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# The Postharvest Safety and Quality of Fresh Basil as Affected by the Use of Cypriot Oregano (*Origanum dubium*) Extracts

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**Abstract:** The use of natural products (including essential oil—EO from medicinal and aromatic plants) on fresh commodities such as leafy greens has gained a lot of attention due to the beneficial effects of those products. However, fresh herbs are highly perishable commodities, and very little is known for their postharvest preservation. The present study aimed to (i) investigate the effects of Cypriot oregano (*Origanum dubium*) EO and hydrosol (at different concentrations and times of application) on fresh basil's quality attributes and preservation and (ii) examine the efficacy of selected doses of *O. dubium* EO and hydrosol against two major foodborne pathogens inoculated on fresh basil stored at 4 °C for six days. The results of the current study indicated that the application of *O. dubium* EO at high concentrations in combination with a longer dipping time presented a less aromatic (less basil-like aroma) and preferable commodity, whereas hydrosol application resulted in a more acceptable and marketable commodity. In addition, an increase in antioxidant capacity and ascorbic acid content were observed with the EO, whilst hydrosol application was found to decrease basil's antioxidant capacity. Both investigated products (EO and hydrosol) where found to present great antibacterial activity against *Salmonella enterica* and *Listeria monocytogenes* inoculated on fresh basil even six days after the application. Overall, the investigated natural products (i.e., *O. dubium* EO and hydrosol) could be considered alternative sanitizing agents during the postharvest processing of fresh basil, whilst preserving and/or improving its nutritional value (i.e., an increase in antioxidants or flavonoids). However, caution should be taken when using it at high concentrations; thus, further research is needed for future commercial-scale use and on other fresh produce.

**Keywords:** basil; essential oil; fresh herbs; hydrosol; dittany; postharvest safety and quality

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## 1. Introduction

Basil (*Ocimum basilicum*), also called sweet basil, is a well-known and used culinary herb of the Lamiaceae family with a distinct and pleasant aroma [1]. Fresh basil is used in the food industry and in everyday cooking to enhance the aroma and flavor of food and drinks [2,3]. As with many other herbs, basil is a rich source of phytochemicals (i.e., polyphenols, flavonoids, vitamins, etc.) that gives this medicinal plant a vast variety of properties and many health benefits [1]. These properties (i.e., antioxidant, anti-inflammatory, antimicrobial, antiviral, antidiabetic, hypocholesterolemic, and anticancer, among others) are linked to its composition, which includes the following: phenolic compounds (cinnamic, caffeic, rosmarinic and ferulic acid), flavonoids (catechin, apigenin), and chicoric acid [1,4,5].

It is well known that fresh culinary herbs (including basil) are highly perishable and delicate commodities with a very short shelf life [1]. Rapid senescing and decay occur during the storage of fresh basil due to increased weight loss, respiration and leaf discoloration (i.e., browning and/or yellowing). The use of low temperatures for storage might work on other commodities and slow down processes leading to decay. However, this is not the case for basil since low temperatures might result to the development of

chilling injuries [1,4]. Moreover, mishandling during harvesting, transfer, processing, packaging and storage could have negative impacts on fresh basil's quality. It has been previously shown that mechanical injuries and improper storage conditions could result in negative alterations to the aroma and flavor of fresh commodities [2,3].

One of the main steps of the postharvest processing of leafy vegetables (including fresh herbs) is the washing with chlorinated water (using chlorine and other chlorine-based products). This process is essential for lowering the commodity's microbial load, which could elongate the commodities' shelf life. However, the use of chlorine as a disinfectant in the food industry (including fruits and vegetables) has been linked to the presence and residues of harmful by-products (i.e., trihalomethanes and haloketones) caused by the reaction of chlorine with organic matter found in water and fresh produce [6,7]. These compounds have been characterized as potentially carcinogenic. Therefore, the food industry and the scientific community are researching the use of natural products that present antimicrobial activities and do not cause a threat to human health as alternative postharvest means of sanitation and preservation.

