean values. The SH3 (200:200:200) and SH5 (300:200:200) treatments recorded the highest fresh weight of leaves/plant (116.41 g and 113.58 g, respectively), whereas plants treated with SH6 and the control treatment had the significantly lowest fresh weight. On the other hand, the highest dry matter content was observed for the control treatment, while the leaves obtained from plants treated with SH4 and SH5 had the lowest overall dry matter content. Our results indicate that apart from the increased amounts of nitrogen, the application of nutrient solution with balanced composition in P and K had a beneficial effect on fresh biomass yield, whereas excessive amount of macronutrients resulted in fresh yields similar to the untreated plants due to reduction in leaves number. These findings corroborate the aspect of minimum nutrients requirements for wild edible species, especially for the case of P and K, which were also confirmed by Polyzos et al. [39] in *Cichorium spinosum* cultivation. Moreover, Papadimitriou et al. [60] suggested that a high ratio of N:K (e.g., 2.38 mol/mol) in nutrient solution resulted in increased yields of leaves and roots in hydroponically grown *Scolymus hispanicus* compared to lower ratios (e.g., 1.59 mol/mol). This contradiction could be attributed to differences in the cropping systems (open hydroponic system vs. pot cultivation), since the availability and uptake of N are not comparable. Regarding the dry matter content, Polyzos et al. [39] also reported increased values of dry matter in untreated plants *C. spinosum* plants, suggesting stressful conditions due to nutrients deprivation, a finding that is in agreement with the results of our study.

Table 1. The effect of the type of substrate on the weight of plant (g), the weight of leaves/plant (g), the number of leaves/plant and the dry matter of leaves (%) of *S. hispanicus* plants.

Treatments	Number of Leaves/Plant	Weight of Leaves/Plant (g)	Dry Matter of Leaves (%)
SHC	15.57 ± 2.28 (c)	94.24 ± 8.71 (c)	11.67 ± 1.51 (a)
SH1	15.00 ± 2.45 (d)	102.27 ± 11.24 (b)	9.73 ± 2.70 (d)
SH2	16.17 ± 1.59 (bc)	99.49 ± 8.64 (b)	10.52 ± 1.13 (b)
SH3	15.75 ± 1.29 (c)	116.41 ± 7.28 (a)	10.16 ± 2.32 (c)
SH4	19.46 ± 1.14 (a)	99.36 ± 4.52 (b)	8.81 ± 0.57 (e)
SH5	16.83 ± 2.25 (b)	113.58 ± 8.09 (a)	8.69 ± 0.25 (e)
SH6	15.11 ± 0.78 (d)	90.67 ± 10.73 (c)	10.02 ± 1.50 (c)

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

Table 2 presents the results of chlorophyll content (SPAD index, leaf area and specific leaf area). SPAD index values were the highest when plants were treated with SH4 and SH1 treatments. Similarly, the plants that received SH4 treatment formed the highest leaf area (1721.63 cm^2), without being significantly different from SH1 treatment, while all the tested fertilization regimes had higher SAPD index and leaf area values than the untreated plants (SHC treatment). This finding is in line with the highest number of leaves per plant, which was recorded for the SH4 treatment, thus indicating that the leaf area increased due to the formation of more leaves instead of the development of bigger leaves. Similarly, control treatment recorded the lowest overall value of specific leaf area (12.65 m^2/kg), while the significantly highest values were measured for plants treated with SH5 and SH4 treatments (16.82 and 16.42 m^2/kg, respectively). The significant effect of fertilization regime on SPAD index value of wild edible leafy greens has been also reported by Polyzos et al. [39] and Fidimundy et al. [46], who studied the growth parameters of *Cichorium spinosum* and *Sanguisorba minor* plants, respectively. On the other hand, Tzortzakis and Klados [64] and Papadimitriou et al. [59] did not record any differences in *C. spinosum* and *S. hispanicus* plants treated with nutrient solutions of different salinity levels. According to Di Mola et al. [65], SPAD index values of baby spinach and lamb's lettuce leaves were positively correlated with nitrogen availability, while Karkanis et al. [66] highlighted the importance of harvesting stage on this parameter. Moreover, Fontana et al. [55] suggested that apart from total nitrogen availability, the nitrogen form may also affect chlorophyll content in purslane plants cultivated in a soilless hydroponic system. Based on our results, the increased amounts of nitrogen combined with low amounts of P and K (SH4) or a balanced solution of N:P:K (SH1) were beneficial to chlorophyll content and leaf area, whereas Polyzos et al. [39] suggested that the untreated plants or those that received a nutrient solution that contained 200:200:200 ppm of N, P and K had the highest overall SPAD values. Therefore, it could be assumed that each species may respond differently to fertilization regime, while in our case, the excessive inputs of micronutrients in *S. hispanicus* (e.g., SH6) were not positively correlated with visual quality and fresh yield of leaves.

