

## Article

# The Effect of Cropping System and Irrigation Regime on the Plant Growth and Biochemical Profile of *Cichorium spinosum*

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**Abstract:** This study evaluated the effects of three irrigation treatments (control (rain-fed plants), deficit irrigation (DI: 50% of maximum field capacity), full irrigation (FI: 100% of maximum field capacity)), and two crop-management treatments (with or without crop rotation with bean, CR, and NCR, respectively) on the plant growth and chemical composition of *C. spinosum*. The results indicated that deficit irrigation combined with crop rotation increased the weight of leaves per plant, followed by rain-fed plants for the same crop-management treatment. Additionally, these two factors significantly influenced the nutritional profile, free sugars, and organic acid content in a variable manner. Moreover, the control treatment and deficit irrigation increased the content of K, Na, and Mg, which are highly mobile nutrients, whereas the levels of moderately mobile nutrients such as Fe, Mn, Cu, and Zn decreased. Deficit irrigation without crop rotation significantly increased the content of total tocopherols, followed by deficit irrigation with crop rotation and full irrigation without crop rotation. The main fatty acids were  $\alpha$ -linolenic acid (C18:3n3), followed by palmitic acid (C16:0) and linoleic acid (C18:2n6), while the control and deficit irrigation treatments combined with crop rotation increased PUFA and decreased SFA content. Furthermore, deficit irrigation and crop rotation induced the accumulation of phenolic compounds, flavonoids, and phenolic acids, especially the content of the major compounds (e.g., chicoric acid, quercetin-*O*-hexuronoside, and luteolin-*O*-hexuronoside). The leaf extracts exhibited varied antioxidant activity (assessed by TBARS and OxHLIA assays), and antimicrobial activity. On the other hand, no antifungal, antiproliferative (except for AGS cell line), hepatotoxic, or anti-inflammatory effects were recorded. In conclusion, the combination of deficit irrigation and crop rotation with bean positively affected the quality traits and the fresh weight of leaves, thus suggesting that such eco-friendly practices could have beneficial effects in the cultivation of *C. spinosum* plants within the context of climate-change mitigation strategies.

**Keywords:** wild edible species; deficit irrigation; antimicrobial properties; crop rotation; cytotoxic effects; bioactive properties; phenolic compounds; antioxidant activity



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## 1. Introduction

The long history of wild edible plants in the Mediterranean culture can be traced back to ancient times, and a strong bond has been created between human societies and wild plants, which have been exploited for food, medicinal, and religious purposes from time to time [1,2]. Wild greens were highly appreciated during famine periods because they offered a valuable and nutritive food source [3,4]. During recent times and as the standards of living improved, consumers tended to neglect these species and shifted towards unhealthy and “modern” dietary habits; however, nowadays, there is a renewed interest for the exploitation of these plants, which are considered functional foods due to their rich phytochemical content and their high nutritional value [5].

*Cichorium spinosum* L. is a wild edible species valued for its tender edible leaves and is often consumed in many local Mediterranean recipes [6]. The species thrives in many Mediterranean countries, especially in coastal and semi-arid regions, and it is highly acknowledged as a culinary trademark of the Cretan diet [7,8]. Regarding the chemical composition and nutritional value of *C. spinosum* leaves, reports in the literature highlight its increased content in vitamins (namely C, E, and K1), phenolic acids (chicoric and 5-O-Caffeoylquinic acid), total glutathione, proteins, fatty acids, carotenoids ( $\beta$ -carotene and lutein) and minerals [9,10]. The leaves of the plant are usually hand-picked from the wild and consumed raw in leafy salads, boiled with meat or even consumed in pickled form [11]. However, the seasonal availability, limited access to wild habitats, and the increasing market needs for high-added-value and functional food products have created a niche for the commercial exploitation of the plant due to its rich phytochemical properties and its high nutritional value [12]. For this purpose, several recent research studies have focused on optimizing the agronomic practices that will facilitate its commercial exploitation without compromising the health benefits of the edible product [13].

The implications of the ongoing climate change have raised serious obstacles to the cultivation of conventional crops with a severe impact on crop production [14,15]. Water is a key natural resource for food production, being essential for high-yield crops due to its involvement in plant functions and physiological processes [16,17]. Net crop water-use requirements are expected to increase globally by 25% at the end of 2080 due to changing environmental conditions and increased irrigation cost [18]. Likewise, the recharging of groundwater in the Mediterranean countries is expected to decrease by approximately 30% in the near future, while crop water-use requirements are expected to increase due to climate change [19]. To overcome these challenges, it is of great importance to exploit and valorize alternative crops that can adapt to harsh conditions but also to improve water-use efficiency and develop new technological irrigation tools [20,21]. In this context, deficit irrigation (DI) could be a valuable tool to address irrigation water scarcity [22]. This strategy may have a beneficial effect on crop production by improving the marketable yield per unit of water used, reducing nutrient loss near to the root area and excessive vegetative vigor, as well as by decreasing the incidence of diseases related to high humidity [23].

Recent research has focused on highlighting the importance of suggesting alternative/complementary crops that can withstand adverse environmental conditions, especially in arid and semi-arid regions that are mostly affected by climate adversities and drought in particular, as well as to reveal the mechanisms involved in this adaptation [24,25]. In this context, the commercialization of wild edible species as high-added-value substitutes of conventional crops could be a key pillar of a sustainable strategy aiming to mitigate the severe impact of climate change on food security [26–29], as well as in the diversification of food systems [30–32]. Moreover, Polyzos et al. [33] found that deficit irrigation could be used as a sustainable tool to manage irrigation water in *Scolymus hispanicus* cultivation without compromising crop yield and the bioactive profile of the edible portion of plants.

However, it should be noted that this irrigation technique (e.g., deficit irrigation) should be tailored to each species, since a varied response has been recorded and various mechanisms of adaptation and tolerance have been reported in the literature [22,29,34].

On the other hand, crop rotation is a very old agronomic practice with profound advantages such as improved climate resilience of crops through the enhancement of water dynamics by increased soil water storage and crop water-use efficiency, the improvement of physical and chemical properties of soil, the increase in soil enzyme activity and nutrient use efficiency, the improvement of soil microbiome, the reduction in weed competitiveness, effective pest and disease management, and the increased biodiversity of agroecosystem [35–40].

So far, there is a scarcity in the literature reports regarding the integration of wild edible plants in modern cropping systems. Therefore, the aim of the present study was to evaluate the potential inclusion of *C. spinosum* plant in a rotation scheme with common bean (*Phaseolus vulgaris* L.) under deficit irrigation conditions and further evaluate the effects of cropping system and irrigation regime on crop performance, and the chemical profile and bioactive properties of the edible leaves of the species. Our results could contribute to the commercial exploitation of wild edible greens and also highlight the importance of implementing sustainable agronomic tools in modern agriculture as a means to mitigate climate change and ensure food security.

## 2. Materials and Methods

### 2.1. Experimental Conditions

At the experimental farm of the University of Thessaly in Velesino (Greece; 39°37'18.6" N, 22°22'55.1" E), young seedlings of *C. spinosum* L. (Asteraceae family) were transplanted to the field when they reached the stage of 3–4 true leaves. Seedlings were prepared by seeds obtained from Geniki Fytotechniki S.A. (Athens, Greece) after sowing them in seed trays containing peat (pH 5.5–6.5, base substrate with no fertilizers added; Klasmann-Deilmann GmbH, Geeste, Germany). The species is depicted in Figure 1. In the current study, there were 3 irrigation treatments, namely deficit irrigation (DI: 50% of field capacity), full irrigation (FI: 100% of field capacity) and the control treatment where the plants were rain-fed. Moreover, the effect of crop rotation was also tested by applying two treatments, namely crop rotation (CR) with plants of *Phaseolus vulgaris* L. and no crop rotation (NCR). The plants were placed at distances of 0.50 cm between the rows and 0.33 cm within the rows, in which each treatment included 3 rows with a plant density of 45 plants per treatment. The plants were fertigated with a base dressing of N-P-K (Atlas 20-20-20 + TE; Gavriel S.A., Volos, Greece) in amounts varying based on the plant growth and the environmental circumstances; whereas, during the growing period of the plants, irrigation was performed once or twice per week, depending on the environmental conditions via a drip irrigation system. Weed management was carried out manually, while pests and pathogens were controlled with chemical management according to the recommended cultivation practices. The soil was sandy clay loam (38% sand, 36% silt, and 26% clay), with pH = 7.4 (1:1 soil/H<sub>2</sub>O) and organic matter content = 1.3%. The meteorological data throughout the experimental period (mean air temperature, mean highest and lowest temperature, relative humidity and rainfall) are presented in Table 1. The harvest of the plants took place in 14 May 2021 and the fresh weight of leaves/plant (g) and number of leaves/plant were calculated per treatment.



**Figure 1.** *Cichorium spinosum* plants cultivated in the field before harvest (left photo) and at the end of the growth cycle (right photo). (Source: personal record of Dr. Spyridon A. Petropoulos).

**Table 1.** Meteorological data during the cultivation period of *C. spinosum* L. plants.

Month	Mean Temperature (°C)	Mean Highest Temperature (°C)	Mean Lowest Temperature (°C)	Relative Humidity (%)	Rainfall (mm)
March	9.15	15.47	3.07	51.75	55.90
April	13.56	20.16	7.08	52.63	21.90
May	20.95	28.52	12.91	45.81	21.50
Mean	14.55	21.38	7.69	50.06	33.10

## 2.2. Nutritional Characterization

The nutritional characterization was determined following official methods of analysis (AOAC methods) in terms of total fat (n° 989.05), crude protein (n° 991.02), ash (n° 935.42), total dietary fiber (n° 991.43 and 992.16) and carbohydrates by difference. The results were expressed in g/100 g dw. Energy value in the dried powder samples of *C. spinosum* leaves was calculated following the European Regulation n° 1169 as described below: energy (kcal/100 g dw) = ((9 × total fat content) + (4 × (crude protein content + carbohydrate content)) + (2 × total dietary fiber content)). The outcomes were expressed in kcal/100 g dw.

## 2.3. Organic Acids

For the extraction of organic acids, 1 g of *C. spinosum* samples were placed in a 25 mL solution of metaphosphoric acid (4.5% v/v) under magnetic stirring at room temperature for 20 min and subsequently filtered, following the protocol as detailed by Polyzos et al. [41]. The identification of organic acids and their respective quantification was determined by comparing retention times, spectra with commercial standards, and respective safety rates (five-level lines). The results were expressed in mg/100 g dw.

## 2.4. Tocopherols

The extraction procedure and chromatographic characterization of tocopherols were carried out in accordance with the methods outlined by Polyzos et al. [33]. The chromatographic analysis was performed using an HPLC system, comprising a quaternary pump, degasser, automatic sampler, and fluorescence detector. The separation process was accomplished on a Polyamide II normal-phase column (250 × 4.6 mm, 5 μm) at 35 °C, with the detector set to 290 nm excitation and 330 nm emission. The mobile phase comprised a mixture of hexane and ethyl acetate (70:30, v/v), with a flow rate of 1 mL/min. Identification and quantification were conducted using Clarity 2.4 software.

### 2.5. Sugars Composition

Sugars composition was determined using high-performance liquid chromatography (HPLC) with a refractive index (RI) detector, according to the methodology described by Polyzos et al. [41]. The analysis was carried out by HPLC-RI at 35 °C, using a Knauer Smartline system equipped with an RI detector (Knauer Smartline 2300, Knauer, Berlin, Germany) and a Eurospher NH2 100-5 column (4.6 × 250 mm, 5 µm, Knauer). The mobile phase consisted of a mixture of acetonitrile–deionized water (70:30, *v/v*) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparison with authentic standards, while quantification was carried out using the internal standard method. The sugar content was expressed in g/100 g dw.

### 2.6. Mineral Composition

The mineral composition of *C. spinosum* leaves was determined in accordance with the AOAC protocol [42]. In summary, 10 mL of nitric acid was utilized for the digestion of the dry powder leaves in a microwave system at 200 °C and 1600 watts for a duration of 30 min. Thereafter, the solution was diluted to a volume of 50 mL with distilled water. Concentrations of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were then quantified by atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA, USA).

### 2.7. Fatty-Acid Composition

The fatty-acid profile was determined by gas chromatography coupled to a flame ionization detector (GC-FID, DANI instrument model GC 1000, Milan, Italy), in accordance with the methodology described by Polyzos et al. [33]. The identification of the fatty acids was based on the comparison of the relative retention times of the peaks of the standard mixture of 37 FAMES with those of the samples. The data were processed using Clarity 4.0.1.7 software (DataApex, Podohradska, Czech Republic) and expressed as a relative percentage of each fatty acid.

