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Effect of Saline Conditions on Chemical Profile and the Bioactive Properties of Three Red-Colored Basil Cultivars

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Abstract: The present study investigated the effect of salinity (Control: 1.8 dS/m, S1: 3.0 dS/m and S2: 4.5 dS/m) on the chemical composition and bioactive properties of three basil cultivars (Red Basil, Dark Opal Red and Basilico Rosso). Crop performance was not affected by increasing salinity in DoR and BaR. Fat, ash and carbohydrates content increased by salinity in DoR, whereas energetic value was negatively affected. Free sugars (total and individual compounds) increased under saline conditions (S2) in BaR, whereas reducing trends were observed for the main organic acids and tocopherols in all the cultivars. The major fatty acids were α-linolenic, linoleic and palmitic acids with no consistent salinity effects, while the richest polyphenols were sagerinic acid and eriodictyol-*O*-malonylhexoside. Finally, basil extracts showed moderate antioxidant and strong antifungal activity. In conclusion, salinity showed a genotype dependent effect on the chemical profile and bioactivities of the tested cultivars.

Keywords: antimicrobial properties; antioxidants; *Ocimum basilicum* L.; organic acids; polyphenols; salinity stress; sweet basil; tocopherols

1. Introduction

Basil (*Ocimum basilicum* L.) is an aromatic herb of the *Lamiaceae* family and, over the past few centuries, there have been many cultivation records. Today, it still has a high commercial value for having multiple purposes, such as medicinal, nutrition, ornamental, cosmetics, religious and insecticidal or insect repellant purposes [1].

Basil is a species that, due to its genetic variability, is difficult to characterize chemically, with numerous cultivars that do not differ significantly in morphological terms but present differences from the chemical composition point of view [1,2]; leaves contain between 0.5 and 1.5% essential oil, flavonoids, phenolic acids, triterpenes, steroids, among other compounds considered nutraceuticals,

and they are also abundant in vitamins and minerals [2]. Basil leaves possess various bioactive properties such as anticarcinogenic, antioxidant, antibacterial, while it is supposed to fortify the human body immune system [3], among other properties.

Nowadays, there is a growing concern with the nutritional and bioactive quality of food sources, while, at the same time, there are increasing environmental and economic concerns regarding sustainable crop management. This paradigm forced us to find solutions that are both profitable, sustainable, ecological and nutritionally advantageous in a clearly expanding market through the use of simple and cost effective cultivation practices and the valorization of the existing genetic diversity that may increase the quality of horticultural products.

It is widely recognized that agronomic conditions at pre-harvest level may affect the crop, both in quantitative and qualitative terms. These conditions range from the choice of genotype, the climatic conditions and the growing location, the cultivation season, the agronomic techniques during cultivation and their interactions [4]. It is also agreed that "genetic improvement" through breeding alters the expression of certain genes that in turn alter the chemical and bioactive profile of the plant, hence the importance of genotype selection [5]. In this sense, it is also known that there is a growing need for soil, not only for agriculture but for all human activities, which in its turn pushes the agriculture to inferior and degraded soils that contain high amounts of salts resulting in salinity stress; soil salinization is already one of the biggest threats to world agriculture as the result of irrational cultivation and the non-compliance to sustainability principles [6].

Due to the edaphoclimatic changes that are happening in the world, it becomes more and more pertinent to understand which plants adapt to the new reality, in particular, the increase in soil salinity, without losing their nutritional/organoleptic/nutraceutical quality and/or therapeutic potential, while at the same time crop performance remains profitable. It is well established that environmental stressors, such as drought and salinity, are associated with significant changes in plant growth and the biosynthetic pathways of several secondary metabolites in basil—e.g., phenolic compounds [7], essential oils [8], photosynthetic pigments [9] and antioxidant activity [10,11]. Moreover, Babalar et al. [12] suggested a significant effect of salinity on phenolic acids and anthocyanins content of basil leaves after storage, while Scagel et al. [7] reported a significant increase in quercetin-rutinoside and rosmarinic acid and a decrease in rosmarinic acid in basil plants grown under saline conditions. The study of Omer et al. [13] showed that saline conditions may affect not only crop performance and essential oil yield and composition, but also the total carbohydrates, total flavonoids and proline content of basil leaves. In addition, Bernstein et al. [13] reported that among the observed changes in basil leaves saline conditions induced essential oil production and carotenoids biosynthesis. According to Akbari et al. [14] there is a great genetic diversity in basil crop which may result in variable response to environmental stressors, especially in regard to crop performance. Therefore, it is worth investigating basil genotypes to identify tolerant or resistant genotypes to abiotic stress that could be used for valorizing degraded soils.

Several works have examined the impact of salinity stress on plant growth and essential oil yield and volatile compounds composition. However, limited literature exists regarding the impact of environmental stressors on phytochemicals profile. Considering the above, the present study aims to examine the effect of different salinity levels on the nutritional value and chemical composition of three red-colored basil cultivars, namely Red Basil, Dark Opal Red and Basilico Rosso. Finally, the bioactive properties (antioxidant activity and antimicrobial properties) of hydroethanolic extracts were assessed.

2. Materials and Methods

2.1. Samples and Samples Preparation

Seeds from three colored basil cultivars (*Ocimum basilicum* L.) were used, namely Red Basil (Geniki Fytotechniki S.A.; Athens, Greece), Dark Opal (De Corato Sementi; Andria, Italy) and Basilico Rosso (Larosa Emanuele Sementi; Andria, Italy). Seeds of basil were sown in seed trays containing peat

on 4 April 2019 and seedlings were transplanted in 2 L plastic pots containing peat and perlite (1:1, *v/v*) on 23 April 2019. Three salinity treatments were applied, namely Control (1.8 dS/m, no salt addition), 3.0 dS/m and 4.5 dS/m. The saline solutions were prepared with the addition of the adequate amounts NaCl in the Control solution until the desired electrical conductivity was achieved. All plants received fertigation with nutrient solution containing 200 ppm of Nitrogen, Phosphorus and Potassium with the application of 20–20–20 (N–P–K) fertilizer control treatment until plant establishment (approximately 3 weeks after transplantation) [15]. After that, plants received the salinity treatments which resulted after the addition of the required amount of NaCl to the control treatment. For each treatment, 15 pots were used with one plant per pot (45 pots in total).

Harvest took place on 14 June 2019 and just before anthesis initiation. Hunter color parameters (L*, a* and b*) were measured on the blades of the upper surface of leaves using a chroma meter (Chroma Meter CR400, Konica Minolta, Tokyo, Japan) [16]. Chroma (C*: relative saturation) and hue angle values (h°) were calculated according to the formulas previously described in the literature [17] following CIELab color space readings (L, a and b values) that were measured through the computerized system.

After harvest, plants were weighed and samples of fresh leaves were freeze-dried, ground to powder and stored at deep-freezing conditions until further analysis.