Table 2. The effect of the fertilization regime on chlorophyll content of the leaves (SPAD values), leaf area (cm^2) and specific leaf area (m^2/kg) of *S. hispanicus* plants.

Treatments	Chlorophyll Content	Leaf Area (cm^2)	Specific Leaf Area (m^2/kg)
SHC	32.14 ± 1.15 (f)	1298.02 ± 179.51 (e)	12.65 ± 1.18 (d)
SH1	37.26 ± 1.77 (a)	1715.79 ± 153.24 (a)	14.85 ± 1.17 (b)
SH2	34.83 ± 1.39 (e)	1402.48 ± 171.05 (c)	13.72 ± 1.29 (c)
SH3	35.72 ± 1.59 (c)	1579.85 ± 202.66 (b)	13.45 ± 1.09 (c)
SH4	37.58 ± 1.25 (a)	1721.63 ± 152.60 (a)	16.42 ± 1.21 (a)
SH5	36.19 ± 2.15 (b)	1614.04 ± 140.34 (b)	16.82 ± 0.29 (a)
SH6	35.35 ± 1.31 (d)	1381.79 ± 169.87 (d)	15.18 ± 1.52 (b)

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

The nutritional profile of *S. hispanicus* leaves in relation to fertigation regime is presented in Table 3, where a variable response was detected. Total fat, crude protein and ash content were negatively affected by fertigation, since the highest overall values were recorded for the untreated plants. In contrast, dietary fiber and carbohydrates content were significantly higher for plants treated with SH3 and SH5 treatments, resulting in a higher energy content for the latter treatment. These results are within the range of the values reported by García-Herrera et al. [56] who evaluated the proximate composition of wild golden thistle plants collected from different sites and in different years. However, the authors of that study detected a great variation among the tested samples, which indicates a significant effect of the genotype and the growing conditions on the nutritional value of the species. Wild *S. hispanicus* plants are considered a rich source of dietary fiber [3,56] and total carbohydrates [23], which in the case of our study, its content was significantly increased by fertigation regimes, whereas fat and ash contents were low compared to other wild edible species [23]. According to the literature, commercial cultivation practices may affect the nutritional value of wild species depending on the species [61,67], allowing us to regulate the quality of the final product and improve the content of beneficial compounds. However, this is not always the case, as for example, Disciglio et al. [18] did not observe a significant difference in protein content between wild and cultivated plants of *C. intybus*, *Borago officinalis* and *Diplotaxis tenuifolia*, whereas Polyzos et al. [39] recorded a decrease in protein and ash content when *C. spinosum* plants were treated with nutrient solutions similar to our study.

Table 3. Nutritional profile and energy content of *Scolymus hispanicus* leaves in relation to fertigation regime (Mean ± SD).