### 2.8. Phenolic Compounds and Bioactive Properties

To evaluate the phenolic compounds and bioactive properties, the leaves of *C. spinosum* were extracted with ethanol–water (80:20 *v/v*). Briefly, 1 g of *C. spinosum* leaves was added to 30 mL of ethanol–water solution (80:20, *v/v*; 30 mL) at room temperature, under constant magnetic stirring (150 rpm) for 1 h. The solution was filtered, and the process was repeated with the same conditions. The alcoholic fraction was evaporated under reduced pressure, while the aqueous fraction was frozen and freeze-dried, and the extract was kept at room temperature until further analysis.

#### 2.8.1. Phenolic Compounds Profile

The lyophilized hydroethanolic extracts of *C. spinosum* leaves were redissolved in an ethanol–water mixture (20:80 *v/v*) to yield a final concentration of 10 mg/mL for chromatographic analysis. The phenolic profile was evaluated through chromatographic analysis following the procedure described by Polyzos et al. [33]. To identify the phenolic compounds, a comparison of the retention times was conducted with the literature and/or, when possible, with the UV–21 Vis mass spectra of authentic commercial standards. The results were expressed in mg/g of extract.

#### 2.8.2. Antioxidant Properties

Two *in vitro* antioxidant activities were carried out, namely the inhibition of lipid peroxidation (TBARS) and inhibition of oxidative hemolysis (OxHLIA). Trolox was used as positive control in both antioxidant activities evaluated. The antioxidant potential

was evaluated via the TBARS assay, with hydroethanolic extracts of *C. spinosum* leaves following the method described by Polyzos et al. [33]. The results obtained were expressed as IC<sub>50</sub> values (µg/mL, the concentration of the extract required to provide 50% of the antioxidant activity).

For the OxHLIA assay, a 2.8% (*v/v*) erythrocyte solution (200 µL) in phosphate-buffered saline (PBS, pH 7.4) was combined with 400 µL of one of the following: extract solution (20–770 µg/mL), Trolox (3.91–4000 µg/mL), PBS (negative control), or distilled water (baseline) [33]. Optical density was measured at 690 nm using an ELx800 microplate reader until hemolysis was complete. IC<sub>50</sub> values (µg/mL) were calculated for a 60 min interval using GraphPad Prism<sup>®</sup> (version 8.1). These values indicate the extract concentration required to protect 50% of the blood-cell population from oxidative hemolysis within the specified time frame.

### 2.8.3. Antibacterial and Antifungal Properties

To evaluate the antibacterial and antifungal activities, the obtained extract of *C. spinosum* leaves from hydroethanolic extraction was prepared at a stock concentration of 10 mg/mL and serially diluted [33]. The evaluation focused on the antibacterial activity of the extracts against a range of Gram-positive and Gram-negative bacterial strains, against clinical isolated strains obtained from patients hospitalized in various departments at the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal), including *Escherichia coli* (VRU12881), *Klebsiella pneumoniae* (VRI17214), *Morganella morganii* (VRU14272), *Proteus mirabilis* (VRU17684), *Pseudomonas aeruginosa* (VRU14123), *Enterococcus faecalis* (VRU14041), *Listeria monocytogenes* (VRU17684), Methicillin-resistant *Staphylococcus aureus* (MRSA; VRI17654), as well as foodborne pathogens (*Enterobacter cloacae* ATCC 49741, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* ATCC 13076; *Yersinia enterocolitica* ATCC 8610, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 11632), that were purchased at Frilabo, Porto, Portugal. The minimum inhibitory concentration (MIC) values were defined as the lowest concentration that indicate bacterial growth inhibition, while the minimum bactericidal concentration (MBC) values corresponded to the concentration required to kill the bacteria. Ampicillin and Streptomycin were used for all bacteria tested, and Methicillin was also used for *Staphylococcus aureus* as a positive control.

Antifungal assays were performed using two microfungi (*Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404); both obtained from Frilabo, Porto, Portugal), according to the method previously described in the literature [33]. The Minimum Fungicidal Concentration (MFC) was established through a process of subculturing the tested compounds and subsequent observation for a duration of 72 h at a temperature of 25 °C. The concentration at which no visible growth was observed was designated as the MFC, thereby indicating a 99.5% eradication of the initial inoculum. Ketoconazole was utilized as positive control.

### 2.8.4. NO-Production Inhibition, Antiproliferative and Hepatotoxic Properties

The nitric oxide (NO)-production inhibition of hydroethanolic extracts was evaluated by lipopolysaccharide (LPS)-stimulated murine macrophage cell line (RAW 264.7) by measuring NO production [41]. The quantification of NO levels was performed using the Griess reagent and a nitrite calibration curve. The results obtained were expressed as IC<sub>50</sub> values, representing the concentration required to inhibit 50% of NO production.

The antiproliferative and hepatotoxic potentials were also evaluated using the sulforhodamine B (SRB) colorimetric assay as described by Polyzos et al. [41]. The cell lines utilized included four tumor cell lines (AGS—gastric adenocarcinoma, Caco-2—colorectal

adenocarcinoma, MCF-7—breast adenocarcinoma, and NCI-H460—non-small cell lung cancer) and one non-tumor cell line (PLP2—porcine liver primary culture for hepatotoxicity). The results were expressed as the extract concentration required to inhibit 50% of cell proliferation ( $GI_{50}$ ). Ellipticine was utilized as a positive control, and cells without extracts as a negative control. All procedures were conducted under aseptic conditions in a vertical laminar flow chamber.

### 2.9. Statistical Analysis

The experimental design was a split plot, with each plot consisting of water-deficit stress with fully randomized sub-plots comprising the crop-rotation treatments. Statistical analysis was conducted with the aid of JMP v 16.1 (SAS Institute Inc., Cary, NC, USA). Data were evaluated for normal distribution according to the Shapiro–Wilk test, whereas the means of values were compared by Duncan’s Multiple Range test at  $p = 0.05$ .

## 3. Results and Discussion

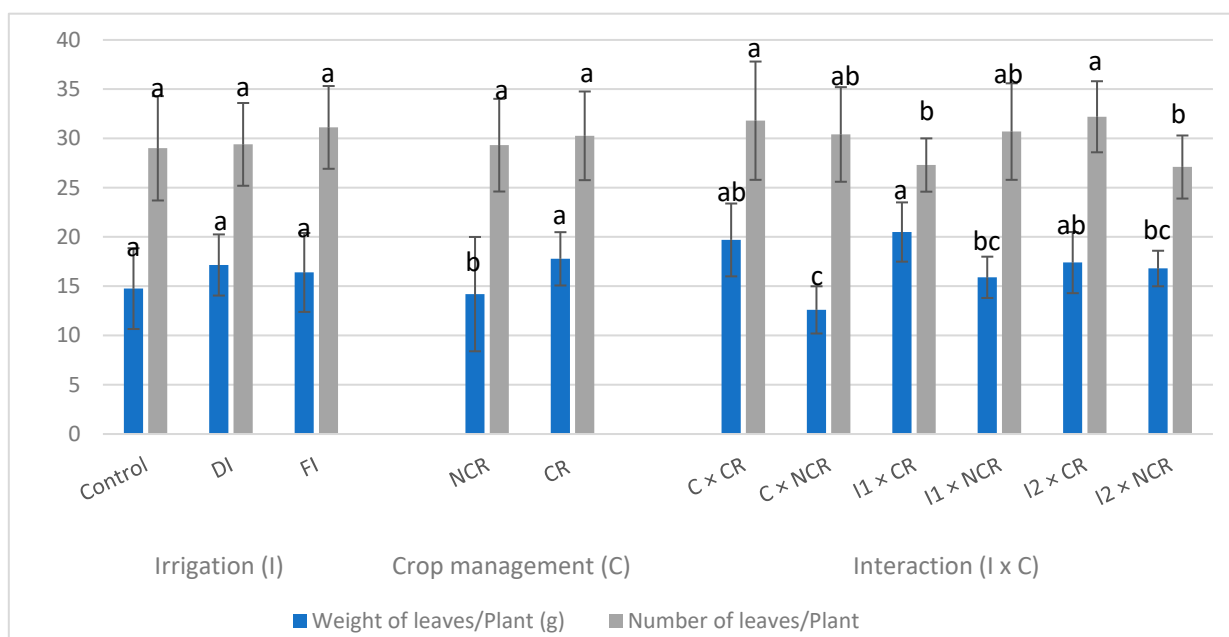
### 3.1. Growth Parameters

The results regarding the effects of irrigation treatments and crop management on growth parameters of *C. spinosum* L. are presented in Figure 2. Deficit irrigation (DI) achieved the highest weight of leaves/plant (18.4 g) followed by full irrigation (FI) (17.2 g) and the control treatment (C; rain-fed plants) (16.1 g), while the highest number of leaves/plants was recorded for the control treatment (31.1 g), followed by FI (29.4 g) and DI (29.0 g) treatments without any significant differences among the treatments. On the other hand, crop rotation had a beneficial effect on the weight of leaves/plant (CR; 19.2 g), while no significant differences were recorded for the number of leaves/plant between crop rotation and the control treatment (no rotation implemented). The interaction of the tested factors showed that deficit irrigation and crop rotation resulted in the highest weight of leaves (20.5 g) being significantly different from all the treatments where no rotations were applied, whereas the lowest overall weight of leaves was recorded for the rain-fed plants combined with no rotation (12.6 g). On the other hand, the highest number of leaves was recorded for fully irrigated plants subjected to crop rotation (32.2), whereas the lowest values were recorded for DI  $\times$  CR and FI  $\times$  NCR treatments (27.3 and 27.1, respectively).

Our findings are in total accordance with the study of Guarise et al. [28], who evaluated the potential of growing *Sisymbrium officinale* (L.) Scop. as a novel leafy vegetable under drought conditions, and they reported that reduction in irrigation water by 50% did not compromise plant yield. Similar observations have also been reported by Polyzos et al. [33] who assessed the crop performance of golden thistle (*Scolymus hispanicus* L.) under drought-stress conditions, and they suggested that deficit irrigation did not significantly affect fresh yield compared to the rain-fed plants, where a significant reduction was recorded, while the number of leaves/plant varied among the studied irrigation treatments. According to the study of Mohamed et al. [43], drought-stress conditions at 80% of field capacity also did not have a detrimental effect on the growth of purslane plants (*Portulaca oleracea* L.) compared to the plants grown at 100% of field capacity, while Saheri et al. [44] mentioned a gradual reduction in plant growth and yield of purslane with increasing drought stress. Furthermore, Delfine et al. [45], who studied the effect of drought stress on chicory plants (*C. intybus* L.), observed a significant reduction in fresh biomass with increasing drought stress, especially at 50 days after transplantation; nonetheless, the authors suggest the cultivation of the species under proper rain-fed conditions as soon as the plants are subjected to a moderate water stress.

Crop rotation with *P. vulgaris* L. had a clear positive effect on the fresh biomass yield of *C. spinosum* plants, a finding which has also been recorded in other studies [40,46,47]. Ac-

According to the meta-analysis of Zhao et al. [48], the integration of legumes in crop-rotations schemes increased the yield by up to 20%, for a large variety of crops including wheat (*Triticum aestivum* L.), vegetables, and oilseed crops. Jensen et al. [49] also observed that crop rotation of legumes and oat (*Avena sativa* L.) with winter barley (*Hordeum vulgare* L.) had positive effects on barley yield, although they suggested that this increase varied depending on the irrigation regime and the soil type (sand or loam). Similarly, Sánchez-Navarro et al. [50] evaluated the effect of rotating two different varieties of cowpea (*Vigna unguiculata* (L.) Walp.) with broccoli (*Brassica oleracea* var. *italica*) and they reported that legumes not only enhanced the yield of broccoli but they also increased the soil nutrient content and reduced the fertilizer inputs by 20%. Moreover, Cui et al. [51] studied the effect of a long-term crop-rotation scheme using flax (*Linum usitatissimum* L.), wheat (*Triticum aestivum* L.), and potato (*Solanum tuberosum* L.) in various combinations and they suggested that the effect of rotation on the annual crop yield and water-use efficiency varied depending on crop combinations. Crop rotation was suggested by Ghadirnezhad Shiade et al. [52] as a sustainable agronomic tool towards the mitigation of negative effects of drought stress on crops, since the resilience of crops against weather extremities may increase through the increase in ecosystem diversification and the improvement of soil properties which are associated with rotational schemes.