2.2. Nutritional Value and Energy

According to the AOAC methods, the proximate composition (ash, protein, fat, carbohydrates, and energy) was determined in the lyophilized material and expressed in g/100 g of dry weight (dw). Crude protein was estimated by macro-Kjeldahl method (N × 6.25) using an automatic distillation and titration unit (model Pro-Nitro-A, JP Selecta, Barcelona), and the crude fat by Soxhlet extraction with petroleum ether throughout 7 h. Incineration at 550 ± 10 °C was the technique applied to determine ash batch [5]. Total carbohydrates were determined by difference and the energetic value was calculated according to the Regulation (EC) No. 1169/2011 of The European Parliament and of the Council as follows: energy (kcal/100 g dried weight (dw)) = 4 × (weight of protein (g)+ weight of carbohydrates (g)) + 9 × (weight of fat (g)).

2.3. Chemical Characterization

Free sugars. Free sugars were estimated according to a formerly described procedure by Spréa et al. [18] and were analyzed in a high-performance liquid chromatography (HPLC) system coupled to a refractive index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany), using the internal standard (IS, melezitose, Sigma-Aldrich, St Louis, MO, USA). Data were recorded and processed using Clarity 2.4 software and the results were expressed as g per 100 g of dry weight (dw).

Tocopherols. The extraction of tocopherols from the lyophilized material was carried out following the procedure described by Spréa et al. [18]. The analysis was achieved by HPLC and a fluorescence detector (HPLC-FL; Knauer, Smartline system 1000, Berlin, Germany) as described by the authors. The compounds were acknowledged via chromatographic assessments with authentic standards and the quantification was grounded on the fluorescence signal response of each standard, using the IS (tocol, Matreya, Pleasant Gap, PA, USA) method and using calibration curves gotten from commercial standards of each compound. Data were recorded and processed using Clarity 2.4 software and the results were given as mg per 100 g of dw.

Organic acids. Organic acids were analyzed by ultra-fast liquid chromatography coupled to a diode-array detector (UFLC-DAD; Shimadzu Corporation, Kyoto, Japan) operating in the optimized conditions described in detail by Pereira et al. [19]. Identification of the compounds was done by comparing the spectra and the retention time of the standards, and quantification was performed based on calibration curves, linking the peaks of the recognized compounds and the standards. The results were recorded and processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan). Data were recorded and processed using LabSolutions Multi LC-PDA software and the results were given as g per 100 g of dw.

Fatty acids. Fatty acid methyl esters (FAME) was explored next to trans-esterification of the lipid fraction attained through Soxhlet extraction as previously described by Spréa et al. [18] and determined by gas-liquid chromatography with flame ionization detection, using a YOUNG IN Crhomass 6500 GC System instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Zebron-Fame column. Fatty acids identification and quantification was achieved by relating the comparative retention times of FAME peaks from samples with standards (reference standard mixture 37 (47885-U), Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using the Software Clarity DataApex 4.0 Software (Prague, Czech Republic) and stated in relative percentage of each fatty acid.

2.4. Preparation of Hydroethanolic Extracts

Extracts Preparation

To prepare the hydroethanolic extracts, each sample (2.5 g) was mixed with ethanol/water solution (80:20, *v/v*; 30 mL) and stirred for 1 h at room temperature. After filtering the supernatant through Whatman filter paper No 4 (Sigma-Aldrich, St Louis, MO, USA), the residue was re-extracted under the same conditions and the combined filtrates were concentrated under reduced pressure (rotary evaporator) at 40 °C and subsequently lyophilized. The freeze drying of the plant biomass was carried out by using a stainless steel pilot scale Sublimator model EKS (Christian Zirbus Co, Germany). The equipment has a 3.5 m^2 surface allocated to 7 shelves and it is furthermore equipped with a cooling trap operating at −45 °C and having a capacity of 20 Kg of water per freeze drying cycle and with a dual stage vacuum pump.

The applied lyophilization program for drying of the plant biomass was the following:

Step 1 −35 °C for 2 h at atmospheric pressure (1000 mbar);
Step 2 From −35 °C to −20 °C in 6 h under vacuum (0.150 mbar);
Step 3 From −20 °C to 0 °C in 12 h under vacuum (0.150 mbar);
Step 4 From 0 °C to 10 °C in 12 h under vacuum (0.150 mbar);
Step 5 From 10 °C to 25 °C in 12 h under vacuum (0.150 mbar).

2.5. Analysis of Phenolic Compounds

Hydroethanolic extracts prepared above, were redissolved in ethanol/water (80:20, *v/v*), to a final concentration of 10 mg/mL. The resulting extracts were after assessed through high performance liquid chromatography coupled with a diode-array and mass spectrometer detector (HPLC-DAD-ESI-MS/MS) working below the settings systematically defined by Bessada et al. [5]. Phenolic compounds identification was made through comparison of their retention times, UV–Vis and mass spectra with existing standard compounds and by comparing the collected information with available data described in the literature, giving a tentative identification of the spotted compounds. For quantitative analysis, a calibration curve for each available phenolic compound standard was created based on the UV signal, whereas when no commercial standards were available, the quantification was done through the calibration curve of the most similar available standard [5]. The results were given as mg per g of extract.

2.6. Evaluation of Bioactive Properties

2.6.1. Antioxidant Activity

Two cell-based assays were performed to measure the in vitro antioxidant activity of the extracts. Lipid peroxidation inhibition in porcine (Sus scrofa) brain homogenates was evaluated by the decline in thiobarbituric acid reactive substances (TBARS) and the color concentration of malondialdehyde–thiobarbituric acid (MDA–TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the formula: [(A−B)/A] × 100%, where A and B

were the absorbance of the control and the sample solutions, respectively [5]. The results were expressed as delayed time of haemolysis (Δt), calculated as follows: Δt (min) = Ht50 (sample) − Ht50 (control), where Ht50 is the 50% haemolysis time (min) graphically obtained from the haemolysis curve of each sample concentration. The inhibitory concentrations of the extract able to promote a Δt haemolysis delay of 60 min and 120 min were calculated (IC50 values μg/mL) and translate the extract concentration required to keep 50% of the erythrocyte population intact.

2.6.2. Antimicrobial Properties

For the determination of the antimicrobial properties of the extracts, the microdilution method was used [20]. The antibacterial properties were tested against the Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* (food isolate), *Listeria monocytogenes* NCTC 7973, as well as the following Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Salmonella enterica* serovar Typhimurium ATCC 13311 and *Enterobacter cloacae* ATCC 35030 were used. For antifungal assays, six micromycetes were used: *Aspergillus fumigatus* ATCC 9197, *A. niger* ATCC 6275, *A. versicolor* ATCC 11730, *Penicillium funiculosum* ATCC 36839, *Trichoderma viride* IAM 5061 and *P. verrucosum* var. *cyclopium* (food isolate). The minimum extract concentrations that completely inhibited bacterial growth (MICs) were determined by a colorimetric microbial viability assay, and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were also calculated. E211 (sodium benzoate) and E 224 (potassium metabisulphite) were used as positive controls and 5% DMSO were used as a negative control.