	SHC	SH1	SH2	SH3	SH4	SH5	SH6
	Nutritional profile (g/100 g dw)						
Total fat	2.9 ±0.1 ^a^	2.8 ± 0.1 ^a^	2.3 ± 0.1 ^c^	2.6 ±0.1 ^b^	2.61 ±0.02 ^b^	2.73 ± 0.02 ^a,b^	2.2 ± 0.1 ^c^
Crude protein	11.84 ± 0.03 ^a^	10.6 ± 0.1 ^b^	10.73 ± 0.41 ^b^	10.8 ±0.5 ^b^	9.8 ± 0.1 ^d^	9.8 ± 0.1 ^d^	10.4 ± 0.1 ^c^
Ash	11.8 ± 0.4 ^a^	11.5 ± 0.4 ^b^	9.856 ± 0.003 ^d^	11.4 ± 0.08 ^b^	10.04 ± 0.09 ^d^	9.9 ± 0.1 ^d^	11.02 ± 0.18 ^c^
Total dietary fiber	37.1 ± 0.1 ^e^	37.0 ± 0.2 ^e^	40.2 ± 1.1 ^b^	40.7 ± 0.2 ^a^	38.81 ± 0.04 ^d^	36.269 ± 0.002 ^f^	39.6 ± 0.6 ^c^
Carbohydrates	36.36 ± 0.35 ^d^	38.04 ± 0.57 ^c^	36.9 ± 1.1 ^d^	34.5 ± 0.6 ^e^	38.7 ± 0.1 ^b^	41.3 ± 0.2 ^a^	36.8 ± 0.6 ^d^
	Energy (kcal/100 g dw)						
	292.9 ± 1.6 ^c^	294.2 ± 1.1 ^b^	291.8 ± 1.4 ^c^	285.7 ± 0.1 ^f^	295.3 ± 0.4 ^b^	301.4 ± 0.3 ^a^	287.9± 1.0 ^e^

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. dw: dry weight.

Organic acid composition is presented in Table 4. The main detected compounds were oxalic, quinic and malic acid followed by shikimic and citric acid, while traces of fumaric acids were also present in the studied samples. A varied response to fertigation regime was observed, with higher amounts of oxalic, quinic and total organic acids being detected in the untreated plants. Malic acid was the highest in SH treatment, while the SH3 treatment resulted in the highest amounts of shikimic and citric acid. On the other hand, the SH4 treatment resulted in the lowest values for all the detected compounds (except for the case of quinic acid where the lowest content was recorded for SH2 treatment) and consequently in the lowest content of total organic acids. According to the literature, the increased inputs of nitrogen are associated with high amounts of oxalic acid [61,67], while the nitrogen form may affect the accumulation of this particular organic acid or total organic acids [68]. Moreover, Dias et al. [69] reported a decrease in oxalic acid content in cultivated *Achillea millefolium* plants compared to unattended ones. This contradiction could be due to the fact that in our study, the control plants were subjected to stress conditions due to nutrient deprivation and P in particular. According to Le Roux et al. [70], P deficiency is associated with the synthesis and accumulation of organic acids which tend to decrease nitrogen assimilation. Another possible explanation could be related with the ratios and the total amounts of macronutrients applied in the tested fertigation regimes, which may result in synergistic or antagonistic effects that consequently affect nutrient assimilation and impair plant physiology and metabolism [71]. According to Aboyeji et al. [72], high amounts of P may have detrimental effects on the yield of groundnut plants due to antagonism between P and Zn that may affect plant growth and development. Moreover, excessive amounts of nitrogen are associated with reduced uptake of other nutrients, which result in stressful conditions and consequently in the accumulation of organic acids [73,74]. Therefore, based on our results and the literature reports, further studies are needed to reveal uptake and translocation of nutrients from roots to upper parts in order to reveal the mechanisms involved in organic acids biosynthesis as part of the antioxidant and osmoregulatory mechanisms of plants. In any case, the reduction of organic acids and oxalic acid in particular after the application of the tested fertigation regimes can be considered a positive impact on the quality of the final product, since oxalic acid is an antinutritional factor, and high intake (> 5 g per day) may have severe health effects [51].

Table 4. Organic acids (mg/100 g dw) content in *S. hispanicus* leaves in relation to fertigation regime (mean ± SD).