**Figure 2.** Growth parameters of *C. spinosum* L. plants in relation to irrigation and crop management (mean  $\pm$  SD). Different Latin letters above bars of the same color and for the same factor or their interaction are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ . DI: deficit irrigation; FI: full irrigation; NCR: no crop rotation; CR: crop rotation.

### 3.2. Chemical Composition

#### 3.2.1. Nutritional Characterization

The nutritional profile of *C. spinosum* leaves is displayed in Table 2. Crop management did not significantly affect most parameters, except for crude protein, which was higher when no crop rotation was implemented, whereas irrigation significantly affected all the nutritional parameters apart from crude protein. In particular, rain-fed conditions increased total fat and fiber content (3.34 g/100 g dw and 49.37 g/100 g dw, respectively), while DI and FI treatments increased total carbohydrate and ash content (15.5 g/100 g dw and 19.1 g/100 g dw, respectively). Finally, FI treatment resulted in a decrease in the

energetic value of leaves being significantly lower from control and DI treatments. Regarding the combinatorial effect of the tested factors, significant interactions were recorded (Table 2). In terms of total fat and total fiber content, the highest values (3.5 g/100 g dw and 50.4 g/100 g dw, respectively) were observed in rain-fed plants grown in a crop-rotation scheme, while deficit irrigation (DI) for the same crop management resulted in the highest carbohydrate content and energetic value (20.3 g/100 g dw and 276.3 kcal/100 g dw, respectively). Finally, plants grown under rain-fed conditions and full irrigation with no crop rotation recorded the highest values of crude protein and ash content (23.4 g/100 g dw and 19.76 g/100 g dw, respectively).

A previous study by Polyzos et al. [41], reported similar values for the nutritional profile of *C. spinosum* leaves. Moreover, according to Polyzos et al. [33], deficit irrigation also had a significant effect on the nutritional profile of *S. hispanicus*, thus suggesting that irrigation management can be a practical tool towards the improvement of the nutritional profile of wild edible greens. In the study of Yousefvand et al. [53], it was reported that drought-stress conditions significantly reduced ash content in shallot plants (*Allium hirtifolium*), while similar findings were recorded by Allamine et al. [54] for the ash content in grain sorghum (*Sorghum bicolor* L.). The same authors also observed that water stress did not affect protein content, a finding which is in accordance with our study [54]. Similarly to our study, drought stress led to increased carbohydrate content in black cumin plants (*Nigella sativa* L.) acting as osmoprotectants against the adverse effects of water stress [55]. Moreover, in previous studies from Petropoulos et al. [56,57], it was reported that abiotic stress conditions may significantly affect the nutritional profile of wild edible species such as *C. spinosum* and *Centaurea raphanina* subsp. *mixta*, either due to the concentration effect and the consequent increase in dry matter content or due to the induced biosynthesis of osmolytes as part of plant's stress-defense mechanism.

### 3.2.2. Organic Acids

The main organic acids detected in the studied samples were quinic acid, oxalic acid, and succinic acid in descending order, while shikimic acid and ascorbic acid were only detected in traces (Table 3). According to the literature, the abovementioned compounds have been previously detected in *C. spinosum* leaves [41,58,59], while these reports also detected malic and citric acid, which were not detected in our study. In contrast, succinic acid was recorded in *C. spinosum* leaves for the first time in our study. Full irrigation resulted in a decrease in the content of the major organic acids (e.g., quinic and oxalic acid) and consequently in total organic acid content, while no significant differences were recorded between the control and DI treatments. Similarly, Muhairi et al. [60] and Skrypnik et al. [61], who evaluated the effect of drought stress on lettuce (*Lactuca sativa* L.), salvia (*Salvia officinalis* L.), and oregano (*Origanum vulgare* L.), reported an increase in organic acid content in response to increasing stress levels, as part of the plants' defense mechanism. Zhao et al. [62] also reported an increase in titratable acidity and vitamin C content in tomato fruit (*Solanum lycopersicum* L.) when plants were subjected to moderate drought-stress conditions due to the concentration effect. Regarding the effect of crop rotation on the quality traits of *C. spinosum* leaves, our results suggest that this agronomic practice did not significantly affect the content of the major and total organic acids (Table 3). However, other cultivation practices such as fertilization and growth stage may have a significantly impact on organic acid content as already reported by Petropoulos et al. [63] and Polyzos et al. [41], while the genotype may also affect the content of individual and total organic acid in the leaves of the species [64].

**Table 2.** Nutritional profile (g/100 g dw) and energy content (kcal/100 g dw) of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean  $\pm$  SD).

Treatments		Total Fat	Crude Protein	Ash	Total Dietary Fiber	Carbohydrates	Energy (kcal/100 g dw)
Irrigation (I)	Control (C)	3.34 $\pm$ 0.16 a	21.25 $\pm$ 2.45 a	15.22 $\pm$ 0.69 b	49.37 $\pm$ 1.22 a	10.82 $\pm$ 0.78 b	257.10 $\pm$ 4.16 a
	Deficit Irrigation (DI)	3.04 $\pm$ 0.24 b	21.81 $\pm$ 0.15 a	15.22 $\pm$ 0.13 b	44.40 $\pm$ 5.51 b	15.5 $\pm$ 5.2 a	265.5 $\pm$ 11.7 a
	Full Irrigation (FI)	2.75 $\pm$ 0.06 c	20.37 $\pm$ 0.71 a	19.1 $\pm$ 0.8 a	48.5 $\pm$ 1.3 a b	9.32 $\pm$ 0.37 b	240.5 $\pm$ 1.2 b
Crop Management (CM)	Crop Rotation (CR)	3.17 $\pm$ 0.32 a	20.23 $\pm$ 1.26 b	16.49 $\pm$ 1.42 a	46.47 $\pm$ 5.34 a	13.6 $\pm$ 5.0 a	256.93 $\pm$ 15.47 a
	No Crop Rotation (NCR)	2.91 $\pm$ 0.22 a	22.06 $\pm$ 1.17 a	16.5 $\pm$ 2.5 a	48.37 $\pm$ 0.92 a	10.15 $\pm$ 0.89 a	251.81 $\pm$ 9.38 a
I $\times$ CM	C $\times$ CR	3.5 $\pm$ 0.1 a	19.1 $\pm$ 0.9 d	15.8 $\pm$ 0.2 c	50.4 $\pm$ 1.1 a	11.21 $\pm$ 0.03 b	253.4 $\pm$ 0.8 c
	C $\times$ NCR	3.19 $\pm$ 0.03 b	23.4 $\pm$ 1.1 a	14.6 $\pm$ 0.5 e	48.3 $\pm$ 0.2 c	10.44 $\pm$ 1.03 b	260.8 $\pm$ 1.3 b
	DI $\times$ CR	3.2 $\pm$ 0.1 b	21.7 $\pm$ 0.2 bc	15.3 $\pm$ 0.1 d	39.4 $\pm$ 0.1 e	20.3 $\pm$ 0.1 a	276.3 $\pm$ 0.5 a
	DI $\times$ NCR	2.8 $\pm$ 0.1 c	21.8 $\pm$ 0.2 b	15.1 $\pm$ 0.1 d	49.4 $\pm$ 0.1 b	10.8 $\pm$ 0.2 b	254.8 $\pm$ 0.3 c
	FI $\times$ CR	2.8 $\pm$ 0.1 cd	19.7 $\pm$ 0.2 d	18.4 $\pm$ 0.2 b	49.7 $\pm$ 0.4 b	9.4 $\pm$ 0.5 c	241.1 $\pm$ 1.5 d
	FI $\times$ NCR	2.7 $\pm$ 0.1 d	20.9 $\pm$ 0.6 c	19.76 $\pm$ 0.01 a	47.3 $\pm$ 0.3 d	9.2 $\pm$ 0.1 c	239.8 $\pm$ 0.2 d

Means in the same column for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ .

**Table 3.** Organic acid composition (mg/100 g dw) of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean  $\pm$  SD).

Treatments		Oxalic Acid	Quinic Acid	Shikimic Acid	Ascorbic Acid	Succinic Acid	Total Organic Acids
Irrigation (I)	Control (C)	4.90 $\pm$ 0.23 a	6.65 $\pm$ 0.19 a	tr	tr	1.55 $\pm$ 0.04 a	13.11 $\pm$ 0.27 a
	Deficit Irrigation (DI)	5.04 $\pm$ 0.08 a	6.8 $\pm$ 0.21 a	tr	tr	1.40 $\pm$ 0.19 a	13.25 $\pm$ 0.24 a
	Full Irrigation (FI)	4.2 $\pm$ 0.6 b	4.32 $\pm$ 1.75 b	tr	tr	1.20 $\pm$ 0.48 a	9.71 $\pm$ 2.84 b
Crop Management (CM)	Crop Rotation (CR)	4.83 $\pm$ 0.14 a	6.43 $\pm$ 0.43 a	tr	tr	1.60 $\pm$ 0.05 a	12.86 $\pm$ 0.49 a
	No Crop Rotation (NCR)	4.60 $\pm$ 0.72 a	5.41 $\pm$ 2.03 a	tr	tr	1.18 $\pm$ 0.34 b	11.19 $\pm$ 3.05 a
I $\times$ CM	C $\times$ CR	4.8 $\pm$ 0.2 bc	6.6 $\pm$ 0.2 a	tr	tr	1.57 $\pm$ 0.01 b	12.97 $\pm$ 0.42 b
	C $\times$ NCR	5.04 $\pm$ 0.3 a	6.7 $\pm$ 0.3 a	tr	tr	1.52 $\pm$ 0.05 b	13.26 $\pm$ 0.03 a
	DI $\times$ CR	4.97 $\pm$ 0.02 ab	6.8 $\pm$ 0.3 a	tr	tr	1.57 $\pm$ 0.04 b	13.3 $\pm$ 0.3 a
	DI $\times$ NCR	5.11 $\pm$ 0.04 a	6.8 $\pm$ 0.4 a	tr	tr	1.2 $\pm$ 0.1 c	13.11 $\pm$ 0.3 ab
	FI $\times$ CR	4.7 $\pm$ 0.1 c	5.9 $\pm$ 0.3 b	tr	tr	1.64 $\pm$ 0.05 a	12.24 $\pm$ 0.3 c
	FI $\times$ NCR	3.65 $\pm$ 0.04 d	2.69 $\pm$ 0.09 c	tr	tr	0.75 $\pm$ 0.01 d	7.09 $\pm$ 0.07 d

Organic acids (mg/mL)—oxalic acid ( $y = 8 \times 10^6x + 331,789$ ,  $R^2 = 0.9912$ ); quinic acid ( $y = 692,575x + 11,551$ ;  $R^2 = 0.9983$ ); shikimic acid ( $y = 5 \times 10^7x + 567,119$ ,  $R^2 = 0.9903$ ); ascorbic acid ( $y = 5 \times 10^7x + 449,262$ ,  $R^2 = 0.9813$ ); succinic acid ( $y = 493,834 - 33106$ ,  $R^2 = 0.9986$ ). Means in the same column for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ .

Regarding the interaction effects between the studied factors on organic acid content, a varied response was recorded (Table 3). However, it should be noted that reduced irrigation in the form of deficit irrigation or the rain-fed conditions resulted in an increase in the content of major organic acids and consequently in total organic acid content, whereas full irrigation, especially when no crop rotation was implemented, recorded the lowest overall values for the content of all the detected organic acids.

### 3.2.3. Tocopherols

The only detected tocopherols in *C. spinosum* leaves were  $\alpha$ - and  $\beta$ -tocopherol, while deficit irrigation resulted in the highest content of  $\beta$ -tocopherol and consequently of total tocopherols (Table 4). On the other hand, crop management affected only  $\beta$ -tocopherol, which was significantly higher when no crop rotation was implemented. Regarding the combinatorial effect of the tested factors, the highest concentration of  $\beta$ - and total tocopherols (0.158 and 0.214 mg/100 g dw, respectively) was observed in plants grown under deficit irrigation without crop rotation, while  $\alpha$ -tocopherol was the highest in rain-fed plants grown in the crop-rotation scheme. The literature reports showed a varied tocopherol composition in *C. spinosum* leaves, including  $\alpha$ - and  $\delta$ -tocopherol [56,64],  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherols [41], or all the forms of tocopherols [65], suggesting that several factors such as the genotype, the growing conditions, or the harvesting stage may affect the tocopherol profile. Muhairi et al. [60], who studied the effect of drought stress on the content of antioxidant compounds in lettuce (*L. sativa* L.), also reported that  $\alpha$ -tocopherol content increased when the irrigation interval increased up to 96 h and it served as a scavenger of reactive oxygen species that may disrupt the membranes of plant leaves. Similarly, Polyzos et al. [33] observed that deficit irrigation significantly increased  $\alpha$ -tocopherol content in the leaves of *S. hispanicus* plants and they also highlighted the important its protective role against abiotic stressors. According to several studies, tocopherols are essential for plant adaption to stress conditions, since they may effectively scavenge lipid peroxides, oxygen radicals, and singlet oxygen, thereby enhancing plant tolerance to stressors [66–68]. Furthermore, tocopherols are not only involved in the defense mechanisms of plants but they are also essential in several metabolic processes and functions that promote plant growth and development under stress or non-stress conditions [69].