2.7. Statistical Analysis

The experiment was carried out according to the randomized compete block (RCB) with three repetitions. Crop performance and color parameters were evaluated in fifteen individual plants (n = 15), whereas for chemical analysis assays, three batch samples were prepared for each treatment and each assay was performed in triplicate (n = 3). All the data were subjected to two-way ANOVA considering as factors the cultivars and the salinity treatments, while means were compared according to Tukey's HSD test (p = 0.05). All the analyses were performed with the statistical package SPSS v. 23.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Crop Performance and Color Parameters

The results of crop performance of the tested cultivars in relation to the salinity treatment are presented in Table 1. The tested cultivars differed significantly in the fresh weight parameter, where Red Basil had the highest fresh weight for all the tested salinity levels, except for the highest level (S2), where it did not differ significantly from the Dark Opal Red cultivar. On the other hand, Dark Opal Red and Basilico Rosso cultivars were not affected by increasing salinity. In contrast, plants of Red Basil exhibited a significant decrease in fresh weight of leaves when the highest level of salinity (4.5 dS/m) was applied. These results are in agreement with literature reports where a variable response to salinity was observed when various basil cultivars were tested under the same conditions [21]. Moreover, Bione et al. [22] reported a linear decrease in basil fresh weight (leaves) with increasing salinity, which was not observed in our study. This contradictory results could be due to the fact that Bione et al. [22] used a control treatment with very low salinity (0.29 dS/m) and a broader range of salinity treatments than our study (1.45 dS/m to 8.43 dS/m).

Table 1. Crop performance (fresh weigh per plant, g/plant), color parameters (*L*, *a*, *b*, *Chroma* and *Hue angle*, mean ± SD; $n = 15$), nutritional (g/100 g dw) and energetic value (kcal/100 g dw) of the tested basil cultivars in relation to salinity level (mean ± SD; $n = 3$).

		Crop Performance	Color Parameters					Nutritional Value				
Cultivar	**Treatment ***	**Fresh Weight**	**L**	**a**	**b**	**Chroma (C)**	**Hue Angle (H)**	**Fat**	**Proteins**	**Ash**	**Carbohydrates**	**Energy**
Dark Opal Red	Control	26 ± 3^{Ba}	28 ± 2^{Bc}	-0.9 ± 0.3^{Ba}	4.0 ± 0.7^{Bc}	4.2 ± 0.9^{Bc}	98 ± 4^{Ab}	1.70 ± 0.04^{b}	46.4 ± 0.2^{d}	12.4 ± 0.5^{cd}	39.5 ± 0.6^{c}	359 ± 1^{a}
	S1	26 ± 2^{Ba}	33 ± 1^{Bb}	-5 ± 2^{Bb}	10 ± 1^{Bb}	11 ± 2^{Bb}	114 ± 18^{Aa}	2.08 ± 0.04^{a}	41.2 ± 0.2^{e}	15.5 ± 0.2^{a}	41.22 ± 0.02^{b}	348.4 ± 0.4^{c}
	S2	27 ± 3^{Aa}	34 ± 1^{Aa}	-6 ± 1^{Cc}	11 ± 1^{Aa}	12 ± 1^{Aa}	120 ± 3^{Aa}	2.10 ± 0.03^{a}	38.4 ± 0.3^{f}	15.2 ± 0.1^{a}	44.3 ± 0.2^{a}	349.7 ± 0.4^{c}
Red Basil	Control	30 ± 3^{Aa}	31 ± 2^{Ac}	-3.5 ± 1^{Ca}	8 ± 4^{Ac}	8.8 ± 0.4^{Ac}	103 ± 14^{Ab}	1.38 ± 0.02^{e}	52.2 ± 0.6^{a}	11.8 ± 0.3^{e}	34.6 ± 0.6^{f}	359.7 ± 0.9^{a}
	S1	29 ± 2^{Aa}	34.4 ± 0.5^{Aa}	-7 ± 1^{Cb}	11 ± 2^{Aa}	13 ± 2^{Aa}	118 ± 10^{Aa}	1.57 ± 0.02^{d}	50.9 ± 0.8^{b}	13.1 ± 0.1^{b}	34.4 ± 0.5^{f}	355.3 ± 0.1^{b}
	S2	2 ± 3^{Ab}	33 ± 1^{Bb}	-4 ± 2^{Ba}	9 ± 2^{Ab}	10.1 ± 0.6^{Bb}	110 ± 6^{Bab}	1.55 ± 0.02^{d}	49.2 ± 0.8^{c}	12.8 ± 0.2^{bc}	36.5 ± 0.4^{d}	356.7 ± 0.5^{b}
Basilico Rosso	Control	22.4 ± 0.8^{Ca}	25.0 ± 0.7^{Cc}	1.1 ± 0.3^{Aa}	1.4 ± 0.6^{Cc}	1.8 ± 0.4^{Cc}	50 ± 18^{Cc}	1.59 ± 0.08^{cd}	51.10 ± 0.07^{ab}	12.2 ± 0.1^{de}	35.2 ± 0.2^{ef}	359.3 ± 0.1^{a}
	S1	18 ± 2^{Cb}	28 ± 2^{Cb}	-0.5 ± 0.7^{Ab}	5 ± 1^{Cb}	5 ± 1^{Cb}	86 ± 13^{Bb}	1.67 ± 0.04^{bc}	49 ± 1^{c}	13.2 ± 0.2^{b}	36.4 ± 0.8^{de}	355.7 ± 0.7^{b}
	S2	22 ± 2^{Ba}	31 ± 2^{Ca}	-2.2 ± 0.8^{Ac}	7 ± 1^{Ba}	8 ± 2^{Ca}	104 ± 11^{Ba}	1.70 ± 0.01^{b}	46.5 ± 0.2^{d}	12.9 ± 0.1^{b}	38.9 ± 0.2^{c}	356.9 ± 0.3^{b}

* Treatments: Control: 1.8 dS/m, S1: 3.0 dS/m and S2: 4.5 dS/m. Different capital Latin letters in the same column indicate significant differences between the tested cultivars for the same salinity treatment, whereas small Latin letters in the same column indicate significant differences between the tested salinity treatments for the same cultivar, according to Tukey's HSD test ($p = 0.05$). L: lightness from black (0) to white (100); a: green (−) to red (+), b: blue (−) to yellow (+); Chroma (C): chroma, relative saturation; Hue angle (H): angle of the hue in the CIELab colour space.