	SHC	SH1	SH2	SH3	SH4	SH5	SH6
Oxalic acid	3.13 ± 0.02 a	2.28 ± 0.01 d	2.08 ± 0.01 e	2.92 ± 0.02 b	1.8 ± 0.01 g	2.440 ± 0.001 c	1.980 ± 0.008 f
Quinic acid	2.86 ± 0.05 a	2.37 ± 0.02 c	2.15 ± 0.01 e	2.8 ± 0.1 b	2.2 ± 0.1 d	2.39 ± 0.02 c	2.2 ± 0.02 d
Malic acid	2.250 ± 0.002 b	2.37 ± 0.02 a	1.660 ± 0.004 e	1.88 ± 0.02 d	1.61 ± 0.04 f	1.95 ± 0.03 c	1.66 ± 0.01 e
Shikimic acid	0.026 ± 0.006 b	0.021 ± 0.001 e	0.016 ± 0.001 g	0.031 ± 0.001 a	0.018 ± 0.001 f	0.025 ± 0.002 c	0.0223 ± 0.0002 d
Citric acid	0.86 ± 0.02 b	0.84 ± 0.02 c	0.82 ± 0.03 c,e	0.903 ± 0.003 a	0.77 ± 0.03 f	0.83 ± 0.01 c,d	0.82 ± 0.01 d,e
Fumaric acid	tr	tr	tr	tr	tr	tr	tr
Sum	9.13 ± 0.02 a	7.88 ± 0.01 c	6.74 ± 0.06 e	8.5 ± 0.1 b	6.4 ± 0.1 g	7.63 ± 0.01 d	6.68 ± 0.02 f

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Calibration curves for organic acids: oxalic acid ($y = 8E+06x + 331789$, $R^2 = 0.9912$); quinic acid ($y = 692575x + 11551$; $R^2 = 0.9983$); citric acid ($y= 968367x-12295$, $R^2= 0.9974$); shikimic acid ($y = 5E+07x + 567119$, $R^2 = 0.9903$); fumaric acid ($y = 9E+07x-100894$, $R^2 = 0.9986$); tr: traces; dw: dry weight.

Tocopherol and free sugar compositions are presented in Table 5. Alpha-tocopherol was the only detected isoform of vitamin in all the studied samples, while except for the case of SH5 treatment, the rest of the fertigation regimes resulted in a significant decrease compared to untreated plants. The detected amounts were different from those reported by Petropoulos et al. [21] (0.68 μg/100 g fw) and Marmouzi et al. [75] (0.54 mg/100 g) and in the same range with the study of Morales et al. [24] (0.02 mg/100 g fw) and Vardavas et al. [22] (0.038 mg/100 g fw). Moreover, in contrast to our study, β- and γ-tocopherols were also detected in *S. hispanicus* leaves, which could be due to different growing conditions and genotypic variability [76]. The variable response to fertilization regime was also reported in the study of Polyzos et al. [39] who evaluated the response of *C. spinosum* plants as similar to our study fertigation regimes and recorded the highest α- and total tocopherols content for the treatment of 300:200:200 ppm of N:P:K. It seems that high amounts of nitrogen combined with a balanced content of P and K in the nutrient solution may improve tocopherol composition, a finding which has been confirmed in other species [67,77].

Table 5. Tocopherol (mg/100 g dw) and free sugar (g/100 g dw) content in *S. hispanicus* leaves in relation to fertigation regime (mean ± SD).

	SHC	SH1	SH2	SH3	SH4	SH5	SH6
				Tocopherols (mg/100 g dw)			
α-Tocopherol	0.095 ± 0.007 b	0.070 ± 0.003 d	0.050 ± 0.002 e	0.080 ± 0.003 c	0.021 ± 0.001 f	0.25 ± 0.01 a	0.02 ± 0.001 f
				Free sugars (g/100 g dw)			
Fructose	4.2 ± 0.2 b	4.1 ± 0.1 b	3.9 ± 0.1 c	4.75 ± 0.04 a	4.72 ± 0.01 a	3.86 ± 0.04 c	3.6 ± 0.1 d
Glucose	6.6 ± 0.2 a	5.9 ± 0.1 c	6.3 ± 0.2 b	5.9 ± 0.1 c	6.2 ± 0.2 b	5.4 ± 0.1 e	5.48 ± 0.04 d
Sucrose	1.8 ± 0.02 f	1.94 ± 0.01 e	1.77 ± 0.02 f	2.1 ± 0.1 d	2.41 ± 0.02 c	2.5 ± 0.1 b	2.8 ± 0.2 a
Trehalose	1.21 ± 0.03 b	1.34 ± 0.03 a	1.19 ± 0.03 b	0.88 ± 0.03 c	0.66 ± 0.02 d	0.89 ± 0.01 c	1.3 ± 0.2 a
Sum	13.8 ± 0.4 b	13.35 ± 0.04 c	13.1 ± 0.2 d	13.68 ± 0.28 b	13.97 ± 0.19 a	12.65 ± 0.01 e	13.18 ± 0.002 d

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test; dw: dry weight.