**Table 4.** Tocopherol composition (mg/100 g dw) of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean  $\pm$  SD).

Treatments		$\alpha$ -Tocopherol	$\beta$ -Tocopherol	Total Tocopherols
Irrigation (I)	Control (C)	0.047 $\pm$ 0.016 a	0.107 $\pm$ 0.003 b	0.153 $\pm$ 0.013 b
	Deficit Irrigation (DI)	0.04 $\pm$ 0.01 a	0.15 $\pm$ 0.01 a	0.19 $\pm$ 0.02 a
	Full Irrigation (FI)	0.031 $\pm$ 0.005 b	0.109 $\pm$ 0.041 b	0.140 $\pm$ 0.036 b
Crop Management (CM)	Crop Rotation (CR)	0.044 $\pm$ 0.013 a	0.10 $\pm$ 0.03 b	0.149 $\pm$ 0.032 a
	No Crop Rotation (NCR)	0.038 $\pm$ 0.13 a	0.138 $\pm$ 0.022 a	0.176 $\pm$ 0.031 a
I $\times$ CM	C $\times$ CR	0.0617 $\pm$ 0.0003 a	0.104 $\pm$ 0.003 e	0.165 $\pm$ 0.003 c
	C $\times$ NCR	0.0319 $\pm$ 0.0001 d	0.11 $\pm$ 0.003 d	0.142 $\pm$ 0.003 d
	DI $\times$ CR	0.0354 $\pm$ 0.0004 c	0.141 $\pm$ 0.005 c	0.177 $\pm$ 0.005 b
	FI $\times$ NCR	0.055 $\pm$ 0.001 b	0.158 $\pm$ 0.007 a	0.214 $\pm$ 0.008 a
	DI $\times$ CR	0.035 $\pm$ 0.001 c	0.072 $\pm$ 0.001 f	0.107 $\pm$ 0.003 e
	FI $\times$ NCR	0.027 $\pm$ 0.001 e	0.147 $\pm$ 0.004 b	0.173 $\pm$ 0.003 b

Means in the same column for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan’s Multiple Range test (DMRT) at  $p = 0.05$ .

### 3.2.4. Sugars Composition

Glucose and sucrose were the main sugars detected in *C. spinosum* leaves, followed by fructose and trehalose which were detected in lesser amounts (Table 5). A variable response to the irrigation system and crop management was observed. In particular, deficit irrigation and rain-fed conditions increased the content of glucose, sucrose, and total sugars content, while the highest content of fructose and trehalose was detected in FI treatment. Moreover, implementing crop rotation resulted in increased amounts of fructose and sucrose, also contributing to a higher total sugars content, whereas trehalose increased under full irrigation. A significant interaction between the studied factors was also recorded, with DI  $\times$  CR treatment showing the highest content of sucrose and total sugars, while glucose content increased for C  $\times$  NCR treatment. Moreover, fructose content was the highest for FI  $\times$  CR treatment, while trehalose content did not differ between C  $\times$  NCR and full irrigation (regardless of crop management) (Table 5). A similar free sugars composition in *C. spinosum* leaves was recently reported by Liava et al. [59], as well as in previous studies [58], while Polyzos et al. [41] detected the same compounds and higher amounts of fructose compared to our study. All these studies highlighted that agronomic practices, such as fertilization regime, harvesting stage, or the genotype may affect free sugars composition in the leaves of the species and eventually have an impact on the organoleptic properties and the quality of the edible product. Similarly to our study, Polyzos et al. [33], who assessed the effect of different irrigation regimes on *S. hispanicus* plants, also reported that full irrigation resulted in decreased values of glucose and sucrose and increased fructose content. Moreover, Kapur et al. [70] suggested that drought stress (50% of crop-pan coefficient value) increased fructose, glucose, and sucrose contents in strawberry fruit (*Fragaria  $\times$  ananassa* Duch.), while Abdou et al. [55] detected an increase in total soluble sugars under deficit irrigation (50% of crop evapotranspiration) in black cumin (*N. sativa* L.) leaves, highlighting their role as osmoprotectants. Ibrahim et al. [71] also noted an increase in total soluble-solid content in broccoli (*B. oleracea* var. *italica*) flowers under water-deficit stress as part of the osmoprotective mechanism of plants, a finding which was also confirmed by Thomas et al. [72], who suggested an increase in the expression of sugar metabolism genes under water-stress conditions. Finally, Xu et al. [73] reported that deficit irrigation induces the redistribution of photosynthetic assimilates to the most metabolically active tissues to support plant growth. On the other hand, Götze et al. [74] reported that the monoculture of sugar beet (*Beta vulgaris* subsp. *vulgaris* (var. *saccharifera*)) decreased the sugar content, while crop rotation resulted in higher sugar content regardless of the rotation scheme (e.g., winter wheat (*T. aestivum* L.), alfalfa (*Medicago sativa* L.), grain maize (*Zea mays* L.) and potato (*S. tuberosum* L.) combined in different combinations).

**Table 5.** Free sugars composition (mg/100 g dw) of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean  $\pm$  SD).

Treatments		Fructose	Glucose	Sucrose	Trehalose	Total Sugars
Irrigation (I)	Control (C)	0.73 $\pm$ 0.06 ab	2.67 $\pm$ 0.08 a	2.4 $\pm$ 0.2 ab	0.38 $\pm$ 0.02 a	6.15 $\pm$ 0.17 a
	Deficit Irrigation (DI)	0.69 $\pm$ 0.09 b	2.68 $\pm$ 0.09 a	2.75 $\pm$ 0.57 a	0.33 $\pm$ 0.06 b	6.45 $\pm$ 0.52 a
	Full Irrigation (FI)	0.82 $\pm$ 0.12 a	2.23 $\pm$ 0.24 b	2.06 $\pm$ 0.24 b	0.41 $\pm$ 0.01 a	5.51 $\pm$ 0.13 b
Crop Management (CM)	Crop Rotation (CR)	0.83 $\pm$ 0.08 a	2.4 $\pm$ 0.3 a	2.70 $\pm$ 0.45 a	0.35 $\pm$ 0.06 b	6.29 $\pm$ 0.56 a
	No Crop Rotation (NCR)	0.67 $\pm$ 0.04 b	2.64 $\pm$ 0.16 a	2.09 $\pm$ 0.19 b	0.39 $\pm$ 0.0 a	5.8 $\pm$ 0.3 b
I $\times$ CM	C $\times$ CR	0.79 $\pm$ 0.02 b	2.6 $\pm$ 0.1 b	2.55 $\pm$ 0.07 b	0.36 $\pm$ 0.01 c	6.31 $\pm$ 0.01 b
	C $\times$ NCR	0.68 $\pm$ 0.02 d	2.73 $\pm$ 0.04 a	2.20 $\pm$ 0.02 c	0.40 $\pm$ 0.02 ab	6.0 $\pm$ 0.1 c
	DI $\times$ CR	0.78 $\pm$ 0.04 b	2.61 $\pm$ 0.01 b	3.3 $\pm$ 0.1 a	0.27 $\pm$ 0.01 d	6.9 $\pm$ 0.1 a
	FI $\times$ NCR	0.61 $\pm$ 0.01 e	2.75 $\pm$ 0.11 a	2.23 $\pm$ 0.09 c	0.39 $\pm$ 0.01 b	5.97 $\pm$ 0.01 c
	DI $\times$ CR	0.94 $\pm$ 0.04 a	2.01 $\pm$ 0.11 d	2.28 $\pm$ 0.06 c	0.41 $\pm$ 0.03 a	5.63 $\pm$ 0.01 d
	FI $\times$ NCR	0.71 $\pm$ 0.01 c	2.44 $\pm$ 0.08 c	1.84 $\pm$ 0.04 d	0.40 $\pm$ 0.01 ab	5.39 $\pm$ 0.04 e

Means in the same column for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ .

### 3.2.5. Mineral Composition

The mineral composition of *C. spinosum* leaves is displayed in Table 6. The irrigation regime significantly affected macro- and micro-mineral content in a varied manner. In particular, K, Na, Ca and Mg content significantly decreased under the full irrigation regime, while the same treatment resulted in the highest content of Fe and Mn. On the other hand, Cu and Zn content was the lowest under rain-fed conditions. Regarding the crop-management effect, only K, Na and Mg content was affected, where K increased when crop rotation was implemented, whereas Na and Mg showed the highest content when no rotation was applied. The combination of the two factors also resulted in significant differences between the treatments in a variable manner. For example, the highest content Ca was recorded for the C × CR treatment, while Na increased for the C × NCR treatment; K content was the highest for C and DI treatments under crop rotation, while Mg content increased in rain-fed conditions (for both crop-management treatments) and for DI × NCR treatment. Finally, full irrigation and no crop rotation resulted in the highest content of Fe, Mn and Zn, while Cu increased when deficit irrigation was combined with crop rotation. According to literature reports, *C. spinosum* leaves are rich in macro- and micro-nutrients, while their content can be affected by several factors including the genotype and the growing conditions [64,75]. Previously, Polyzos et al. [33] who studied the effect of different irrigation regimes on the mineral composition of *S. hispanicus* plants reported that deficit irrigation did not affect K, Fe, and Mn, while it increased the content of Ca, Mg, Cu, and Zn. Moreover, the same authors observed that rain-fed plants had the lowest content of Ca, Mg, Cu, and Zn and the highest Na value [33]. In contrast, deficit irrigation did not affect the content of Ca, Cu, Fe, Ca, Mg, Na, and N in bean (*P. vulgaris* L.) pods, although it resulted in increased P and Zn values [76], while drought stress reduced N, P, and K content in purslane (*P. oleracea* L.) leaves, tomato (*S. lycopersicum* L.) fruits, and broccoli (*B. oleracea* var. *italica*) leaves [43,71,77]. The negative effects of deficit irrigation on the content of specific nutrients could be attributed to a limited uptake of moderately mobile elements due to the severe effects of water stress on soil enzyme activity and soil microbiome, which interfere in the soil–plant interaction [78], as well as to the reduced uptake of water from roots due to increased values of soil-matrix potential [79,80]. Regarding the effect of crop-rotation on nutrients' status, Yfantopoulos et al. [81], who studied the effect of crop rotation on a cabbage (*B. oleracea* var. *capitata*) crop, observed that nutrient content was not influenced by rotation, while Haruna and Nkongolo [82] reported that soil nutrients were not affected by the continuous monocropping of corn (*Z. mays* L.) and soybean (*Glycine max* (L.) Merr.), as well as by corn–soybean and soybean–corn rotation. According to Adesanya et al. [83], the positive effects of crop rotation on nutrient availability are nutrient-specific and they may also vary in the long term due to weather conditions.

### 3.2.6. Fatty-Acid Composition

In terms of the profile of fatty acids, twenty individual compounds were identified as presented in Table 7. The most abundant fatty acid was  $\alpha$ -linolenic acid (C18:3n3) with values ranging from 37.1% to 45.4%, followed by palmitic acid (C16:0; 27.84% to 20.88%), and linoleic acid (C18:2n6; 16.7% to 21.1%). The leaves also had a high content of polyunsaturated fatty acids (PUFA; 54.6% to 65.6%), followed by saturated fatty acids (SFA; 29.5% to 39.6%) and monounsaturated fatty acids (MUFA; 4.8% to 5.9%). Irrigation regime had a variable effect on the major fatty acids, where rain-fed and deficit irrigation conditions increased the content of  $\alpha$ -linolenic acid, while the highest content of linoleic acid was recorded for full irrigation. In contrast, irrigation regime had no effect on palmitic acid content. Additionally, crop rotation resulted in a reduction in palmitic acid and an increase in  $\alpha$ -linolenic and linoleic acid content, which contributed to higher PUFA/SFA ratios. The

combined effect of the tested factors also showed a variable response to the composition of fatty acids. In particular, crop rotation under rain-fed and deficit irrigation conditions resulted in the highest  $\alpha$ -linolenic acid content, whereas palmitic acid increased when no crop rotation was implemented and plants were treated with deficit and full irrigation. Moreover, linoleic acid benefitted from the DI  $\times$  NCR treatment. Finally, rain-fed and deficit irrigation treatments combined with crop rotation had the highest and lowest values of PUFA and SFA, respectively.