Regarding color parameters, lightness (*L*) increased when plants were subjected to salinity treatments compared to the control treatment in all the tested cultivars, while significant differences were observed among the cultivars with Basilico Rosso, presenting the lowest *L* values (Table 1). Parameter *a* decreased with increasing salinity in Red Opal Red and Basilico Rosso, whereas fluctuating trends were observed for the Red Basil cultivar. This result indicates that leaves of Dark Opal Red and Basilico Rosso plants subjected to salinity were less red than the control treatment, whereas fluctuating trends were observed for Red Basil plants. The comparison between the cultivars showed that Basilico Rosso had the highest *a* values compared to the other two cultivars, meaning that the red color of the leaves of this specific cultivar was more intense than the other two. Similar trends were observed for Chroma (*C*) and Hue angle (*H*) values which increased with increased salinity, while the lowest values for both parameters were recorded in Basilico Rosso plants. Similar to our study, Elhindi [23] reported that salinity may affect foliage color of herbs. In contrast, Scagel et al. [7] reported no significant effects of salinity on leaf color probably because they tested only green-colored cultivars.

3.2. Chemical Composition

For all the tested parameters the two-way ANOVA revealed a significant interaction for the factors in study. Therefore, all the possible combinations of treatments were compared with each other and the results are presented in the corresponding tables.

Nutritional and energetic value results are presented in Table 1. Fat content ranged between 1.38 and 2.10 g/100 g dw for the control treatment in Red Basil and the S2 treatment of Dark Opal Red, respectively. In general, the increased salinity resulted in an increase in fat content for all the cultivars, while the highest fat amounts were recorded in Dark Opal Red. Similarly, ash content values where within the range of 12.2 (Basilico Rosso; Control treatment) and 15.5 g/100 g dw (Dark Opal Red; S1 treatment), while increasing trends were observed for all the cultivars when salinity increased especially in the Dark Opal Red cultivar where the highest increase was recorded (25.0 and 22.5% for the S1 and S2 treatments, respectively). Proteins content ranged between 38.4 (Dark Opal Red; S2 treatment) and 52.2 g/100 g dw (Red Basil; Control treatment), while in contrast to fat and ash a significant decrease was recorded when salinity increased (up to 17% in Dark Opal Red cultivar). Carbohydrates content was also affected by both factors and values ranged between 35.2 (Basilico Rosso; Control treatment) and 44.3 g/100 g dw (Dark Opal Red; S2 treatment), while increasing salinity resulted in a significant increased content of carbohydrates (up to 12.1% for Dark Opal Red). Regarding the energetic value, the highest energetic value was recorded in the control treatment for all the tested cultivars, while the increase in salinity resulted in concomitant reduction in energetic value. Similarly to our study, Kaur et al. [24] suggested protein and carbohydrates contents within the same range, whereas fat and protein content was higher and lower than that of our study. Moreover, Ghoora et al. [25] reported a protein content in French basil micro-greens within the same range (38 g/100 g dw) although ash content was significantly higher than that of our study (22.7 g/100 g dw). Other studies where different *Ocimum* species (*O. viride* and *O. gratissimum*) or different basil cultivars were studied reported a different nutritional profile compared to our study [26–29], while Naiji and Souri [30] who tested the effect of the fertilization regime (chemical fertilization vs. organic cultivation) on sweet basil leaves also suggested a different nutritional composition. These results indicate a significant effect of the genotype and the cultivation practices on the nutritional status of basil, while according to Ribas et al. [31], the differences among the various studies could be attributed to the presence of branches within the samples. Regarding the salinity effect, according to the literature, salt stress induces the accumulation of various osmolytes, such as carbohydrates, for the protection of cytoplasm and cellular structures and the retention of water absorption, as was also observed in our study [9].

Free sugars composition is presented in Table 2 where only three compounds were identified. Glucose was the main sugar in most of the cases, followed by sucrose and fructose. The same sugar profile was reported by Carocho et al. [32], whereas Fernandes et al. [33] additionally detected the presence of trehalose in similar amounts to sucrose and fructose. Basilico Rosso contained higher

amounts of total and individual free sugars that the other two cultivars, especially in the S2 treatment where the highest overall values were recorded. It is interesting to highlight that total and individual free sugars increased with increasing salinity for all the tested cultivars, except for the case of Dark Opal Red where a significant reduction in glucse was recorded. This variable response of the tested cultivars to salinity treatments could be associated with the results of fresh weight per plant (see Table 1) where Dark Opal Red fresh weight slightly increased at high salinity (S2 treatment). Considering that free sugars are considered the main osmolytes that plants use to increase cytoplasm potential as a protective mechanism against salinity stress [9], the findings of our study indicate a possible higher tolerance to salinity for the Dark Opal Red cultivar compared to the other two cultivars. Salinity stress is associated with the induction of essential oil biosynthetic pathways in various aromatic plants [34], with sugars being considered as the main precursors, especially sucrose [35]. Moreover, abiotic stressors, such as water deficit or high salinity, may induce sugars accumulation as a means to mitigate negative effects on cellular metabolism [9,36].

Tocopherols' profile is presented in Table 2. Three compounds were detected, namely α-, γ- and δ-tocopherol with the former being the most abundant in all the tested samples. The results of our study are in contrast with the study of Fernandes et al. [33] who not only detected all tocopherol isomers but also suggested γ-tocopherol as the most abundant compound, whereas Inoue et al. [37] detected only α-, β- and γ-tocopherol These contradictory results could be assigned to the fact that different genotypes were tested in the referred studies. The highest contents of γ- and δ-tocopherols were detected in the control treatment of Dark Opal Red, while α-tocopherol was most abundant in Red basil for the same treatment. Regarding the salinity effect, increased salinity resulted in a decrease in total and individual tocopherols in all the cultivars, which is in contrast with the study of Tarchoune et al. [29] who reported the increase in tocopherols in basil leaves as part of the non-enzymatic detoxification mechanism of plants. However, the same authors reported a variable response depending on the cultivar (cv. "Genovese" and "Fine"), the duration of stress (15 or 30 days) and the type of salt (NaCl or Na_2SO_4) responsible which could partly justify the different findings compared to our study. Another explanation for this difference could be the contribution of other bioactive compounds such as polyphenols and organic acids in plant protection against oxidative stress [38], or the fact that the plants in our study were harvested at flower initiation where according to Petropoulos et al. [39] developmental stage may have an impact on tocopherols composition.

The composition of organic acids is presented in Table 2 with quinic acid being the main organic acid followed by oxalic and shikimic acids, while ascorbic acid was only detected in traces. Quinic acid was reported being the abundant organic acid by Fernandes et al. [33] who studied the chemical composition of red rubin basil leaves (*Ocimum basilicum* var. *purpurascens*), while they also identified oxalic, malic, citric, shikimic, fumaric and ascorbic acid. The same main organic acids were also suggested by Carocho et al. [32] who also identified malic and citric acid in lower amounts. The highest contents of oxalic, quinic and total organic acids were recorded for the control treatment of Basilico Rosso cultivar, while for all the tested cultivars increasing salinity resulted in a decrease in the main organic acids (quinic and oxalic acid) as well as of total organic acids content. The same trends were reported by Petropoulos et al. [36,40] and Carvalho et al. [41] in other leafy vegetables, who also observed a decrease in organic acids with increasing salinity. Moreover, considering the results for sugars and tocopherols content, it seems that the main protective non-enzymatic mechanism of basil against salinity stress is mostly related with sugars accumulation which are the main osmolytes, while tocopherols and organic acids have a less profound role.