Regarding the free sugar composition, glucose was the main detected free sugar, followed by fructose, sucrose and trehalose. A variable response was recorded in relation to the tested fertigation regimes. In particular, the highest glucose content was detected in control plants, while fructose content was the highest in SH3 and SH4 treatments. Similarly, sucrose and trehalose were significantly higher for the SH6 and SH1 and SH6 treatments, respectively. Finally, the highest and lowest total free sugar content was recorded for SH4 and SH% treatments, respectively. To the best of our knowledge, this is the first report regarding the free sugar composition and no references are available for comparison purposes. However, Polyzos et al. [39] who studied the same fertigation regimes in *C. spinosum* plants also reported a varied response, while similar fluctuations have been suggested in other crops due to growing conditions and nutrients availability [78,79]. Regulation of free sugar composition through the application of tailor-made fertigation regimes could be a cost-effective means to improve the quality of the final products of wild edible species, since the increased sugar content could be associated with improved taste and organoleptic properties.

Mineral composition of *S. hispanicus* leaves is presented in Table 6. A varied response was recorded without specific trends in the effects of the tested fertigation regimes being observed. In particular, the untreated plants had the highest content of K and Zn, while plants treated with SH4 treatment recorded the highest content of Na, Ca and Mg. Moreover, SH1 and SH2 treatments had significantly higher Fe content compared to the rest of the treatments, while Mn and Cu content was significantly higher for SH1 treatment. The range of minerals detected in our study was in the same range as the values reported by García-Herrera et al. [56] with slight variations in the case of Ca and Cu, while Papadimitriou et al. [59] reported higher values for Na, K, Ca and Mg. However, it has to be noted that García-Herrera et al. [56] recorded a high variability in minerals profile depending on the collection site and year, and they suggested a significant impact on growing conditions, while they determined the mineral content of the midribs *S. hispanicus* instead of whole leaves, which were evaluated in our study. According to Rietra et al. [72], significant interaction may occur among plant macro- and micronutrients, which may negatively or positively affect plant growth and crop yield. This is evident in our study where the varied amounts of N:P:K applied through fertigation resulted in a varied response regarding the mineral composition of *S. hispanicus* leaves. According to Fageria and Oliveira [80], P is the most influential nutrient since its imbalance may severely affect crop yield, a finding that is in agreement with our study where the highest overall fresh yield was recorded for the treatments where 200 ppm of P was applied (SH3 and SH5). Moreover, other studies report significant antagonistic effects between P and Mg or Ca [81,82], which coincide with the findings of our study where the highest Mg and Ca contents were recorded in SH4 treatment where 300:100:100 ppm of N:P:K was applied. Therefore, it could be suggested that the regulation of fertilization regime may favor crop yield and improve the mineral profile of *S. hispanicus* leaves at the same time. However, the impact of growing conditions and genotype should be further investigated.

Table 6. Mineral composition in *S. hispanicus* leaves in relation to fertigation regime (mean ± SD).

