The effects of fertilization regime on growth parameters and bioactive properties of pot grown *Cichorium spinosum* L. plants

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GENERAL SECRETARIAT FOR RESEARCH AND TECHNOLOGY

The PRIMA programme is supported and funded under Horizon 2020, the Framework European Union's Programme for Research and Innovation

INTRODUCTION

- *Cichorium spinosum* L. is a wild edible species, commonly known as stamnagkathi found in many parts of Greece especially in Crete area and other Mediterranean countries.
- It is an integral part of the Mediterranean diet with people from rural communities usually hand picking the rosettes of the plant and use them in many traditional dishes.
- It is a plant that presents a wide adaptability that can be grown even coastal areas with low soil fertility, exhibits considerable tolerance to salt stress, while its cultivation demands are quite minimal regarding to its nutrition.
- Stamnagkathi presents a high content in vitamins E (α- and γ-tocopherols) and K1, antioxidants, ω-3 fatty acids and several mineral elements; hence for that reason has been acknowledged as functional healthy food.
- The aim of the current study was to evaluate the effects of fertilization regime on growth parameters, chemical composition and bioactive properties of pot grown *C. spinosum* plants.

MATERIALS AND METHODS

- Young seedlings of *Cichorium spinosum* after emergence were transplanted in 2 L plastic pots containing peat and perlite (1:1, v/v).
- Seven treatments were used which varied in the amounts of N:P:K namely 100:100:100 (C111), 200:100:100 (C211), 200:200:200 (C222), 300:100:100 (C311), 300:200:200 (C322) and 300:300:300 (C333) ppm ratio of N:P:K and control (C0) where no fertilizers were added were applied via nutrient solution in the *C. spinosum* plants.
- Each treatment contained fifteen pots (n=15) and in total they were used 105 pots. All the treatments received the same amount of nutrient solution in which the plants were fertigated manually once a week comprising of 150 mL of N-P-K per plastic plot.
- Before harvest, it was recorded the chlorophyll content of leaves (SPAD index values) per treatment and after harvest the growth traits namely number of leaves/plant, weight of leaves/plant (g), dry matter of leaves (%), leaf area index (cm^2) and specific leaf area index (m^2/kg).
- Regarding the chemical composition and bioactive properties of the plants they were evaluated the identification and quantification of the phenolic compounds, antioxidant, anti-inflammatory, hepatotoxic and cytotoxic activities.

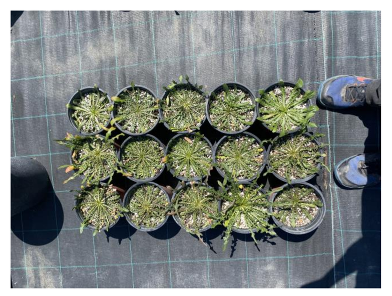

Image 1. The effects of fertilization regime on growth parameters and bioactive properties of pot grown *Cichorium spinosum* L. plants.

RESULTS

Table 1. The effects of fertilization regimes on number of leaves/plant, weight of leaves/plant (g), dry matter of leaves (%), chlorophyll content of leaves (SPAD index values), leaf area index (cm^2) and specific leaf area index (m^2/kg) of *C. spinosum* (Mean ± SD).

Treatments	Traits					
	Number of leaves/plant	Weight of leaves/plant (g)	Dry matter (%)	SPAD index values	Leaf area index (cm^2)	Specific leaf area index (m^2/kg)
C0	29.54 ± 1.35 (a)	11.49 ± 0.97 (c)	8.27 ± 2.16 (a)	94.81 ± 12.21 (a)	297.12 ± 8.50 (b)	27.09 ± 1.69 (e)
C111	29.14 ± 1.13 (a)	11.59 ± 1.22 (c)	6.55 ± 1.02 (e)	82.82 ± 7.79 (bc)	282.82 ± 7.86 (c)	31.17 ± 1.71 (bc)
C211	24.21 ± 1.38 (c)	12.90 ± 1.35 (a)	6.69 ± 0.09 (c)	74.19 ± 6.61 (c)	324.75 ± 8.57 (a)	28.20 ± 1.73 (e)
C222	27.33 ± 0.73 (b)	9.91 ± 1.12 (e)	6.08 ± 1.24 (e)	98.14 ± 13.10 (a)	260.23 ± 11.39 (d)	37.61 ± 1.98 (a)
C311	27.29 ± 1.27 (b)	12.02 ± 1.69 (b)	5.56 ± 2.55 (f)	62.10 ± 7.00 (d)	250.42 ± 6.76 (d)	39.20 ± 1.45 (a)
C322	30.36 ± 1.73 (a)	11.54 ± 1.26 (c)	7.93 ± 1.05 (b)	87.89 ± 7.24 (ab)	278.37 ± 8.28 (c)	30.82 ± 1.64 (cd)
C333	29.73 ± 1.30 (a)	10.85 ± 0.77 (d)	6.92 ± 2.57 (c)	76.08 ± 7.79 (c)	255.81 ± 7.99 (d)	29.78 ± 1.46 (d)

*Mean values and standard deviations in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Student's t-test.