Table 2. Free sugars (g/100 g dw), organic acids (g/100 g dw), main fatty acids (%), fatty acid groups (%), and composition in tocopherols (mg/100 g dw) identified in the tested basil cultivars in relation to salinity level (mean ± SD, n = 3).

		Free Sugars				Organic Acids				
Cultivar	**Treatment**	**Fructose**	**Glucose**	**Sucrose**	**Total Free Sugars**	**Oxalic Acid**	**Quinic Acid**	**Shikimic Acid**	**Ascorbic Acid**	**Total Organic Acids**
Dark Opal Red	Control	0.93 ± 0.03 ^{e}	2.59 ± 0.02 ^{h}	0.95 ± 0.01 ^{h}	4.47 ± 0.01 ^{i}	5.23 ± 0.08 ^{c}	9.87 ± 0.03 ^{c}	0.090 ± 0.001 ^{f}	tr	15.2 ± 0.1 ^{c}
	S1	1.65 ± 0.01 ^{bc}	2.80 ± 0.01 ^{f}	1.73 ± 0.04 ^{e}	6.17 ± 0.03 ^{e}	4.08 ± 0.01 ^{g}	8.19 ± 0.08 ^{f}	0.100 ± 0.001 ^{e}	tr	12.38 ± 0.08 ^{h}
	S2	1.69 ± 0.01 ^{b}	1.35 ± 0.06 ^{i}	1.79 ± 0.02 ^{d}	4.84 ± 0.08 ^{h}	4.81 ± 0.02 ^{f}	8.11 ± 0.03 ^{f}	0.180 ± 0.002 ^{a}	tr	13.11 ± 0.01 ^{g}
Red Basil	Control	1.52 ± 0.04 ^{d}	2.67 ± 0.02 ^{g}	0.88 ± 0.02 ^{i}	5.08 ± 0.01 ^{g}	5.49 ± 0.03 ^{b}	10.5 ± 0.2 ^{b}	0.110 ± 0.001 ^{d}	tr	16.0 ± 0.2 ^{b}
	S1	1.61 ± 0.04 ^{c}	2.90 ± 0.01 ^{e}	1.11 ± 0.01 ^{g}	5.63 ± 0.05 ^{f}	4.97 ± 0.03 ^{e}	8.77 ± 0.05 ^{e}	0.100 ± 0.001 ^{e}	tr	13.85 ± 0.02 ^{f}
	S2	1.92 ± 0.01 ^{a}	3.15 ± 0.01 ^{d}	1.57 ± 0.03 ^{f}	6.64 ± 0.02 c	4.97 ± 0.02 ^{e}	7.4 ± 0.1 ^{g}	0.130 ± 0.001 ^{b}	tr	12.5 ± 0.1 ^{h}
Basilico Rosso	Control	0.52 ± 0.04 ^{f}	3.71 ± 0.02 ^{c}	2.09 ± 0.04 ^{c}	6.32 ± 0.03 ^{d}	5.80 ± 0.05 ^{a}	11.97 ± 0.01 ^{a}	0.120 ± 0.002 ^{c}	tr	17.89 ± 0.06 ^{a}
	S1	1.61 ± 0.02 ^{c}	3.76 ± 0.01 ^{b}	2.30 ± 0.01 ^{b}	7.67 ± 0.02 ^{b}	5.09 ± 0.07 ^{d}	9.71 ± 0.05 ^{c}	0.110 ± 0.001 ^{d}	tr	14.90 ± 0.03 ^{d}
	S2	1.94 ± 0.02 ^{a}	3.97 ± 0.03 ^{a}	2.48 ± 0.03 ^{a}	8.39 ± 0.04 ^{a}	5.17 ± 0.06 ^{cd}	9.33 ± 0.09 ^{d}	0.090 ± 0.001 ^{f}	tr	14.60 ± 0.04 ^{e}
		Main Fatty Acids			**Fatty Acid Groups**			**Tocopherols**			
Cultivar	**Treatment ***	**C16:0**	**C18:2n6c**	**C18:3n3**	**SFA**	**MUFA**	**PUFA**	**α-Tocopherol**	**γ-Tocopherol**	**δ-Tocopherol**	**Total Tocopherols**
Dark Opal Red	Control	21.17 ± 0.01 ^{f}	14.94 ± 0.02 ^{e}	42.14 ± 0.05 ^{f}	33.65 ± 0.05 ^{c}	8.46 ± 0.01 ^{a}	57.91 ± 0.06 ^{f}	6.11 ± 0.04 ^{b}	1.35 ± 0.04 ^{a}	1.15 ± 0.09 ^{a}	8.61 ± 0.08 ^{b}
	S1	21.8 ± 0.4 ^{e}	18.29 ± 0.02 ^{b}	40.5 ± 0.3 ^{g}	32.8 ± 0.5 ^{d}	7.8 ± 0.3 ^{c}	59.3 ± 0.3 ^{d}	3.49 ± 0.05 ^{f}	1.04 ± 0.01 ^{c}	0.49 ± 0.02 ^{cd}	5.02 ± 0.04 ^{d}
	S2	20.9 ± 0.2 ^{g}	18.72 ± 0.03 ^{a}	44.0 ± 0.1 ^{d}	29.1 ± 0.2 ^{f}	7.7 ± 0.1 ^{c}	63.2 ± 0.1 ^{b}	3.84 ± 0.05 ^{e}	1.07 ± 0.04	0.40 ± 0.01 ^{ef}	5.32 ± 0.08 ^{c}
Red Basil	Control	19.6 ± 0.1 ^{i}	14.4 ± 0.3 ^{f}	49.2 ± 0.1 ^{a}	28.7 ± 0.2 ^{f}	7.3 ± 0.1 ^{d}	64.1 ± 0.2 ^{a}	7.07 ± 0.02 ^{a}	1.12 ± 0.01 ^{b}	1.12 ± 0.05 ^{a}	9.31 ± 0.02 ^{a}
	S1	22.85 ± 0.01 ^{c}	15.3 ± 0.1 ^{d}	42.2 ± 0.1 ^{f}	33.8 ± 0.1 ^{c}	8.2 ± 0.1 ^{b}	58.0 ± 0.1 ^{f}	4.11 ± 0.02 ^{c}	0.83 ± 0.02 ^{d}	0.43 ± 0.02 ^{de}	5.37 ± 0.02 ^{c}
	S2	22.07 ± 0.33 ^{d}	16.3 ± 0.3 ^{c}	42.4 ± 0.1 ^{e}	32.4 ± 0.4 ^{d}	8.2 ± 0.1 ^{b}	59.4 ± 0.4 ^{d}	3.98 ± 0.07 ^{d}	0.77 ± 0.01 ^{e}	0.35 ± 0.01 ^{f}	5.10 ± 0.06 ^{d}
Basilico Rosso	Control	19.84 ± 0.01 ^{h}	12.6 ± 0.1 ^{g}	49.2 ± 0.1 ^{a}	29.6 ± 0.1 ^{e}	7.8 ± 0.1 ^{c}	62.6 ± 0.1 ^{c}	1.29 ± 0.02 ^{g}	0.49 ± 0.04 ^{f}	0.90 ± 0.02 ^{b}	2.68 ± 0.03 ^{e}
	S1	30.16 ± 0.06 ^{a}	10.3 ± 0.1 ^{i}	47.3 ± 0.1 ^{c}	36.5 ± 0.1 ^{a}	5.4 ± 0.1 ^{f}	58.1 ± 0.1 ^{f}	1.07 ± 0.01 ^{h}	0.47 ± 0.01 ^{f}	0.53 ± 0.02 ^{c}	2.07 ± 0.02 ^{f}
	S2	29.86 ± 0.01 ^{b}	10.67 ± 0.04 ^{h}	47.59 ± 0.06 ^{b}	35.7 ± 0.1 ^{b}	5.7 ± 0.1 ^{e}	58.7 ± 0.1 ^{e}	0.75 ± 0.01 ^{i}	0.41 ± 0.01 ^{g}	0.38 ± 0.01 ^{ef}	1.54 ± 0.01 ^{g}