.	SHC	SH1	SH2	SH3	SH4	SH5	SH6
[K]/(g/Kg)	27.1 ± 0.7 ^{a}	20.4 ± 0.7 ^{b}	13.4 ± 0.4 ^{e}	19 ± 1 ^{c}	12.70 ± 0.02 ^{f}	13.98 ± 0.17 ^{d}	18.9 ± 0.7 ^{c}
[Na]/(mg/Kg)	5757 ± 182 ^{d}	5713 ± 228 ^{d}	6139 ± 75 ^{c}	6174 ± 166 ^{c}	7396 ± 110 ^{a}	6717 ± 103 ^{b}	6768 ± 302 ^{b}
[Ca]/(g/Kg)	8.02 ± 0.06 ^{c}	8.5 ± 0.4 ^{b}	8.2 ± 0.1 ^{b,c}	8.3 ± 0.4 ^{b}	9.6 ± 0.4 ^{a}	7.2 ± 0.3 ^{e}	7.70±0.02 ^{d}
[Mg]/(g/Kg)	1.98 ± 0.04 ^{f}	2.30 ± 0.03 ^{e}	2.6 ± 0.1 ^{b}	2.4 ± 0.1 ^{c,d}	3.6 ± 0.1 ^{a}	2.44 ± 0.01 ^{c}	2.36 ± 0.02 ^{d,e}
[Fe]/(mg/Kg)	172 ± 6 ^{e}	222 ± 2 ^{a,b}	223 ± 8 ^{a}	218 ± 7 ^{b}	194 ± 8 ^{c}	185 ± 3 ^{d}	184 ± 7 ^{d}
[Mn]/(mg/Kg)	116.8 ± 0.2 ^{f}	155 ± 9 ^{a}	142 ± 3 ^{c}	123 ± 5 ^{e}	135 ± 2 ^{d}	122 ± 6 ^{e}	148 ± 2 ^{b}
[Cu]/(mg/Kg)	4.3 ± 0.1 ^{g}	5.7 ± 0.2 ^{a}	5.2 ± 0.2 ^{c,e}	5.3 ± 0.2 ^{b,c}	5.4 ± 0.2 ^{b}	4.71 ± 0.02 ^{f}	5.2 ± 0.1 ^{e}
[Zn]/(mg/Kg)	48.1 ± 0.8 ^{a}	33.9 ± 0.7 ^{c}	28 ± 1 ^{d}	35.5 ± 0.5 ^{b}	20.5 ± 0.6 ^{f}	20.8 ± 0.3 ^{f}	25.8 ± 0.4 ^{e}

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

The fatty acid composition is presented in Table 7. The most abundant compounds were α-linolenic acid (47.8–59.4%), palmitic acid (19.4–24.5%) and linoleic acid (10.44–12.72%), while the polyunsaturated fatty acids (PUFA) were the major class of fatty acids (60.3–70.8%). A varied response was recorded in relation to the fertigation regime without specific trends being observed among the tested treatments. The highest content of the major fatty acids was recorded either for untreated plants (e.g., α-linolenic acid) or for the treatments with low nutrient amounts (e.g., SH1 and SH2 in the case of linoleic and palmitic acid, respectively). The recorded composition was in the same range as the fatty acids profile reported by Morales et al. [83] who detected high amounts of PUFA (57.66%), followed by monounsaturated and saturated fatty acids (MUFA: 34.16% and SFA: 8.19%) in peeled basal leaves. However, the content of individual compounds varied compared to our study, with α-linolenic acid being the most abundant one (30.55%), followed by linoleic and palmitic acids (26.44 and 20.65%, respectively). In contrast to our study, Vardavas et al. [25] reported a different composition of the main fatty acids groups, suggesting MUFA (54.8%) as the most abundant class, followed by SFA (33.7%) and PUFA (11.4%). Moreover, they recorded a balanced ratio of n6/n3 (1.06) and different amounts of individual fatty acids (linoleic acid 33.8%; α-linolenic acid 32% and palmitic acid 30.3%). The variable-reported results could be associated with the tested raw material (whole leaves in our study were compared to peeled basal leaves) as well as to growing conditions and agronomic practices. Considering that the plants in our study were grown in a growth substrate under different fertigation regimes, this could also be a possible explanation for the observed differences as already confirmed by the literature reports [39,45].

Table 7. Fatty acid composition (relative percentage %) in *S. hispanicus* leaves in relation to fertigation regime (mean ± SD).