Essential oils (EOs) from aromatic and medicinal plants could be considered potential eco-friendly sanitizing agents due to their antioxidant and antimicrobial properties. EOs are mixtures of secondary plant metabolites (i.e., hydrocarbons and oxygenated derivatives) and can be found in different parts of plant tissues such as leaves, flowers and bark, among others [8]. One of the most common processes that is used to obtain Eos is hydrodistillation, where its by-product called hydrosol (the remaining water) has also been proven to possess antioxidant and antimicrobial activities [9,10]. Previous studies on leafy greens showed the positive effects of EO and hydrosol application of different plant origins on fresh commodities' preservation as well as the significant reduction in foodborne pathogens' occurrence in such products [11–14]. For instance, Poimenidou et al. showed that washing spinach and lettuce with oregano aqueous extract reduced *Escherichia coli* O:157:H7 and at the same time did not negatively affect their color [12]. The aim of the present study was to evaluate the effects of *Origanum dubium* EO and hydrosol (at different concentrations and times of application via dipping) on fresh basil's quality attributes and preservation. Moreover, the efficacy of the selected doses (combination of concentration and time of application selected from a preliminary screening) of these products against two major foodborne pathogens (i.e., *Salmonella enterica* and *Listeria monocytogenes*) inoculated on fresh basil stored at 4 °C for six days was also assessed.

## 2. Materials and Methods

### *2.1. Chemical Reagents and Media*

All chemical reagents (analytical grade) and media were purchased from Merck (Darmstadt, Germany), unless otherwise stated.

### *2.2. Plant Material*

Fresh basil (*Ocimum basilicum*), of BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) code 48, was obtained from the Cyprus University of Technology's experimental farm/greenhouse (Limassol, Cyprus) where it was grown in a nutrient film technique (NFT) hydroponic cultivation system. The hydroponic infrastructure and nutrient solution has been reported previously [15]. The plant material was transferred to the laboratory immediately after harvest and inspected for no physical defects (leaf wilding or damage).

Fresh *Origanum dubium* (Cypriot oregano) plant tissue was obtained from the experimental farm/greenhouse of Cyprus University of Technology (Limassol, Cyprus) where it was grown in soil (clay–loam texture, 2.45% organic matter; pH 8.28; EC 0.79 mS/cm; total nitrogen—N 0.73 g/kg) without any pesticides or chemical applications. The collected plant tissue (whole stems with leaves) before the flowering stage (BBCH code 59), was transferred to the laboratory and air-dried at 42 °C in an air-ventilated oven. The essential oil was obtained by the hydrodistillation (3 h) of the dried plant tissue using a Clevenger

apparatus, and the collected EO was stored at −20 °C until use. After hydrodistillation, the hydrosol (i.e., the remaining water) was collected, filtered with a cheesecloth, and stored at 4 °C until use. The composition of *O. dubium* EO was determined with Gas Chromatography–Mass Spectrometry (GC/MS; Shimadzu GC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010 plus mass spectrometer, Kyoto, Japan) following the procedure according to Chrysargyris et al. [15]. From this, the main compounds identified in *O. dubium* EO were: carvacrol (54.09%), γ-terpinene (8.21%) and p-cymene (7.13%).

### 2.3. Preliminary Screening

Fresh basil (whole stems with leaves, approx. 17 cm height) (approx. 40 g per replication) was submerged for different times (1, 5 and 10 min) in 1.5 L of treatment solutions (*O. dubium* EO and hydrosol) of different concentrations (0%, 0.001%, 0.01% and 0.1%) (Table 1 and Figure 1). The application took place at room temperature (25 °C). Distilled water was used for the control. The EO was homogenously diluted into distilled water with the use of Tween 20 (Scharlau, Spain) (an emulsifier) at a concentration of 0.1% *v*/*v*. After application, basil was left to dry for approx. 30 min at room temperature and then placed in polypropylene plastic containers (capacity of 5 L; dimensions: 18.5 × 18.5 × 17.5 cm). Two bundles (approx. 40 g each and placed in two small plastic cups, respectively) were placed in each container. Prior to closing the container's lid, a piece of wet paper was placed inside each box in order to maintain a high humidity level (90% relative humidity). Afterwards, the containers were stored at 4 °C for six days and quality parameters were determined on appropriate days (day 0, 3 and 6) as mentioned below. Containers were ventilated every three days.

**Table 1.** Treatments and concentrations examined during the preliminary screening.

| Treatment | Concentration | Time |
|---|---|---|
| Control | 0.00% | 1 min |
| EO | 0.001%, 0.01%, 0.1% | 5 min |
| Hydrosol | 0.001%, 0.01%, 0.01% | 10 min |

#### 2.3.1. The Effects on Basil's Weight Loss and Respiration Rate

The weight and production of carbon dioxide ($CO_2$) were monitored and recorded during storage (day 0, 3 and 6) in order to estimate fresh basil's weight loss and respiration rate, respectively. For weight loss, the weight of each replication was recorded on day 0, 3 and 6, and the results were expressed as percentages (%) of total weight loss according to the following formula: weight loss percentage (%) = $[(m_0 - m_d)/m_0] \times 100$, where $m_0$ is the initial weight and $m_d$ is the weight after 3 or 6 days of storage. The determination of basil's respiration rate as affected by the applied treatments was performed by the procedure mentioned by Xylia et al. [16]. Briefly, samples were enclosed hermetically in 5 L polypropylene plastic containers and left at room temperature for 1 h and 30 min. The air from each container was sucked for 40 s with a dual gas analyzer (GCS 250 Analyzer, International Control Analyser Ltd., Kent, UK), and results were expressed as mL of carbon dioxide ($CO_2$) produced per kg per h (mL $CO_2$/kg/h). For this, the weight and volume of each sample were also recorded.