A similar fatty-acid profile was recorded by Petropoulos et al. [64], who suggested genotypic differences among various *C. spinosum* ecotypes, as well as a significant impact of agronomic practices and harvesting stage [64]. Moreover, Liava et al. [59] highlighted the high ratios of PUFA/SFA in *C. spinosum* leaves, which are associated with high nutritional value and health benefits. In contrast to our study, water deficit did not affect fatty-acid content in grain sorghum (*Sorghum bicolor* (L.) Moench) [54] and groundnut (*Arachis hypogaea* L.) [84], whereas Ghaffari et al. [85] reported a significant increase in palmitic and linoleic acid content in sunflower (*Helianthus annuus* L.) seeds under drought conditions. Similarly, deficit irrigation (50% of evapotranspiration) decreased the oil quality in chia seeds due to increased palmitic acid and decreased linoleic and  $\alpha$ -linolenic acid content compared to full irrigation (100% of evapotranspiration) [86], while Abdou et al. [55] suggested an increase in SFA and a decrease in polyunsaturated fatty acids in *N. sativa* seed oil due to the activation of the lipase enzyme under water-stress conditions. According to Upchurch [87], the acclimatization of plants to stress conditions is associated with increases in unsaturated fatty-acid content which help cells to retain membrane fluidity and avoid oxidative damage. This is also evident in our study with the increased content of  $\alpha$ -linolenic acid under rain-fed and deficit irrigation conditions, whereas the lack of effect of drought stress on the overall content of PUFA suggests the drought tolerance of the species [88]. Regarding the effect of the cropping system, Stepien et al. [89] suggested that integrating canola (*B. napus* L.) in a crop-rotation scheme resulted in increased content of linoleic and  $\alpha$ -linolenic acid, while Mohammadi et al. [90] reported a variable effect of crop rotation on the canola fatty-acid profile depending on the rotation scheme (e.g., the succession and diversification of crops included in the scheme). On the other hand, Wacal et al. [91] reported an increase in  $\alpha$ -linolenic and linoleic acids in sesame (*Sesamum indicum* L.) seeds grown under continuous monocropping, which indicates the variable impact of cropping system depending on the crop, the rotation scheme, and the growing conditions.

**Table 6.** Mineral composition (mg/100 g dw) of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean  $\pm$  SD).

Treatments		K (g/Kg)	Na (mg/Kg)	Ca (g/Kg)	Mg (g/Kg)	Fe (mg/Kg)	Mn (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)
Irrigation (I)	Control (C)	36.6 $\pm$ 6.6 ab	6004 $\pm$ 1052 a	11.98 $\pm$ 0.49 a	3.70 $\pm$ 0.07 a	849 $\pm$ 47 b	105.7 $\pm$ 18.6 c	9.37 $\pm$ 0.54 b	45.8 $\pm$ 1.3 b
	Deficit Irrigation (DI)	37.8 $\pm$ 6.6 a	5259 $\pm$ 694 a	11.1 $\pm$ 0.3 b	3.5 $\pm$ 0.2 a	537 $\pm$ 32 b	135.7 $\pm$ 9.7 b	12.9 $\pm$ 0.5 a	58.4 $\pm$ 1.4 a
	Full Irrigation (FI)	29.5 $\pm$ 3.4 b	3481 $\pm$ 572 b	9.5 $\pm$ 0.7 c	3.1 $\pm$ 0.4 b	2915 $\pm$ 645 a	183.3 $\pm$ 18.4 a	12.3 $\pm$ 0.4 a	58.8 $\pm$ 9.9 a
Crop Management (CM)	Crop Rotation (CR)	39.6 $\pm$ 5.4 a	4212 $\pm$ 959 b	10.7 $\pm$ 1.5 a	3.3 $\pm$ 0.4 b	1260 $\pm$ 113 a	133.3 $\pm$ 14.9 a	12.3 $\pm$ 3.1 a	51.8 $\pm$ 5.9 a
	No Crop Rotation (NCR)	29.6 $\pm$ 2.5 b	5617 $\pm$ 1300 a	11.1 $\pm$ 0.7 a	3.6 $\pm$ 1.2 a	1608 $\pm$ 142 a	149.8 $\pm$ 17.6 a	10.8 $\pm$ 1.5 a	56.8 $\pm$ 9.8 a
I $\times$ CM	C $\times$ CR	43 $\pm$ 2 a	5049 $\pm$ 170 c	12.3 $\pm$ 0.4 a	3.7 $\pm$ 0.1 a	888 $\pm$ 44 c	88.7 $\pm$ 0.4 f	8.9 $\pm$ 0.4 e	46 $\pm$ 1 d
	C $\times$ NCR	30.6 $\pm$ 0.4 c	6960 $\pm$ 154 a	11.7 $\pm$ 0.6 b	3.6 $\pm$ 0.1 a	809 $\pm$ 4 d	122.7 $\pm$ 1.3 e	9.8 $\pm$ 0.4 d	45 $\pm$ 2 d
	DI $\times$ CR	44 $\pm$ 3 a	4627 $\pm$ 82 d	10.78 $\pm$ 0.06 c	3.35 $\pm$ 0.13 b	565 $\pm$ 25 e	144.5 $\pm$ 0.3 c	16.1 $\pm$ 0.5 a	59 $\pm$ 1 b
	FI $\times$ NCR	31.9 $\pm$ 1.1 bc	5891 $\pm$ 62 b	11.4 $\pm$ 0.1 b	3.7 $\pm$ 0.2 a	510 $\pm$ 10 f	126.9 $\pm$ 2 d	9.8 $\pm$ 0.2 d	57 $\pm$ 1 b
	DI $\times$ CR	32.6 $\pm$ 0.5 b	2962 $\pm$ 117 f	8.9 $\pm$ 0.2 e	2.7 $\pm$ 0.1 c	2327 $\pm$ 74 b	166.7 $\pm$ 5.7 b	11.9 $\pm$ 0.3 c	50 $\pm$ 1 c
	FI $\times$ NCR	26.3 $\pm$ 0.4 d	4000 $\pm$ 89 e	10.2 $\pm$ 0.3 d	3.45 $\pm$ 0.05 b	3504 $\pm$ 20 a	199.9 $\pm$ 2.3 a	12.7 $\pm$ 0.03 b	68 $\pm$ 1 a

Means in the same column for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan’s Multiple Range test (DMRT) at  $p = 0.05$ .

**Table 7.** Fatty-acid composition (relative percentage) of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean  $\pm$  SD).