	SHC	SH1	SH2	SH3	SH4	SH5	SH6
C8:0	nd	0.496 ± 0.018 ^{e}	0.523 ± 0.001 ^{d}	0.62 ± 0.03 ^{c}	0.84 ± 0.03 ^{a}	0.66 ± 0.02 ^{b}	nd
C13:0	0.93 ± 0.01 ^{e}	0.43 ± 0.01 ^{f}	1.23 ± 0.05 ^{b}	1.15 ± 0.01 ^{c}	1.33 ± 0.04 ^{a}	1.14 ± 0.04 ^{c}	1.11 ± 0.01 ^{d}
C14:0	1.256 ± 0.003 ^{e}	0.83 ± 0.03 ^{f}	2.5 ± 0.08 ^{a}	1.63 ± 0.01 ^{d}	1.86 ± 0.05 ^{b}	1.76 ± 0.06 ^{c}	1.87 ± 0.07 ^{b}
C14:1	0.48 ± 0.01 ^{e}	0.21 ± 0.01 ^{f}	0.62 ± 0.01 ^{c}	0.58 ± 0.03 ^{d}	0.72 ± 0.02 ^{a}	0.616 ± 0.005 ^{c}	0.69 ± 0.03 ^{b}
C15:0	0.263 ± 0.001 ^{a}	0.17 ± 0.01 ^{d}	0.14 ± 0.01 ^{g}	0.2 ± 0.01 ^{c}	0.238 ± 0.003 ^{b}	0.157 ± 0.003 ^{f}	0.162 ± 0.003 ^{e}
C16:0	19.4 ± 0.1 ^{f}	24.34 ± 0.03 ^{b}	24.5 ± 0.2 ^{a}	23.1 ± 0.6 ^{c}	23.3 ± 0.2 ^{c}	19.9 ± 0.2 ^{e}	20.4 ± 0.1 ^{d}
C16:1	1.76 ± 0.01 ^{b,c}	2.24 ± 0.01 ^{a}	1.67 ± 0.02 ^{d}	1.74 ± 0.01 ^{c}	1.77 ± 0.08 ^{b}	1.607 ± 0.004 ^{e}	1.487 ± 0.003 ^{f}
C17:0	0.54 ± 0.01 ^{d}	1.251 ± 0.001 ^{b}	1.16 ± 0.04 ^{c}	1.25 ± 0.04 ^{b}	1.58 ± 0.06 ^{a}	0.39 ± 0.01 ^{f}	0.448 ± 0.004 ^{e}
C18:0	2.02 ± 0.07 ^{f}	2.4 ± 0.1 ^{d}	3.9 ± 0.1 ^{a}	3.1 ± 0.1 ^{b}	3.1 ± 0.1 ^{b}	2.25 ± 0.03 ^{e}	2.5 ± 0.1 ^{c}
C18:1n9c	1.373 ± 0.001 ^{c}	1.31 ± 0.04 ^{d}	2.07 ± 0.07 ^{a}	1.4 ± 0.06 ^{b,c}	1.44 ± 0.03 ^{b}	1.2 ± 0.02 ^{e}	1.4 ± 0.1 ^{c}
C18:2n6c	11.04 ± 0.35 ^{c}	12.72 ± 0.03 ^{a}	10.44 ± 0.05 ^{e}	11.13 ± 0.09 ^{c}	10.77 ± 0.09 ^{d}	11.81 ± 0.04 ^{b}	11.9 ± 0.7 ^{b}
C18:3n3	59.4 ± 0.4 ^{a}	51.9 ± 0.1 ^{d}	47.8 ± 0.1 ^{g}	49.9 ± 0.3 ^{e}	48.9 ± 0.4 ^{f}	56.3 ± 0.2 ^{b}	54.97 ± 1.14 ^{c}
C22:0	0.62 ± 0.02 ^{e}	0.67 ± 0.01 ^{e}	1.32 ± 0.06 ^{c}	1.77 ± 0.05 ^{a}	1.52 ± 0.01 ^{b}	0.84 ± 0.01 ^{d}	1.23 ± 0.36 ^{c}
C23:0	0.36 ± 0.01 ^{g}	0.39 ± 0.01 ^{f}	0.85 ± 0.03 ^{c}	0.9 ± 0.04 ^{b}	1.01 ± 0.01 ^{a}	0.61 ± 0.02 ^{e}	0.76 ± 0.03 ^{d}
C24:0	0.617 ± 0.002 ^{f}	0.68 ± 0.01 ^{e}	1.271 ± 0.004 ^{b}	1.58 ± 0.06 ^{a}	1.58 ± 0.03 ^{a}	0.82 ± 0.03 ^{d}	1.06 ± 0.01 ^{c}
SFA	25.7 ± 0.3 ^{g}	31.5 ± 0.2 ^{d}	37.3 ± 0.5 ^{a}	35.1 ± 1 ^{c}	36.1 ± 0.5 ^{b}	28.3 ± 0.4 ^{f}	29.4 ± 0.7 ^{e}
MUFA	2.498 ± 0.025 ^{c}	2.63 ± 0.02 ^{b}	2.43 ± 0.03 ^{d}	2.52 ± 0.05 ^{c}	2.73 ± 0.1 ^{a}	2.38 ± 0.01 ^{e}	2.34 ± 0.04 ^{f}
PUFA	71.8 ± 0.7 ^{a}	65.9 ± 0.1 ^{d}	60.3 ± 0.2 ^{g}	62.4 ± 0.4 ^{e}	61.1 ± 0.5 ^{f}	69.3 ± 0.2 ^{b}	68 ± 2 ^{c}
n6/n3	0.186 ± 0.005 ^{f}	0.245 ± 0.001 ^{a}	0.218 ± 0.001 ^{d}	0.223 ± 0.001 ^{b}	0.220 ± 0.001 ^{c}	0.209 ± 0.001 ^{e}	0.216 ± 0.008 ^{d}
PUFA/SFA	2.794 ± 0.005 ^{a}	2.09 ± 0.01 ^{d}	1.61 ± 0.02 ^{g}	1.76 ± 0.05 ^{e}	1.69 ± 0.02 ^{f}	2.44 ± 0.03 ^{b}	2.32 ± 0.02 ^{c}