Table 2. Retention time (Rt), wavelength of the maximum absorption (λmax), deprotonated ion ($[M-H]^-$), main mass fragments (MS^2) and tentative identification of the phenolic compounds found in the hydroethanolic and aqueous extracts of *C. spinosum* samples.

Peak	Rt	λmax	$[M-H]^-$	MS^2	Tentative identification
1	8.51	292	337	191(100),173(12),163(71),155(3),119(34)	3-O-p-Coumaroylquinic acid
2	10.64	292	337	191(5),173(100),163(39),155(10),119(23)	4-O-p-Coumaroylquinic acid
3	17.24	352	477	301(100)	Quercetin-O-hexuronoside
4	17.82	345	461	285(100)	Luteolin-O- hexuronoside
5	18.97	342	505	463(24),301(100)	Quercetin-O-acetylhexoside
6	20.71	342	461	285(100)	Kaempherol-O- hexuronoside
7	22.07	340	491	315(100)	Isorhamnetin-O- hexuronoside
8	23.19	343	489	285(100)	Kaempherol-O-acetylhexoside
9	24.48	344	519	315(100)	Isorhamnetin-O-acetylhexoside

- The highest number of leaves was recorded by the C322 treatment, the highest fresh weight was noted for the C211 treatment and the highest SPAD index values was achieved by control treatment (C0).
- Related to leaf area index, the treatment C211 had the highest leaf area, while the C311 treatment recorded the highest specific leaf area index and the C0 treatment the lowest one respectively.
- In the case of hydroethanolic extracts, the highest antioxidant activity for the OxHLIA and TBARS assays was recorded by the C111 and C311 treatments, respectively, whereas the treatment C222 showed the best results for OxHLIA and TBARS assays in the aqueous extracts.
- Nine phenolic compounds were detected in both extracts, including two phenolic acids and seven flavonoids, with the major compounds being 4-*O*-*p*-coumaroylquinic acid and isorhamnetin-*O*-hexuronoside, regardless of the extraction method.
- The treatment of C333 contained the highest amounts of total phenolic acids, regardless of the extraction method. In the case of the hydroethanolic extracts, the highest content of total flavonoids was recorded by C311 treatment, total flavonoids, whereas regarding the aqueous extracts the highest content of total flavonoids was recorded by the C222 and C333 treatments
- The C311 treatment achieved the most content of total phenolic compounds in the case of the hydroethanolic extracts, whereas the C333 treatment recorded the highest content for the aqueous extracts
- Both extracts, did not show cytotoxic or anti-inflammatory activities.

Table 3. Antioxidant (IC_{50} values μg/mL), anti-inflammatory (IC_{50} values μg/mL), hepatotoxic (GI_{50} values μg/mL) and cytotoxic (GI_{50} values μg/mL) activities of the hydroethanolic extracts (HE) and aqueous extract (AE) of *C. spinosum* (Mean ± SD).

Bioactive properties		Treatments						
		C0	C111	C211	C222	C311	C322	C333
Antioxidant Activity [A] - (HE/AE)	OxHLIA	322 ± 20b 131 ± 5c	53 ± 3g 97 ± 5d	339 ± 18a 25 ± 2e	61 ± 2f 20 ± 1f	65 ± 2e 207 ± 12b	103 ± 4d 278 ± 9a	123 ± 7c 207±13b
	TBARS	479 ± 9b 357 ± 11a	411±15c 143 ± 2e	408 ± 6c 167 ± 6c	363 ± 16d 116 ± 5f	151 ± 6e 225 ± 8b	465 ± 15b 163 ± 8cd	547 ± 27a 159 ± 7d
Anti-inflammatory Activity [B] - (HE/AE)	RAW 264,7	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400
Hepatotoxicity Activity [C] - (HE/AE)	PLP2	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400
Cytotoxicity Activity [D] - (HE/AE)	AGS	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400
	CaCo2	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400
	VERO	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400
	MCF7	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400	>400 >400

[A] Trolox IC_{50} values: 5.8±0,6 μg/mL (TBARS), 21.8±0.3 μg/mL (OxHLIA 60 min); [B] Dexametaxone IC_{50} value: 6.3 ±0.4 μg/mL; [C, D] Ellipticine GI_{50} values: 1.4 ± 0.1 μg/mL (PLP2), 1.23 ± 0.03 μg/mL (AGS), 1.21 ± 0.02 μg/mL (CaCo2), 1.41 ± 0.06 μg/mL (VERO) and 1.02 ± 0.02 μg/mL (MCF-7).

Table 4. Quantification (mg/g extract) of the phenolic compounds found in the hydroethanolic extracts (HE) and aqueous extracts (AE) of *C. spinosum* samples (Mean ± SD).