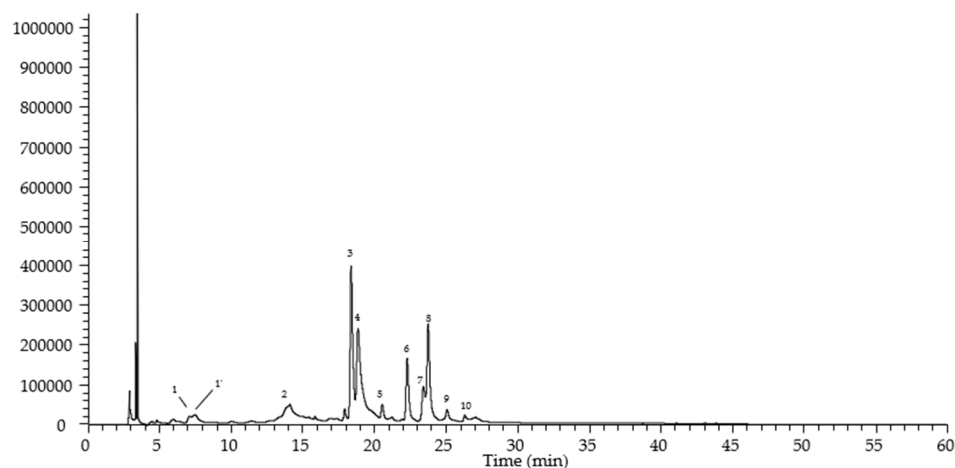
	Irrigation (I)			Crop Management (CM)		I $\times$ CM					
	C	DI	FI	CR	NCR	C $\times$ CR	C $\times$ NCR	DI $\times$ CR	DI $\times$ NCR	FI $\times$ CR	FI $\times$ NCR
C6:0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
C11:0	0.139 $\pm$ 0.016 a	0.26 $\pm$ 0.17 a	0.193 $\pm$ 0.063 a	0.123 $\pm$ 0.012 ba	0.276 $\pm$ 0.118 a	0.125 $\pm$ 0.003 e	0.154 $\pm$ 0.003 v	0.109 $\pm$ 0.003 f	0.423 $\pm$ 0.004 a	0.136 $\pm$ 0.004 d	0.251 $\pm$ 0.008 b
C12:0	0.11 $\pm$ 0.05 a	0.175 $\pm$ 0.101 a	0.122 $\pm$ 0.063 a	0.071 $\pm$ 0.009 b	0.201 $\pm$ 0.051	0.066 $\pm$ 0.002 e	0.156 $\pm$ 0.001 c	0.084 $\pm$ 0.001 d	0.268 $\pm$ 0.007 a	0.065 $\pm$ 0.002 e	0.181 $\pm$ 0.005 b
C13:0	0.79 $\pm$ 0.24 b	1.010 $\pm$ 0.015 a	0.662 $\pm$ 0.054 b	0.875 $\pm$ 0.197 a	0.769 $\pm$ 0.196 a	1.01 $\pm$ 0.02 a	0.576 $\pm$ 0.001 d	1.002 $\pm$ 0.011 a	1.02 $\pm$ 0.03 a	0.613 $\pm$ 0.01 c	0.712 $\pm$ 0.003 b
C14:0	0.994 $\pm$ 0.073 a	1.116 $\pm$ 0.328 a	1.146 $\pm$ 0.087 a	0.94 $\pm$ 0.11 b	1.232 $\pm$ 0.156 a	0.927 $\pm$ 0.004 d	1.061 $\pm$ 0.001 c	0.82 $\pm$ 0.03 e	1.42 $\pm$ 0.03 a	1.07 $\pm$ 0.03 c	1.22 $\pm$ 0.06 b
C14:1	0.33 $\pm$ 0.12 b	0.437 $\pm$ 0.039 a	0.261 $\pm$ 0.015 b	0.37 $\pm$ 0.07 a	0.31 $\pm$ 0.12 a	0.44 $\pm$ 0.01 b	0.218 $\pm$ 0.005 f	0.4 $\pm$ 0.2 c	0.47 $\pm$ 0.02 a	0.27 $\pm$ 0.01 d	0.248 $\pm$ 0.007 e
C15:0	0.168 $\pm$ 0.038 a	0.154 $\pm$ 0.074 a	0.209 $\pm$ 0.027 a	0.151 $\pm$ 0.065 b	0.203 $\pm$ 0.016 a	0.134 $\pm$ 0.001 e	0.204 $\pm$ 0.005 c	0.086 $\pm$ 0.001 f	0.222 $\pm$ 0.005 b	0.234 $\pm$ 0.006 a	0.185 $\pm$ 0.006 d
C15:1	0.222 $\pm$ 0.066 a	0.228 $\pm$ 0.020 a	0.203 $\pm$ 0.027 a	0.23 $\pm$ 0.05 a	0.205 $\pm$ 0.035 a	0.283 $\pm$ 0.004 a	0.162 $\pm$ 0.003 d	0.246 $\pm$ 0.006 b	0.21 $\pm$ 0.003 c	0.163 $\pm$ 0.007 d	0.243 $\pm$ 0.006 b
C16:0	23.39 $\pm$ 2.49 a	24.36 $\pm$ 3.81 a	25.67 $\pm$ 1.98 a	21.97 $\pm$ 1.46 b	27.0 $\pm$ 1.1 a	21.1 $\pm$ 0.2 d	25.6 $\pm$ 0.9 b	20.88 $\pm$ 0.03 d	27.84 $\pm$ 0.07 a	23.8 $\pm$ 0.4 c	27.4 $\pm$ 0.8 a
C16:1	2.68 $\pm$ 0.16 a	2.83 $\pm$ 0.35 a	3.009 $\pm$ 0.471 a	2.54 $\pm$ 0.04 b	3.14 $\pm$ 0.27	2.54 $\pm$ 0.05 d	2.82 $\pm$ 0.1 c	2.51 $\pm$ 0.05 d	3.15 $\pm$ 0.02 b	2.58 $\pm$ 0.03 d	3.4 $\pm$ 0.1 a
C17:0	0.97 $\pm$ 0.22 a	0.85 $\pm$ 0.24 a	1.003 $\pm$ 0.365 a	0.69 $\pm$ 0.06 b	1.19 $\pm$ 0.12 a	0.77 $\pm$ 0.04 d	1.17 $\pm$ 0.02 b	0.64 $\pm$ 0.01 f	1.07 $\pm$ 0.02 c	0.67 $\pm$ 0.01 e	1.34 $\pm$ 0.01 a
C18:0	2.93 $\pm$ 0.41 a	3.12 $\pm$ 0.65 a	3.24 $\pm$ 0.53 a	2.61 $\pm$ 0.11 b	3.58 $\pm$ 0.21 a	2.55 $\pm$ 0.06 d	3.3 $\pm$ 0.07 b	2.525 $\pm$ 0.001 d	3.71 $\pm$ 0.01 a	2.755 $\pm$ 0.004 c	3.73 $\pm$ 0.05 a
C18:1n9c	1.65 $\pm$ 0.33 c	1.865 $\pm$ 0.036 b	1.99 $\pm$ 0.027 a	1.85 $\pm$ 0.15 a	1.82 $\pm$ 0.15 a	1.68 $\pm$ 0.01 c	1.62 $\pm$ 0.03 d	1.86 $\pm$ 0.05 b	1.87 $\pm$ 0.07 b	2.01 $\pm$ 0.02 a	1.97 $\pm$ 0.03 a
C18:2n6c	18.56 $\pm$ 1.52 b	18.05 $\pm$ 1.46 b	20.28 $\pm$ 0.80 a	20.11 $\pm$ 0.72 a	17.81 $\pm$ 1.32 b	19.95 $\pm$ 0.02 b	17.2 $\pm$ 0.2 d	19.4 $\pm$ 0.2 c	16.7 $\pm$ 0.1 e	21.1 $\pm$ 0.2 a	19.6 $\pm$ 0.1 c
C18:3n6	0.350 $\pm$ 0.039 a	0.336 $\pm$ 0.056 a	0.38 $\pm$ 0.04 a	0.338 $\pm$ 0.059 a	0.371 $\pm$ 0.022 a	0.32 $\pm$ 0.01 d	0.386 $\pm$ 0.009 b	0.29 $\pm$ 0.01 e	0.387 $\pm$ 0.002 b	0.41 $\pm$ 0.02 a	0.342 $\pm$ 0.008 c
C18:3n3	43.90 $\pm$ 1.63 a	41.5 $\pm$ 4.4 a b	39.01 $\pm$ 2.13 b	43.95 $\pm$ 2.27 a	39.0 $\pm$ 2.6 b	45.4 $\pm$ 0.1 a	42.4 $\pm$ 0.7 b	45.6 $\pm$ 0.2 a	37.5 $\pm$ 0.2 d	40.9 $\pm$ 0.1 c	37.1 $\pm$ 0.9 d
C22:0	0.843 $\pm$ 0.087 a	0.93 $\pm$ 0.06 a	0.90 $\pm$ 0.18 a	0.904 $\pm$ 0.133 a	0.88 $\pm$ 0.12 a	0.76 $\pm$ 0.01 e	0.92 $\pm$ 0.02 c	0.881 $\pm$ 0.005 d	0.99 $\pm$ 0.01 b	1.07 $\pm$ 0.01 a	0.73 $\pm$ 0.02 f
C22:2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
C23:0	0.631 $\pm$ 0.138	0.952 $\pm$ 0.023 a	0.62 $\pm$ 0.04 b	0.78 $\pm$ 0.12 a	0.69 $\pm$ 0.21 a	0.76 $\pm$ 0.01 c	0.51 $\pm$ 0.01 f	0.93 $\pm$ 0.02 b	0.972 $\pm$ 0.003 a	0.66 $\pm$ 0.01 d	0.59 $\pm$ 0.02 e
C24:0	1.36 $\pm$ 0.15 b	1.758 $\pm$ 0.052 a	1.089 $\pm$ 0.041 b	1.49 $\pm$ 0.25 a	1.312 $\pm$ 0.456 a	1.23 $\pm$ 0.03 d	1.51 $\pm$ 0.02 c	1.8 $\pm$ 0.1 a	1.72 $\pm$ 0.01 b	1.46 $\pm$ 0.06 c	0.72 $\pm$ 0.03 e
SFA	32.32 $\pm$ 3.15 a	34.7 $\pm$ 5.4 a	34.86 $\pm$ 2.48 a	30.61 $\pm$ 1.52 b	37.31 $\pm$ 1.99 a	29.5 $\pm$ 0.2 e	35.2 $\pm$ 0.8 c	29.75 $\pm$ 0.01 e	39.65 $\pm$ 0.04 a	32.6 $\pm$ 0.3 d	37.1 $\pm$ 0.8 b
MUFA	4.878 $\pm$ 0.075 b	5.36 $\pm$ 0.38 a	5.47 $\pm$ 0.48 a	4.994 $\pm$ 0.052 b	5.5 $\pm$ 0.5 a	4.93 $\pm$ 0.07 d	4.82 $\pm$ 0.07 e	5.018 $\pm$ 0.005 c	5.71 $\pm$ 0.07 b	5.03 $\pm$ 0.02 c	5.9 $\pm$ 0.2 a
PUFA	62.8 $\pm$ 3.1 a	59.9 $\pm$ 5.8 a	59.67 $\pm$ 2.96 a	64.40 $\pm$ 1.55 a	57.21 $\pm$ 2.37 b	65.6 $\pm$ 0.1 a	60.001 $\pm$ 0.892 c	65.235 $\pm$ 0.004 a	54.64 $\pm$ 0.03 e	62.3 $\pm$ 0.3 b	57.001 $\pm$ 0.969 d

Fatty acids are expressed as a relative percentage of each fatty acid: C11:0—undecanoic acid; C12:0—lauric acid; C13:0—Tridecanoic acid; C14:0—myristic acid; C14:1—tetradecanoic acid; C15:0—pentadecanoic acid; C15:1—pentadecylic acid; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—heptadecanoic acid; C18:0—stearic acid; C18:1n9c—oleic acid; C18:2n6c—linoleic acid; C18:3n6—gamma linolenic acid; C18:3n3— $\alpha$ -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; dw—dry weight; tr—traces. DI: deficit irrigation; FI: full irrigation; CR: crop rotation; NCR no crop rotation. Means in the same row for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ .

### 3.3. Phenolic Compounds and Bioactive Properties

#### 3.3.1. Phenolic Compounds

Eleven phenolic compounds (Figure 3) were detected in the hydroethanolic extracts of *C. spinosum* leaves (Table 8), including three phenolic acids and six flavonoids, while the profile of phenolic compounds varied among the studied treatments (Table 9). In particular, chicoric acid (peak 2), quercetin-*O*-hexurumoside (peak 3), and luteolin-*O*-hexurumoside (peak 4) were the most abundant compounds, followed by *trans* 5-*O*-Caffeoylquinic acid (peak 1'), isorhamnetin-*O*-hexurunoside (peak 8), apigenin-*O*-hexurunoside (peak 7), and kaempferol-*O*-hexurunoside (peak 6); flavonoids were the richest class of phenolic compounds. The irrigation regime had a significant impact on the phenolic compounds profile with deficit irrigation, resulting in the highest amount for all the detected compounds. On the other hand, crop management did not affect the phenolic compounds profile, except for the content of peaks 9 and 11, which increased when plants were grown under no crop rotation and crop rotation, respectively. Finally, the combined effect of the tested factors was also significant, with seven compounds (including the major ones) having the highest content for the DI  $\times$  CR treatment, while peaks 7, 9, and 10 benefitted from deficit irrigation and no crop rotation and peak 8 from full irrigation and no crop rotation.



**Figure 3.** HPLC spectral phenolic profile of hydroethanolic extracts of *C. spinosum* leaves recorded at 370 nm. See Table 8 for tentative identification of detected peaks (peak 1–10).

**Table 8.** Retention time (Rt), wavelength of the maximum absorption ( $\lambda_{max}$ ), deprotonated ion ( $[M-H]^-$ ), main mass fragments (MS2), and tentative identification.

Peak	Rt	$\lambda_{max}$	$[M-H]^-$	MS2	Tentative Identification
1	7.07	326	353	191 (100), 179 (23), 135(5)	<i>cis</i> 5- <i>O</i> -Caffeoylquinic acid
1'	7.41	326	353	191 (100), 179 (12), 135 (5)	<i>trans</i> 5- <i>O</i> -Caffeoylquinic acid
2	13.98	328	473	391 (39), 311 (100), 293 (28)	Chicoric acid
3	18.22	353	477	301 (100)	Quercetin- <i>O</i> -hexurunoside
4	18.73	343	461	285 (100)	Luteolin- <i>O</i> -hexurunoside
5	20.4	335	505	463 (11), 301 (100)	Quercetin- <i>O</i> -acetylhexoside
6	22.13	346	461	285 (100)	Kaempferol- <i>O</i> -hexurunoside
7	23.32	333	445	269 (100)	Apigenin- <i>O</i> -hexurunoside
8	23.59	345	491	315 (100)	Isorhamnetin- <i>O</i> -hexurunoside
9	24.9	326	489	285 (100)	Kaempferol- <i>O</i> -acetylhexoside
10	26.14	327	519	315 (100)	Isorhamnetin- <i>O</i> -acetylhexoside

**Table 9.** Quantification (mg/g extract) of the phenolic compounds found in the hydroethanolic extracts of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean ± SD).