* Treatments: Control: 1.8 dS/m, S1: 3.0 dS/m and S2: 4.5 dS/m. tr—traces; C16:0—palmitic acid; C18:2n6c—linoleic acid; C18:3n3—alpha-linolenic acid; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; different Latin letters in the same column indicate significant differences according to Tukey's HSD test (p = 0.05).

Eighteen individual fatty acids were detected in all the studied samples (Supplementary Material: Table S1), while the most abundant ones were α-linolenic, linoleic and palmitic acids (Table 2). Moreover, polyunsaturated fatty acids (PUFA) were the prevalent class of fatty acids, followed by saturated and monounsaturated fatty acids (SFA and MUFA, respectively). A similar fatty acids profile was reported for red rubin basil by Fernandes et al. [33] not only for individual compounds but also for fatty acid classes, while Ababutain [42] and Suanarunsawat et al. [43] identified the same compounds in sweet basil and holy basil (*O. sanctum*) leaf extracts, respectively. In contrast, Jensen et al. [44] who tested the effect of the quality of supplemental light on basil physiology reported a different profile with palmitic and oleic acids accounting for approximately 92% of total fatty acids. The effect of salinity on the main compounds content varied among the cultivars, although Tarchoune et al. [45] suggested that fatty acids composition remained unaltered when plants were subjected to high salinity either by applying 25 mM of Na2SO4 or 50 mM of NaCl. Moreover, the highest amounts of PUFA in the control treatment of Red Basil should be associated with the results of tocopherols (see Table 2), since the role of tocopherols against lipid peroxidation is well confirmed [38]. In contrast, Sgherri et al. [46] reported the low contribution of tocopherols in lipophilic antioxidant activity of basil extracts although they suggested that the contents of single antioxidants do not reflect the overall antioxidant activity due to synergistic and redox reactions among the various bioactive molecules.

The results regarding phenolic compounds identification and quantification are presented in Tables 3 and 4, respectively. Six individual compounds were detected in all the tested samples, namely four phenolic compounds and two *O*-glycosylated flavonoids (quercetin and eriodictyol derivatives). The most profound polyphenols were phenolic acids, which accounted for 63.0–83.4% of total phenolic compounds. Similar results were reported by Majdi et al. [47] and Fernandes et al. [33] although the profile of individual compounds differed from that of our study. Literature reports suggest various profiles of phenolic compounds in basil leaves, suggesting several factors that may affect chemical composition, including the nitrogen fertilization regime [48], the extraction method and genotype [47] or the inoculation with mycorrhizal fungi [49].

Table 3. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ max), mass spectral data and tentative identification of the phenolic compounds present in the hydroethanolic extracts of the tested basil cultivars.

Peak	Rt (min)	λmax (nm)	[M − H]− (*m*/*z*)	MS^2 (*m*/*z*)	Tentative Identification
1	8.91	323	179	135(100)	Caffeic acid
2	14.96	323	473	313(61),293(100)	Chicoric acid
3	16.8	334	609	301(100)	Quercetin-*O*-deoxyhexoside-hexoside
4	19.5	290/325	535	491(100),287(34)	Eriodictyol-*O*-malonylhexoside
5	20.76	282/327	719	359(100),197(31),179(42),161(50),135(5)	Sagerinic acid
6	35.36	287/333	313	269(51),203(12),179(5),161(100),135(5)	Salvianolic acid F

The quantification of individual compounds showed a variable profile in the studied samples with sagerinic acid and eriodictyol-*O*-malonylhexoside being the most abundant polyphenols, followed by salvianolic acid F and chicoric acid. In contrast to most of the literature reports, rosmarinic acid was not detected in our study, while eriodictyol-*O*-malonylhexoside and chicoric acid were also identified by Fernandes et al. [33]. The highest amounts of total phenolic compounds, total flavonoids and total phenolic acids were recorded in the S2 treatment of Red Basil due to the highest content of sagerinic acid and eriodictyol-*O*-malonylhexoside, whereas the control treatment of Basilico Rosso contained the lowest amounts of individual and total polyphenols. Moreover, the highest increase in individual and total phenolic compounds was recorded in Basilico Rosso cultivar, and this finding could be attributed to the concomitant increase in reducing sugars content, which are associated with phenolic compound biosynthesis [50].

Table 4. Quantification (mg/g of extract) of the phenolic compounds (total phenolic acids, total flavonoids and total phenolic compounds) present in the hydroethanolic extracts of leaves of the tested basil cultivars in relation to salinity level (mean ± SD, n = 3).