Peak	Treatments						
	C0	C111	C211	C222	C311	C322	C333
1- (HE/EA)	0.54 ± 0.003a 0.46 ± 0.000b	0.47 ± 0.002c 0.38 ± 0.001c	0.45 ± 0.003c 0.37 ± 0.004c	0.45 ± 0.001c 0.34 ± 0.005d	0.51 ± 0.003b 0.44 ± 0.003b	0.31 ± 0.001d 0.38 ± 0.011c	0.46 ± 0.002c 0.52 ± 0.01a
2 - (HE/EA)	0.86 ± 0.004cd 0.57 ± 0.006d	0.938 ±0.008b 0.584 ± 0.001c	0.81 ± 0.001e 0.59 ± 0.001c	0.89 ± 0.016c 0.66 ± 0.002b	0.84 ± 0.004de 0.65 ± 0.014b	0.69 ± 0.001f 0.68 ± 0.007b	1.01 ± 0.005a 0.82 ± 0.001a
3- (HE/EA)	0.49 ± 0.001b 0.53 ± 0.002c	0.46 ± 0.000c nd	0.45 ± 0.0001c 0.47 ± 0.001d	0.46 ± 0.000c 0.60 ± 0.000a	0.53 ± 0.000a 0.53 ± 0.001c	0.49 ± 0.000b 0.49 ± 0.001d	0.48 ± 0.002b 0.56 ± 0.001b
4 - (HE/EA)	0.54 ± 0.000c 0.59 ± 0.001c	0.53 ± 0.000c 0.52±0.000f	0.50 ± 0.000d 0.54 ± 0.00e	0.49 ± 0.000d 0.63 ± 0.001b	0.61 ± 0.001v nd	0.53 ± 0.001c 0.56 ± 0.001d	0.63 ± 0.002a 0.66 ± 0.01a
TPA - (HE/EA)	1.40 ± 0.002b 1.03 ± 0.006d	1.40 ± 0.010b 0.96 ± 0.000f	1.27 ± 0.002d 0.96 ± 0.003f	1.34 ± 0.015c 1.00 ± 0.007e	1.36 ± 0.006c 1.10 ± 0.018b	1.01 ± 0.000e 1.06 ± 0.005c	1.48 ± 0.002a 1.34 ± 0.009a
TF - (HE/EA)	3.67 ± 0.002c 3.03 ± 0.008b	3.53 ± 0.003d 1.55 ± 0.005e	3.41 ± 0.001e 2.81 ± 0.003d	3.44 ± 0.001e 3.27 ± 0.001a	3.97 ± 0.000a 3.04 ± 0.010b	3.75 ± 0.001b 2.94 ± 0.000c	3.75 ± 0.001b 3.27 ± 0.008a
TPC - (HE/EA)	5.08 ± 0.000c 4.07± 0.002d	4.94 ± 0.013d 2.52 ± 0.005g	4.68 ± 0.003f 3.77± 0.000f	4.79 ± 0.015e 4.27 ± 0.008b	5.33 ± 0.007a 4.15 ± 0.027c	4.76 ± 0.001e 4.01 ± 0.005e	5.23 ± 0.003b 4.61 ± 0.001a

nd – not detected. TPA – Total Phenolic Acids; TF – Total Flavonoids; TPC- Total Phenolic Compounds. Standard calibration curves used for quantification: *p*-coumaric acid ($y = 301950x + 6966{,}7$, $R^2 = 1$, LOD = 0.68 μg/mL and LOQ = 1.61 μg/mL, peaks 1 and 2) and quercetin-3-*O*-glucoside ($y = 34.843x - 160.173$, $R^2 = 0.9998$, LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL, peaks 3 to 9).

CONCLUSIONS AND RECOMMENDATIONS

- The application of fertilizers on *C. spinosum* plants had positive effects on plant growth, especially the 200:100:100 treatment where the highest fresh yield was recorded, while variable effects of fertilizer regimes on the chemical composition and bioactive properties were recorded.
- Discovering the optimal cultivation practices regarding the fertilizer management of spiny chicory could provide a promising outcome to the agronomic parameters, chemical composition and bioactive properties of the crop.
- Commercial cultivation of wild edible species is a promising cropping alternative in the climate change conditions as well in degraded soils where conventional crops cannot be cultivated or their yield is severely compromised.
- Further research is demanded in terms of evaluating the cultivation protocol in order to establish the commercial cultivation of the wild edible species.

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ACKNOWLEDGMENTS

- This work was funded by the General Secretariat for Research and Technology of Greece and PRIMA foundation under the project VALUEFARM (PRIMA2019-11). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national founds FCT/MCTES to CIMO (UIDB/00690/2020); For the grant of B.H. Paschoalinotto and for the financial support within the scope of the Project PRIMA Section 2 – Multi-topic 2019: VALUEFARM (PRIMA/0009/2019); and L. Barros and M.I. Dias thank FCT, P.I., through institutional scientific employment program-contract for contracts.