Mean values in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Fatty acids are expressed as relative percentage of each fatty acid. C8:0—caprylic acid; C13:0—tridecanoic acid; C14:0—myristic acid; C14:1 -tetradecanoic acid; C15:0—pentadecanoic acid; C15:1; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—heptadecanoic acid; C18:0—stearic acid; C18:1n9c—oleic acid; C18:2n6c—linoleic acid; C18:3n3—linolenic acid; C22:0—Behenic acid; C23:0 -tricosanoic acid; C24:0—lignoceric acid. SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; nd—not detected.

4. Conclusions

The domestication of wild edible species is pivotal for the introduction and integration of such alternative crops in the existing farming systems, especially in small-scale farms of the Mediterranean. Fertilization is one of the most effective and common agronomic practices that is applied in commercial farming and significantly contributes to yield increase in various crops. Considering the lack of information regarding the best practice guides and fertilization regimes for *S. hispanicus*, this study aimed to evaluate how different amounts of nutrients can affect yield and quality of edible leaves. In particular, treatments with moderate amounts of P and K (SH3 and SH5) recorded the highest fresh yield, whereas the highest inputs (SH6) negatively affected fresh biomass yield. The highest leaf area was recorded for SH4 treatment and coincided with the highest numbers of leaves. Apart from the highest fresh weight, SH3 and SH5 treatments were the most beneficial for dietary fiber and carbohydrates content, which are important nutritional parameters. On the other hand, SH4 treatment recorded the lowest oxalic acid content, which is considered an anti-nutritional factor, while it was beneficial to mineral profile (Ca and Mg content), fructose and total sugars content ~~and significantly reduced oxalic acid content~~. Finally, treatments SHC, SH1 and SH2 were beneficial to the major fatty acids content (α-linolenic, linoleic and palmitic acid, respectively). In conclusion, it could be suggested that the regulation of nutrient solution seems to be an effective practice to increase fresh yield in *S. hispanicus* with low to moderate inputs, without compromising the nutritional profile of the edible product. This is an important finding for introducing sustainable practices in the existing farming systems, since the commercial cultivation of wild species such as *S. hispanicus* reduces the risk of genetic erosion due to irrational harvesting and increases the biodiversity of agroecosystems and its resilience to climate change conditions.

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