		Peak Number *								
	Treatment *	**1**	**2**	**3**	**4**	**5**	**6**	**TPA**	**TF**	**TPC**
Dark Opal Red	Control	1.54 ± 0.04 ^{d}	3.52 ± 0.04 ^{a}	0.34 ± 0.01 ^{g}	8.5 ± 0.2 ^{b}	10.3 ± 0.3 ^{e}	3.34 ± 0.03 ^{b}	18.7 ± 0.3 ^{cd}	8.9 ± 0.2 ^{b}	27.6 ± 0.1 ^{c}
	S1	1.11 ± 0.02 ^{f}	2.94 ± 0.01 ^{c}	0.859 ± 0.001 ^{d}	4.40 ± 0.06 ^{f}	11.6 ± 0.5 ^{d}	1.57 ± 0.01 ^{g}	17.2 ± 0.5 ^{e}	5.3 ± 0.1 ^{f}	22.4 ± 0.6 ^{e}
	S2	1.39 ± 0.04 ^{e}	3.37 ± 0.01 ^{b}	1.62 ± 0.03 ^{a}	5.96 ± 0.03 ^{c}	11.94 ± 0.01 ^{d}	1.37 ± 0.08 ^{i}	18.1 ± 0.1 ^{d}	7.6 ± 0.1 ^{c}	25.7 ± 0.2 ^{d}
Red Basil	Control	2.18 ± 0.04 ^{a}	1.98 ± 0.03 ^{e}	0.429 ± 0.003 ^{f}	8.4 ± 0.1 ^{b}	16.51 ± 0.06 ^{b}	4.99 ± 0.05 ^{a}	25.7 ± 0.1 ^{b}	8.9 ± 0.1 ^{b}	34.5 ± 0.1 ^{b}
	S1	1.69 ± 0.01 ^{c}	1.11 ± 0.03 ^{g}	0.756 ± 0.004 ^{e}	3.0 ± 0.2 ^{g}	14.3 ± 0.6 ^{c}	2.04 ± 0.02 ^{e}	19.1 ± 0.6 ^{c}	3.8 ± 0.2 ^{g}	22.9 ± 0.4 ^{e}
	S2	1.99 ± 0.03 ^{b}	2.23 ± 0.04 ^{d}	1.48 ± 0.01 ^{b}	10.5 ± 0.3 ^{a}	19.8 ± 0.2 ^{a}	3.19 ± 0.02 ^{c}	27.2 ± 0.2 ^{a}	12.0 ± 0.3 ^{a}	39.2 ± 0.1 ^{a}
Basilico Rosso	Control	0.63 ± 0.01 ^{g}	0.84 ± 0.02 ^{i}	0.215 ± 0.003 ^{i}	3.25 ± 0.05 ^{g}	2.96 ± 0.01 ^{g}	1.47 ± 0.04 ^{h}	5.90 ± 0.01 ^{h}	3.47 ± 0.05 ^{h}	9.37 ± 0.06 ^{h}
	S1	1.65 ± 0.02 ^{c}	1.05 ± 0.02 ^{h}	1.12 ± 0.01 ^{c}	5.35 ± 0.03 ^{e}	6.5 ± 0.3 ^{f}	1.82 ± 0.02 ^{f}	11.1 ± 0.3 ^{g}	6.47 ± 0.02 ^{e}	17.5 ± 0.3 ^{g}
	S2	1.66 ± 0.01 ^{c}	1.25 ± 0.02 ^{f}	1.43 ± 0.08 ^{b}	5.67 ± 0.03 ^{d}	6.6 ± 0.3 ^{f}	2.28 ± 0.02 ^{d}	11.8 ± 0.2 ^{f}	7.1 ± 0.1 ^{d}	18.9 ± 0.1 ^{f}

* Treatments: Control: 1.8 dS/m, S1: 3.0 dS/m and S2: 4.5 dS/m. TPA—total phenolic acids; TF—Total flavonoids, TPC—Total phenolic compounds; different Latin letters in the same column indicate significant differences according to Tukey's HSD test (p = 0.05). * Peak numbers correspond to the compounds presented in Table 3.

Antioxidant activity of basil leaves extracts was determined with the TBARS and OxHLIA methods (Table 5). The highest activity for the TBASR assays was observed in the control treatment of the Dark Opal Red cultivar, while the IC_{50} values of the OxHLIA assay after 60 and 120 min were the lowest for the control treatment of Red Basil and the S1 treatments of Dark Opal Red and Basilico Rosso in the first case and the control treatment of Red basil in the second case. None of the tested extracts showed higher activity than Trolox which was the positive control. The variable results depending on the implemented assay is usual in natural matrices since several antioxidants are involved in the overall antioxidant mechanisms of plants, including tocopherols, polyphenols, free sugars and organic acids [36]. The findings of our study could be partly attributed to γ- and δ-tocopherols and chicoric acid in the case of TBARS assay where the highest activity was associated with the highest amounts of these compounds observed in Dark Opal Red (control treatment). On the other hand, α-tocopherol and caffeic and salvianolic acid F could be responsible for the highest activity against OxHLIA at Δt = 120 min recorded in Red Basil (control treatment). Although it is reported that salinity stress and nitrogen deprivation induce the biosynthesis of polyphenols as an adaptation means of basil plants to oxidative stress [7,48], the increase in total and individual phenolic compounds with increasing salinity in the Red Basil cultivar was not accompanied by an increase in the antioxidant activity of leaves extracts indicating a complex antioxidant mechanism.

Table 5. Antioxidant activity of the tested basil cultivars in relation to salinity level (mean ± SD, n = 3).

Cultivar	Treatment *	TBARS(EC_{50}, μg/mL)	OxHLIA (IC_{50} Values, μg/mL)	
			Δt = 60 min	Δt = 120 min
Dark Opal Red	Control	13.0 ± 0.5 ^{i}	66 ± 3 ^{b}	155 ± 5 ^{b}
	S1	14.5 ± 0.4 ^{h}	41 ± 1 ^{e}	104 ± 3 ^{c}
	S2	30 ± 1 ^{f}	50 ± 1 ^{d}	100 ± 2 ^{c}
Red Basil	Control	24.6 ± 0.7 ^{g}	35 ± 1 ^{e}	73 ± 2 ^{d}
	S1	45.3 ± 0.5 ^{c}	69 ± 2 ^{b}	168 ± 13 ^{b}
	S2	49.4 ± 0.6 ^{b}	58 ± 3 ^{c}	112 ± 3 ^{c}
Basilico Rosso	Control	31.4 ± 0.2 ^{e}	86 ± 5 ^{a}	209 ± 4 ^{a}
	S1	35.6 ± 0.9 ^{d}	38 ± 2 ^{e}	99 ± 2 ^{c}
	S2	51.0 ± 0.9 ^{a}	51 ± 1 ^{d}	100 ± 2 ^{c}
Trolox (positive control)		5.4 ± 0.3	19.6 ± 0.7	41 ± 1

* Treatments: Control: 1.8 dS/m, S1: 3.0 dS/m and S2: 4.5 dS/m. Different Latin letters in the same column indicate significant differences according to Tukey's HSD test (p = 0.05).