#### 2.3.2. The Effects on Basil's Sensory Characteristics

For the evaluation of fresh basil's sensory attributes (i.e., aroma, appearance and marketability), four to six panelists were employed (2 males and 4 females, age: 23–33 years old, not professionals, but an introduction/training for the basil storage conditions was conducted) on the appropriate days (day 0, 3 and 6) [16]. The aroma was evaluated with the use of a 10-point scale (1-point interval), where 1: not basil-like and very unpleasant aroma, 3: not basil-like and slightly unpleasant aroma, 5: not basil-like but pleasant aroma, 8: less basil-like aroma, and 10: intense basil-like aroma. Fresh basil's appearance (in terms

of visual quality and color) was assessed with the use of a 10-point scale (1-point interval), where 1: yellow color of 50%, 3: yellow-green, 5: light green, 8: green, and 10: deep green. A 1–10 scale (1-point interval) was used for the assessment of basil's marketability (i.e., overall quality), where 1: not marketable quality (i.e., malformation, wounds, infection), 3: low marketability with malformation, 5: marketable with few defects, i.e., wilting, decolorization (medium quality), 8: marketable (good quality), and 10: marketable with no defects (extra quality).

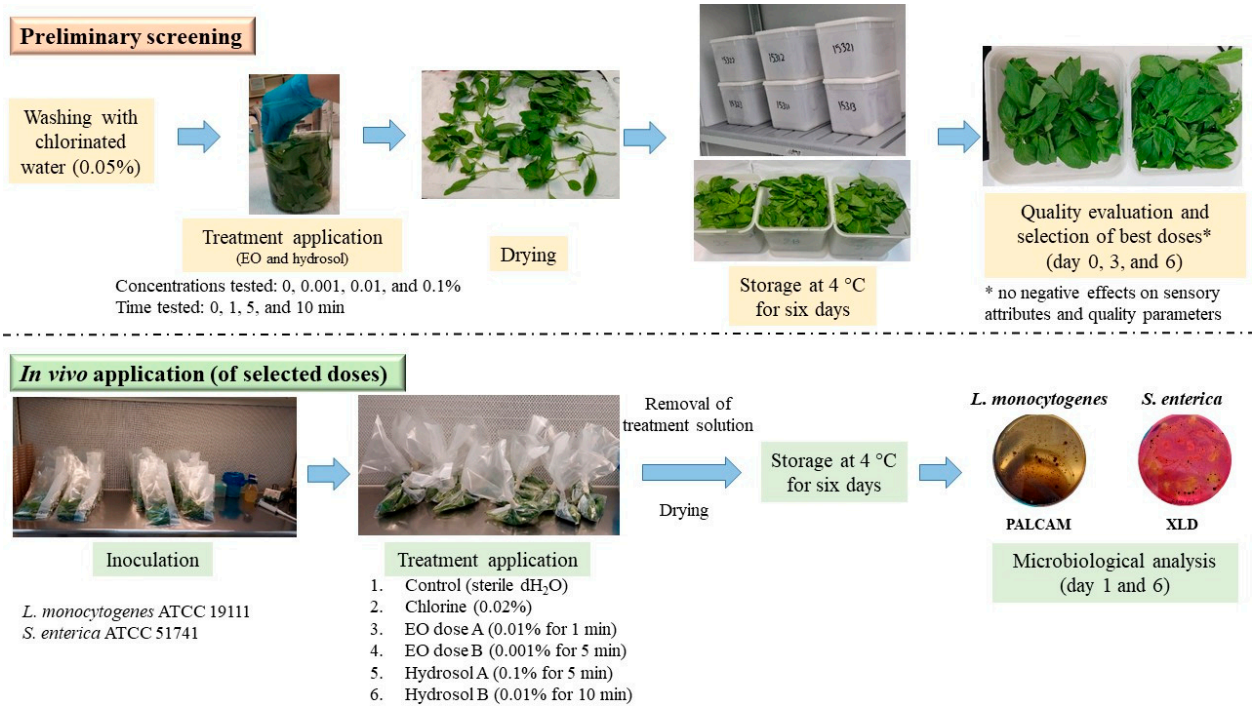



**Figure 1.** Experimental set up for preliminary screening application of *O. dubium* EO and hydrosol on fresh basil's storage, including selected applications against *Salmonella enterica* subsp. *enterica* and *Listeria monocytogenes*.