Tentative Identification	Irrigation (I)			Crop Management (CM)			I × CM				
	Control (C)	Deficit Irrigation (DI)	Full Irrigation (FI)	Crop Rotation (CR)	None Crop Rotation (NCR)	C × CR	C × NCR	DI × CR	DI × NCR	FI × CR	FI × NCR
<i>cis</i> 5- <i>O</i> -Caffeoylquinic acid	0.54 ± 0.13 b	0.91 ± 0.23 a	0.413 ± 0.122 c	0.613 ± 0.38 a	0.63 ± 0.07 a	0.41 ± 0.01 e	0.66 ± 0.01 c	1.13 ± 0.01 a	0.69 ± 0.03 b	0.29 ± 0.01 f	0.53 ± 0.01 d
<i>trans</i> 5- <i>O</i> -Caffeoylquinic acid	0.83 ± 0.25 b	1.92 ± 0.39 a	0.644 ± 0.067 c	1.23 ± 0.78 a	1.03 ± 0.37 a	0.81 ± 0.03 d	0.84 ± 0.02 c	2.301 ± 0.002 a	1.54 ± 0.05 b	0.58 ± 0.03 f	0.71 ± 0.03 e
Chicoric acid	1.095 ± 0.361 b	3.36 ± 0.57 a	0.84 ± 0.18 c	1.90 ± 1.46 a	1.63 ± 0.85 a	1.13 ± 0.01 c	1.07 ± 0.04 d	3.92 ± 0.01 a	2.8 ± 0.05 b	0.67 ± 0.01 f	1.02 ± 0.02 e
Quercetin- <i>O</i> -hexuronoside	1.310 ± 0.006 b	3.38 ± 0.03 a	1.51 ± 0.07 c	2.05 ± 0.98 a	2.08 ± 0.92 a	1.31 ± 0.01 e	1.31 ± 0.01 e	3.41 ± 0.03 a	3.354 ± 0.003 b	1.45 ± 0.01 d	1.575 ± 0.001 c
Luteolin- <i>O</i> -hexuronoside	1.410 ± 0.098 c	2.55 ± 0.02 a	2.074 ± 0.318 b	1.95 ± 0.46 a	2.07 ± 0.55 a	1.51 ± 0.02 e	1.32 ± 0.03 f	2.569 ± 0.004 a	2.53 ± 0.03 b	1.77 ± 0.04 d	2.38 ± 0.02 c
Quercetin- <i>O</i> -acetylhexoside	0.52 ± 0.07 b	0.757 ± 0.027 a	0.512 ± 0.005 b	0.625 ± 0.118 a	0.568 ± 0.122 a	0.585 ± 0.002 c	0.454 ± 0.003 f	0.78 ± 0.02 a	0.73 ± 0.01 b	0.507 ± 0.003 e	0.517 ± 0.004 d
Kaempferol- <i>O</i> -hexuronoside	0.811 ± 0.062 c	1.360 ± 0.006 a	0.917 ± 0.122 b	1.009 ± 0.252 a	1.051 ± 0.255 a	0.872 ± 0.004 d	0.75 ± 0.01 f	1.355 ± 0.004 b	1.365 ± 0.006 a	0.799 ± 0.009 e	1.036 ± 0.008 c
Apigenin- <i>O</i> -hexuronoside	1.066 ± 0.185 b	1.46 ± 0.021 a	1.57 ± 0.38 a	1.369 ± 0.211 a	1.36 ± 0.44 a	1.25 ± 0.03 d	0.89 ± 0.01 f	1.66 ± 0.03 b	1.26 ± 0.01 c	1.2 ± 0.4 e	1.939 ± 0.001 a
Isorhamnetin- <i>O</i> -hexuronoside	1.03 ± 0.03 c	1.47 ± 0.06 a	1.164 ± 0.257 b	1.113 ± 0.223 b	1.336 ± 0.203 a	1.01 ± 0.02 d	1.06 ± 0.01 c	1.42 ± 0.05 b	1.53 ± 0.03 a	0.91 ± 0.01 e	1.41 ± 0.01 b
Kaempferol- <i>O</i> -acetylhexoside	0.48 ± 0.04 b	0.616 ± 0.014 a	0.493 ± 0.033 b	0.529 ± 0.059 a	0.53 ± 0.08 a	0.523 ± 0.002 d	0.436 ± 0.003 f	0.603 ± 0.001 b	0.63 ± 0.004 a	0.461 ± 0.001 e	0.526 ± 0.001 c
Isorhamnetin- <i>O</i> -acetylhexoside	0.425 ± 0.042 b	0.491 ± 0.002 a	0.421 ± 0.003 b	0.459 ± 0.031 a	0.432 ± 0.044 b	0.466 ± 0.001 c	0.383 ± 0.003 f	0.4927 ± 0.0001 a	0.4887 ± 0.0002 b	0.4179 ± 0.0005 e	0.4234 ± 0.0009 d
Total Phenolic Acids	2.46 ± 0.12 b	6.19 ± 1.19 a	1.90 ± 0.37 c	3.75 ± 0.62 a	3.27 ± 0.26 a	2.34 ± 0.05 d	2.57 ± 0.01 c	7.35 ± 0.01 a	5.03 ± 0.03 b	1.54 ± 0.02 f	2.26 ± 0.02 e
Total Flavonoids	7.06 ± 0.47 c	12.09 ± 0.21 a	8.66 ± 1.18 b	9.1 ± 1.3 a	9.43 ± 1.22 a	7.51 ± 0.03 d	6.602 ± 0.001 e	12.29 ± 0.07 a	11.89 ± 0.05 b	7.51 ± 0.03 d	9.81 ± 0.03 c
Total Phenolic compounds	9.516 ± 0.353 c	18.3 ± 1.4 a	10.56 ± 1.55 b	12.8 ± 1.9 a	12.72 ± 3.26 a	9.9 ± 0.1 d	9.174 ± 0.009 e	19.6 ± 0.1 a	16.92 ± 0.02 b	9.06 ± 0.05 f	12.07 ± 0.01 c

Means in the same row for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ . Calibration curves used for quantification: *p*-Coumaric acid ( $y = 301,950x + 6966.7$ ,  $R^2 = 0.9999$ , LOD ( $\mu\text{g/mL}$ ) = 0.62 and LOQ ( $\mu\text{g/mL}$ ) = 1.89, peaks 1 and 1'); caffeic acid ( $y = 388,345x + 406,369$ ,  $R^2 = 0.9939$ , LOD ( $\mu\text{g/mL}$ ) = 8.57 and LOQ ( $\mu\text{g/mL}$ ) = 25.97, peak 2); quercetin-3-*O*-glucoside ( $y = 34,843x - 160,173$ ,  $R^2 = 0.9998$ , LOD ( $\mu\text{g/mL}$ ) = 17.01 and LOQ ( $\mu\text{g/mL}$ ) = 51.54, peaks 3, 4, 5, 6, 8, 9 and 10); apigenin-7-*O*-glucoside ( $y = 10,683x - 45,794$ ,  $R^2 = 0.996$ , LOD ( $\mu\text{g/mL}$ ) = 136.95 and LOQ ( $\mu\text{g/mL}$ ) = 414.98, peak 7).

In the previous studies of Petropoulos et al. [56], it was also mentioned that chicoric acid was the predominant phenolic compound; however, the same authors indicated that 5-O-Caffeoylquinic acid was the second most abundant compound, whereas they did not detect quercetin-O-hexurumoside and luteolin-O-hexurumoside which were predominant in our study. Moreover, Liava et al. [59] detected significant amounts of luteolin derivatives and 5-O-Caffeoylquinic acid in decoctions and hydroethanolic extracts of *C. spinosum* leaves, whereas chicoric acid content was very low. These contradicting results could be associated with the different growing conditions and agronomic practices implemented, as well as the genetic material tested, since according to the literature a significant variation in phenolic profile should be expected among different *C. spinosum* ecotypes [64].

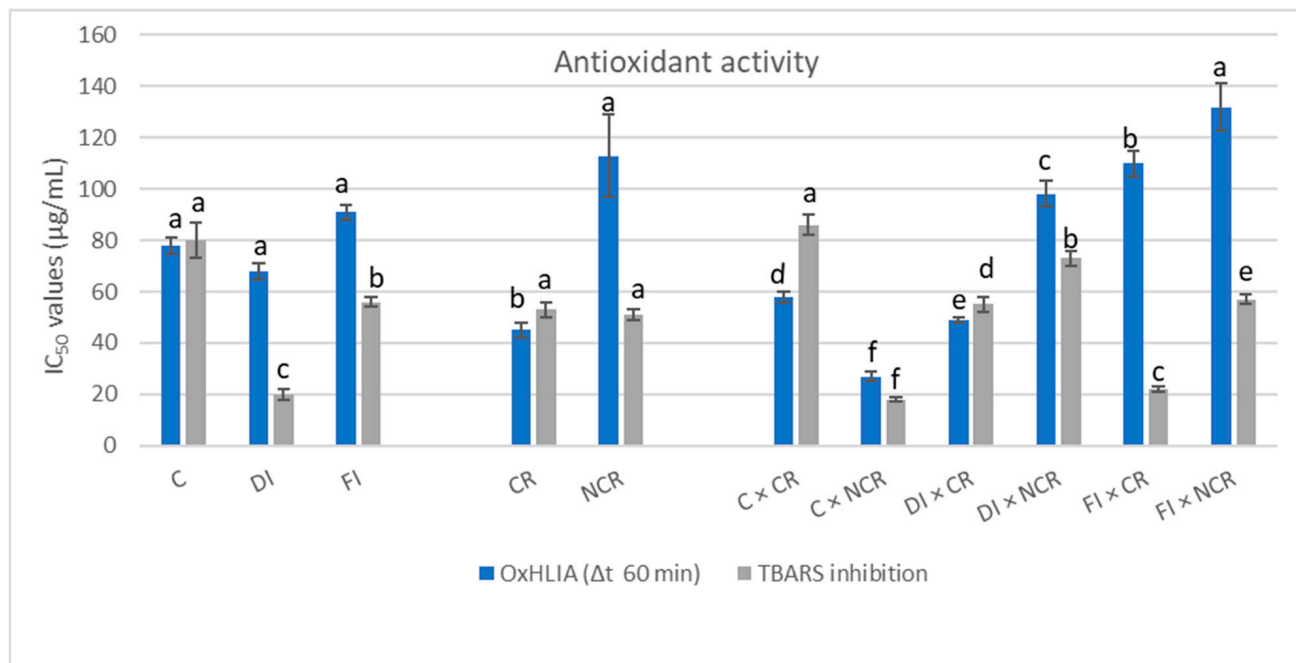
Moreover, Polyzos et al. [33], who studied the effect of deficit irrigation on *S. hispanicus*, observed that water deficit induced the accumulation of phenolic compounds in either rain-fed conditions or deficit irrigation compared to full irrigation. In pepper (*Capsicum annum* L.) fruit, the greatest TPC values were observed in plants grown under deficit irrigation (50%) [92], while low to moderate (50% field capacity) stress also increased TPC content in *Achillea* species [93]. Moreover, Skrypnik et al. [61] reported that TPC increased with the increasing severity of drought stress in salvia (*S. officinalis* L.) and oregano (*O. vulgare* L.), whereas moderate water stress (50% of soil field capacity) induced the accumulation of phenolic compounds in hyssop (*Hyssopus officinalis* L.) and severe stress (25% of soil field capacity) resulted in lower values of TPC. In contrast, water-deficit conditions (50% field capacity) decreased anthocyanin, quercetin, gallic acid, vanillic acid, chlorogenic acid, and cinnamic acid in sunflower (*H. annuus* L.) leaves [94]. Overall, deficit irrigation regulates the biosynthesis of phytochemical, including phenolic compounds, in a species-specific manner, since, apart from phenolic compounds, other antioxidant compounds could contribute to plant's defense against abiotic stressors [73]. Albergaria et al. [95] also highlighted that the increase in secondary metabolites is not uniform across all plant tissue and organs, and the severity of water stress may significantly impact metabolite synthesis. Therefore, mild water stress could be considered as a eustress and promote the biosynthesis of secondary metabolites, whereas severe stress may result in reduced values when the defense mechanisms of plants cannot cope with the severity of stress [96].

Crop-rotation schemes may also have an impact on phenolic compounds content. For example, Mitchell et al. [97] suggested that flavonoid content in tomato (*S. lycopersicum* L.) fruit increased when organic cultivation combined with various rotation schemes over a ten-year period. Moreover, the same authors recorded the largest increase during the last 4 years of the experiments, regardless of the cropping system (e.g., organic vs. conventional) which explains the lack of effect in our experiment, where a short-term rotation was implemented. Similarly, Buczek et al. [98] observed an increase in phenolic acid content in winter wheat (*T. aestivum* L.) grain when crop rotation was integrated into organic cultivation, whereas Siwek et al. [99] indicated that the integration of cover crops in rotation schemes may increase the phenolic compounds content of the succeeding crop depending on the species both of cover crop and succeeding crop.

### 3.3.2. Bioactive Properties

The evaluation of the antioxidant activity was performed with cell-based assays, namely TBARS and OxHLIA (Figure 4). In the OxHLIA assay, irrigation had no significant effect, whereas deficit irrigation resulted in the highest antioxidant activity in the TBARS assay. Conversely, crop rotation increased antioxidant activity in the OxHLIA assay, while no significant effect was observed in the TBARS assay. For the combined effect of the tested factors, the highest activity for OxHLIA and TBARS was recorded for DI × CR and C × NCR treatments, respectively. Previously, Polyzos et al. [33] mentioned that the two

methods showed a varied response in *S. hispanicus* plants grown under deficit irrigation conditions, with deficit irrigation having the highest activity for TBARS assay and control treatment for OxHLIA assay. These variations may also arise from the involvement of different types of secondary metabolites, further emphasizing the complexity of antioxidant activity assessment and the necessity to evaluate it with more than one method [100].



**Figure 4.** Antioxidant activity of *C. spinosum* L. plants in relation to irrigation and crop management (mean  $\pm$  SD), determined with OxHLIA and TBARS assays. The results are expressed as IC<sub>50</sub> values ( $\mu\text{g}/\text{mL}$ ). Different Latin letters above bars of the same color and for the same factor or their interaction are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ . DI: deficit irrigation; FI: full irrigation; NCR: no crop rotation; CR: crop rotation.

Under drought stress, plants activate both enzymatic and non-enzymatic antioxidants to mitigate the adverse effects [34,101]. For example, Gharibi et al. [93], who assessed various *Achillea* species under different irrigation regimes, observed that each species possessed unique characteristics in its antioxidant systems. Additionally, Briudes et al. [11] identified chicoric acid as the primary phenolic compound in *C. spinosum* and *C. intybus* leaves and the main contributor to antioxidant activities. In *C. intybus*, quercetin-3,4-O-diglucoside has also been found to be responsible for increased antioxidant activity [102]. Moreover, Petropoulos et al. [103] indicated a negative linear correlation between phenolic compounds content and EC<sub>50</sub> values of various antioxidant activity assays in several ecotypes of *C. spinosum* which corroborates the importance of phenolic compounds in the antioxidant system of the species. However, besides phenolic compounds and flavonoids, organic acids and tocopherols, particularly  $\alpha$ -tocopherol, are also recognized as potent antioxidant molecules [60,66,104].