The antimicrobial activities of the tested extracts were tested against six bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Enterobacter cloacae*) and six fungi (*Aspergillus fumigatus*, *A. niger*, *A. versicolor*, *Penicillium funicolosum*, *P. verrucosum* var. *cyclopium* and *Trichoderma viride*) strains and the results are presented in Table 6. In most of the cases, positive controls (E211 and E224) were more effective than the extracts obtained from basil leaves, although there were treatments with similar values to the control MIC against *S. aureus* (control and S1 treatments of Red Basil and Basilico Rosso, respectively), *B. cereus* (control and S1 treatment of Basilico Rosso) and *S. enterica* serovar Typhimurium (S2 and control treatment of Red Basil and Basilico Rosso, respectively). In contrast, the activity of the extracts against the tested fungi was more profound than the same positive controls (E211 and E224), especially against *A. fumigatus*, *A. niger* and *A. versicolor*, where all the extracts had lower MIC and MFC values than the controls. Significant activity was also observed against *T. viride* where almost all the extracts had MIC values MFC lower and similar to the control, respectively (except for the S1 treatment of Basilico Rosso). Moreover, Red Basil and Dark Opal Red (only the control treatment) extracts were effective against *P. verrucosum* var. *cyclopium*, while all the extracts (except for the S1 treatment of Red Basil) exhibited lower MIC values than the positive controls against *P. funiculosum*. Most of the published reports refer to the antimicrobial properties of basil essential oils [51,52] suggesting possible applications in the food industry as antimicrobial agents [53]. However, apart from the essential oils, leaf extracts may also exhibit significant antimicrobial effects against various pathogens,

such as *L. monocytogenes* and *P. aeruginosa* [47], where these effects were attributed to the presence of rosmarinic acid, or against *P. aeruginosa*, *Shigella* sp., *L. monocytogenes*, *S. aureus* and *E. coli* where methanolic extracts were most effective compared to the chloroform and acetone ones [54]. Moreover, Kocić-Tanackov et al. [55] suggested that basil extracts may be effective against various *Fusarium* species. According to Ababutain [42] fatty acids composition is related with antimicrobial properties and chain length may affect the activity against bacteria strains with unsaturated fatty acids being more effective than the saturated ones against *S. aureus*, *Helicobacter pylori* and *Mycobacterium.*

Table 6. Antibacterial and antifungal activity (mg/mL) of hydroethanolic extracts of the leaves of the tested basil cultivars in relation to salinity level.

Cultivar	Treatment *	Antibacterial Activity	*S. aureus*	*B. cereus*	*L. monocytogenes*	*E. coli*	*S. enterica* Serovar Typhimurium	*E. cloacae*
Dark Opal Red	Control	MIC	4	1	2	2	2	4
		MBC	8	2	4	4	4	8
	S1	MIC	2	1	2	2	2	2
		MBC	4	2	4	4	4	4
	S2	MIC	2	1	1	2	2	2
		MBC	4	2	2	4	4	4
Red Basil	Control	MIC	1	1	1	1	2	1
		MBC	2	2	2	2	4	2
	S1	MIC	2	1	1	2	2	2
		MBC	4	2	2	4	4	4
	S2	MIC	2	1	1	2	1	2
		MBC	4	2	2	4	2	4
Basilico Rosso	Control	MIC	2	0.5	1	2	1	2
		MBC	4	1	2	4	2	4
	S1	MIC	1	0.5	1	2	2	1
		MBC	2	1	2	4	4	2
	S2	MIC	2	1	1	2	2	2
		MBC	4	2	2	4	4	4
Positive controls	E211	MIC	4	0.5	1	1	1	2
		MBC	4	0.5	2	2	2	4
	E224	MIC	1	2.0	0.5	0.5	1	0.5
		MBC	1	4.0	1	1	1	0.5
Cultivar	**Treatment**	**Antifungal Activity**	***A. fumigatus***	***A. niger***	***A. versicolor***	***P. funiculosum***	***P. v. var. cyclopium***	***T. viride***
Dark Opal Red	Control	MIC	0.5	0.5	0.5	0.5	0.5	0.25
		MFC	1	1	1	1	1	0.5
	S1	MIC	0.5	0.5	0.5	0.5	1	0.25
		MFC	1	1	1	1	2	0.5
	S2	MIC	0.5	0.5	0.5	0.5	1	0.25
		MFC	1	1	1	1	2	0.5
Red Basil	Control	MIC	0.5	0.5	0.5	0.5	0.5	0.25
		MFC	1	1	1	1	1	0.5
	S1	MIC	0.5	0.5	0.5	1	0.5	0.25
		MFC	1	1	1	2	1	0.5
	S2	MIC	0.5	0.5	0.5	0.5	0.5	0.25
		MFC	1	1	1	1	1	0.5
Basilico Rosso	Control	MIC	0.25	0.5	0.5	0.5	0.5	0.25
		MFC	0.5	1	1	1	1	0.5
	S1	MIC	0.5	0.5	0.5	0.5	1	0.5
		MFC	1	1	1	1	2	1
	S2	MIC	0.5	0.5	0.5	0.5	1	0.25
		MFC	1	1	1	1	2	0.5
Positive controls	E211	MIC	1	1	2	1	2	1
		MFC	2	2	2	2	4	2
	E224	MIC	1	1	1	0.5	1	0.5
		MFC	1	1	1	0.5	1	0.5

* Treatments: Control: 1.8 dS/m, S1: 3.0 dS/m and S2: 4.5 dS/m. MIC—minimal inhibition concentration; MBC—minimal bactericidal concentration; MFC—minimal fungicidal concentration.

4. Conclusions

The results of our study showed that the tested cultivars were moderately tolerant to salinity with no significant effects on fresh weight of leaves, whereas a slight discoloration (loss of red color) was also recorded in all the cultivars when the highest salinity level was applied. Varied effects of salinity were observed regarding the nutritional value of basil leaves with an increase in fat, ash

and carbohydrates and a decrease in protein and energetic value for all the cultivars. Moreover, plants of Red Basil and Bassilico Rosso cultivars subjected to salinity stress tended to accumulate free sugars and phenolic compounds as the main osmolytes to contribute to the overall plant antioxidant mechanism, whereas tocopherols and organic acids were negatively affected by salinity in all the cultivars. However, the slight reduction in oxalic acid is deemed beneficial due to the antinutritional effects of this compound. The antioxidant effects varied for the tested assays and for the TBARS leaf extracts from the control treatment showed the highest activity probably due to the reduction in tocopherols for the same treatment. On the other hand, the results of the OxHLIA assay indicate that high salinity may increase the antioxidant activity for specific cultivars (Dark Opal Red and Basilico Rosso). Interestingly, most of the leaf extracts showed antifungal activities against the tested pathogenic fungi with MIC and MFC values similar or lower than the positive controls, whereas the antibacterial effectiveness for most of the tested extract was lower than the tested controls. Therefore, it could be assumed that the cultivation of basil under moderate salinity stress is a viable option allowing for the valorization of slightly salinized soils or the use of brackish water. However, further studies are needed to identify and select those genotypes that are suitable for cultivation under saline conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/11/1824/s1, Table S1: Fatty acids composition (%) of the tested basil cultivars in relation to salinity level (mean ± SD, n = 3).

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