Means in the same row for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan's Multiple Range test (DMRT) at  $p = 0.05$ . The results of the antibacterial properties of hydroethanolic extracts from *C. spinosum* leaves against selected food are presented in Table 10. In terms of food and clinical bacteria, the extracts exhibited moderate inhibitory activity against the studied strains depending on the extract, whereas all of the tested extracts recorded MIC and MBC values higher than the controls. In particular, extracts from plants grown under deficit irrigation conditions showed MIC values against the

food bacteria *Enterobacter cloacae*, *Escherichia coli*, *Yersinia enterocolitica*, *Bacillus cereus* and *Listeria monocytogenes*, as well as against the clinical bacteria *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (the control treatment was similarly effective, recording the same MIC value). Moreover, the lack of crop rotation resulted in extracts with the highest MIC values against the food bacteria *Escherichia coli*, *Salmonella enterica*, *Yersinia enterocolitica* and *Listeria monocytogenes*, as well as the clinical bacteria *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus*. For the combined effect of the tested factors, C × NCR treatment recorded the highest MIC values against *Listeria monocytogenes* (both food and clinical strains) and *Staphylococcus aureus* (food bacterial strains and Methicillin-resistant clinical strains); DI × CR treatment was the most effective against *Enterobacter cloacae*, while DI × CR treatment had the highest MIC values against *Escherichia coli* (both food and clinical strains), *Salmonella enterica*, *Yersinia enterocolitica*, and Methicillin-resistant *Staphylococcus aureus*. Finally, none of the tested extracts showed any antifungal, NO-production inhibition, antiproliferative, or hepatotoxic effects (Table 10). The only exception was observed in the activity against the AGS cell line, where the hydroethanolic extracts from all the treatments showed the capacity to inhibit cell proliferation, especially extracts from plants grown under deficit irrigation, no crop rotation, or the combination of no crop rotation and full irrigation.

Similarly to our study, Polyzos et al. [41] found no inhibitory effects against *Pseudomonas aeruginosa* for *C. spinosum* leaf extracts. In contrast, Petropoulos et al. [105] reported that *E. cloacae*, *Listeria monocytogenes*, and *Salmonella enterica* were the most sensitive bacterium, while they did not record any inhibitory effects against *E. coli*, which was observed in this study. Additionally, Birsa and Sarbu [102] suggested that *C. intybus* extracts possess antimicrobial and antifungal activities, although no such effects (e.g., antifungal) were recorded in our study. Furthermore, Polyzos et al. [33], who studied the antibacterial properties of *S. hispanicus* leaf extracts, also reported varied efficacy depending on the irrigation regime. In particular, they found that rain-fed plants and plants grown under deficit irrigation exhibited higher efficacy against *Listeria monocytogenes* and *Staphylococcus aureus*, a finding that was not recorded in this study. It is important to note that, apart from the plant species used, the extraction method may also influence the antimicrobial properties, since the choice of solvents affects the extracted bioactive phytochemicals and the antimicrobial properties of the obtained extract [12]. For example, the extraction solvent affected the antimicrobial and anticancer activity of *Mentha piperita* and *Catharanthus roseus* extracts, while drought stress decreased these activities due to a reduction in phenolic and flavonoid content [106]. Moreover, Petropoulos et al. [105] suggested that apart from phenolic compounds, other bioactive components may also play an important role in the antimicrobial properties of *C. spinosum*. This finding partly explains the higher antibacterial effects recorded in our study for the extracts obtained from plants grown under deficit irrigation. However, this is not consistent with the effects of the cropping system, where no crop rotation showed better results than crop rotation without significant differences in total phenolic compounds between these two treatments. Therefore, it should be noted that the growing conditions, plant species, and extraction methods may lead to changes in antimicrobial and antifungal properties due to differences in the phytochemical composition of the obtained extracts.

**Table 10.** The antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and hepatotoxic activities of the hydroethanolic extracts of *Cichorium spinosum* leaves in relation to irrigation and crop management (mean ± SD).

Treatments	Irrigation (I)						Crop Management						I × CM											
	Control (C)		Deficit Irrigation (DI)		Full Irrigation (FI)		Crop Rotation (CR)		None Crop Rotation (NCR)		C × CR		C × NCR		DI × CR		DI × NCR		FI × CR		FI × NCR			
Antioxidant activity IC <sub>50</sub> values (µg/mL) <sup>A</sup>																								
OxHLIA (Δt 60 min)	78 ± 3 a		68 ± 3 a		91 ± 3 a		45 ± 3 b		113 ± 16 a		58 ± 2 d		27 ± 2 f		49 ± 1 e		98 ± 5 c		110 ± 5 b		132 ± 9 a			
TBARS inhibition	80 ± 7 a		20 ± 2 c		56 ± 2 b		53 ± 3 a		51 ± 2 a		86 ± 4 a		18 ± 1 f		55 ± 3 d		73 ± 3 b		22 ± 1 e		57 ± 2 c			
Antibacterial activity (mg/mL) <sup>B</sup>																								
Food bacteria																								
Gram-negative bacteria																								
<i>Enterobacter cloacae</i>	10	>10	7.5	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	5	>10	10	>10	>10	>10	>10	>10	10	>10
<i>Escherichia coli</i>	5	>10	3.75	>10	10	>10	6.7	>10	5.8	>10	5	>10	5	>10	5	>10	2.5	>10	10	>10	10	>10	10	>10
<i>Pseudomonas aeruginosa</i>	10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	>10	>10	>10	>10	10	>10
<i>Salmonella enterica</i>	>10	>10	>10	>10	10	>10	>10	>10	5.58	>10	>10	>10	5	>10	>10	>10	2.5	>10	10	>10	10	>10	10	>10
<i>Yersinia enterocolitica</i>	10	>10	7.5	>10	10	>10	10	>10	8.3	>10	10	>10	10	>10	10	>10	5	>10	10	>10	10	>10	10	>10
Gram-positive bacteria																								
<i>Bacillus cereus</i>	10	>10	7.5	>10	>10	>10	>10	>10	10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	>10	>10
<i>Listeria monocytogenes</i>	5	>10	3.75	>10	10	>10	6.7	>10	5.8	>10	>10	>10	2.5	>10	10	>10	5	>10	10	>10	10	>10	10	>10
<i>Staphylococcus aureus</i>	10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	2.5	>10	10	>10	5	>10	10	>10	10	>10	10	>10
Clinical bacteria																								
Gram-negative bacteria																								
<i>Escherichia coli</i>	5	>10	3.75	>10	10	>10	6.7	>10	7.5	>10	5	>10	5	>10	5	>10	2.5	>10	10	>10	10	>10	10	>10
<i>Klebsiella pneumoniae</i>	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10
<i>Morganella morganii</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
<i>Proteus mirabilis</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
<i>Pseudomonas aeruginosa</i>	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10

Table 10. Cont.

Treatments	Irrigation (I)						Crop Management						I × CM										
	Control (C)		Deficit Irrigation (DI)		Full Irrigation (FI)		Crop Rotation (CR)		None Crop Rotation (NCR)		C × CR		C × NCR		DI × CR		DI × NCR		FI × CR		FI × NCR		
Gram-positive bacteria																							
<i>Enterococcus faecalis</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10
<i>Listeria monocytogenes</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	2.5	>10	10	>10	5	>10	10	>10	10	>10
MRSA	6.25	>10	6.25	>10	10	>10	10	>10	5	>10	10	>10	2.5	>10	10	>10	2.5	>10	10	>10	10	>10	
Antifungal activity (mg/mL) <sup>B</sup>																							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	
<i>Aspergillus brasiliensis</i>	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	
<i>Aspergillus fumigatus</i>	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	
NO-production inhibition (IC <sub>50</sub> values µg/mL) <sup>C</sup>																							
RAW 264,7	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	
Antiproliferative Activity (GI <sub>50</sub> values µg/mL) <sup>D</sup>																							
AGS	291 ± 16.04 a		221 ± 21 c		240 ± 46 b		264 ± 32 a		237 ± 42 b		289 ± 28 ab		225 ± 23 c		279 ± 26 b		293 ± 22 a		218 ± 14 c		202 ± 13 d		
Caco-2	>400	>400	>400	>400	>400	>400	>400	>400	>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	>400	>400	>400	>400	>400	>400	>400	>400	
NCI-H460	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	
Hepatotoxicity (GI <sub>50</sub> values µg/mL) <sup>D</sup>																							
PLP2	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	

For antioxidant activity results, means in the same row for the same factor (irrigation and crop management) or their interaction followed by different Latin letters are significantly different according to Duncan’s Multiple Range test (DMRT) at  $p = 0.05$ ; <sup>A</sup> Trolox IC<sub>50</sub> values: 5.8 ± 0.6 µg/mL (TBARS), 21.5 ± 0.2 µg/mL (OxHLIA 60 min); <sup>B</sup> Food bacteria—Streptomycin, 1 mg/mL (*E. cloacae*, *S. enterocolitica*, *Y. enterocolitica*, *B. cereus*, *L. monocytogenes* and *S. aureus*—MIC/MBC 0.007; *E. coli*—MIC/MBC 0.01; *P. aeruginosa*—MIC/MBC 0.06), Methicillin, 1 mg/mL (*S. aureus*—MIC/MIB 0.007), Ampicillin, 10 mg/mL (*E. cloacae*, *E. coli*, *S. enterica*, *Y. enterocolitica*, *L. monocytogenes* and *S. aureus*—MIC/MBC 0.15; —MIC/MBC 0.01; *P. aeruginosa*—MIC/MBC 0.63); clinical bacteria—Ampicillin, 10 mg/mL (*E. coli*, *P. mirabilis*, *E. faecalis*, *L. monocytogenes*, MRSA MIC/MBC—<0.15; *K. pneumoniae*, *M. morgani*, *P. aeruginosa* MIC/MBC—>10); Imipenem, 1 mg/mL (*E. coli*, *K. pneumoniae*, *M. morgani*, *P. mirabilis*, *L. monocytogenes* MIC/MBC—<0.0078; *P. aeruginosa* MIC/MBC—0.5/1); Vancomycin, 1 mg/mL (*E. faecalis* MIC/MBC—<0.0078 and MRSA MIC/MBC—0.25/0.5; antifungal activity—Ketoconazole (*A. brasiliensis* MIC/MFC 0.06/0.125, *A. fumigatus* MIC/MFC 0.5/1); MIC—Minimum Inhibitory Concentration, MBC—Minimum Bactericidal Concentration, and MFC—Minimum Fungicidal Concentration; <sup>C</sup> Dexamethasone IC<sub>50</sub> value: 6.3 ± 0.4 µg/mL; <sup>D</sup> Ellipticine GI<sub>50</sub> values: 1.23 ± 0.03 µg/mL (AGS), 1.21 ± 0.02 µg/mL (Caco-2), 1.01 ± 0.01 µg/mL (NCI-H460), 1.02 ± 0.02 µg/mL (MCF-7), and 1.4 ± 0.1 µg/mL (PLP2).

## 4. Conclusions

The results of this study indicate that *C. spinosum* can be grown under deficit irrigation conditions while being incorporated into a crop-rotation system, without compromising the biomass yield and the quality of the final product. Specifically, the fatty-acid profile was optimized, showing an increase in PUFA and a decrease in SFA, while total phenolic compounds, flavonoids, and phenolic acid content in the leaf extracts increased, thus enhancing their health-promoting properties. Moreover, leaf extracts exhibited inhibitory activity against certain bacteria, including *E. cloacae*, *E. coli*, *L. monocytogenes*, *Y. enterocolitica*, *B. cereus*, and *MRSA*, while all the extracts were moderately effective against AGS cell lines. In conclusion, based on our results, *C. spinosum* is a promising alternative/complementary crop that could be valorized in small-scale farms of the Mediterranean, thus facilitating its commercial exploitation through its integration into sustainable cropping systems. Moreover, agronomic practices such as deficit irrigation and crop rotation may positively affect the growth and quality of *C. spinosum* plants contributing towards the mitigation of climate change adverse effects on crop production, while contributing to saving water and the diversification of crops. However, further research is required to refine crop management through the diversification of crops integrated into rotation schemes and to better study the long-term effects of crop rotation both on plant growth and chemical composition, as well as on soil physicochemical properties and functional microbiome.

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