### 2.3.3. The Effects on Basil's Quality Parameters

#### Color

Fresh basil's quality parameters were assessed on the initial and last day of storage (day 0 and 6, respectively). The evaluation of basil leaves' color was performed with the use of a colorimeter (Chroma meter CR400 Konica Minolta, Tokyo, Japan). The L*, a* and b* values were recorded, where L* represents brightness/lightness (0: black/100: white), a* represents greenness/redness (−a*: greenness and +a*: redness) and b* represents blueness/yellowness (−b*: blueness and +b*: yellowness) (CIELAB uniform color space). Using these values, hue (h), chroma value (C) and color index (CI) were also calculated. Hue (h) is measured in degrees (°) and is estimated with the following equation (if a* textless 0): $h(°) = 180 + \tan^{-1}(b^*/a^*)$. Hue indicates the distinction between the colors positioned around the color wheel, where h = 0°: red-purple; h = 90°: yellow, h = 180°: bluish-green, h = 270°: blue) [17,18]. The chroma value (C) (also called saturation index) shows the quality of a color's purity/intensity (i.e., the degree of departure from grey to the pure chromatic color), and it is calculated as follows: $C = (a^{*2} + b^{*2})^{1/2}$ [17]. Color index (CI) was calculated with the use of the following equation: $CI = (a^* \times 1000)/(L^* \times b^*)$, where CI: ≥−40 and <−20: blue-violet to dark green; ≥−20 and <−2: dark green and yellowish green; ≥+2 <+20: aple yellow and deep orange; ≥+20 and <+40: deep orange and deep red color [18,19].

Leaf Pigments

The extraction and determination of leaf pigment content (chlorophylls and total carotenoids) was performed by the procedure described by Wellburn [20] using methanol (Merck, Darmstadt, Germany) as solvent. Chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (tot chl) and total carotenoids (tot car) contents were estimated by measuring the solution's absorbance at 480, 649 and 665 nm, and results were expressed as mg of chlorophyll (or carotenoids) per g of fresh weight (mg/g).

Polyphenols, Antioxidant Activity, Total Flavonoids and Ascorbic Acid Content

Basil's polyphenols and flavonoids were extracted by the procedure reported by Chrysargyris et al. [21] where methanol 50% (*v*/*v*) was used. The phenolic content of the obtained methanolic extracts was determined with the Folin–Ciocalteu method [21]. The reaction's optical density was measured at 755 nm with the use of a spectrophotometer (Multiskan GO, Thermo Fisher Scientific Oy, Vantaa, Finland), and results were expressed as mg of gallic acid (Scharlab, Sentmenat, Spain) equivalents (GAE) per gram of fresh weight (mg GAE/g).

The antioxidant activity of basil's methanolic extracts was determined with two different assays: (i) the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (ii) the ferric-reducing antioxidant power (FRAP). Both assays were performed according to the procedures described by Chrysargyris et al. [21]. The ability of the methanolic extracts to reduce the purple-colored DPPH free radical was determined by measuring the reaction's absorbance at 517 nm. For the FRAP assay, the absorbance of the blue-colored complex formed was measured at 593 nm. For both assays (i.e., DPPH and FRAP), the results were expressed as mg of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox; Sigma-Aldrich, Germany) per gram of fresh weight (mg trolox/g). Total flavonoid content was determined by the colorimetric method reported by Meyers et al. [22], with adapted modifications as mentioned by Chrysargyris et al. [21]. The reaction's absorbance was measured at 510 nm, and results were expressed as mg of rutin per gram of fresh weight (mg rutin/g). Basil's ascorbic content (AA) was estimated with the 2,6-dichloroindophenol (DCPIP) titrimetric method [23], and results were expressed as mg of ascorbic acid per 100 g of fresh weight (mg AA/100 g).

2.3.4. The Determination of Damage Indexes

The damage index caused by abiotic and/or biotic stress six days after the applied treatments and storage at 4 °C was estimated by measuring the production of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA). Those molecules are products of the primary and secondary peroxidation of unsaturated fatty acids from the phospholipid layer of plant cells [24]. For the determination of $H_2O_2$ levels, the method mentioned by Loreto and Velikova was used [25]. The reaction's absorbance was measured at 390 nm, and the results were expressed as μmol of $H_2O_2$ per g of fresh weight (μmol $H_2O_2$/g). The production of MDA was assessed with the 2-thiobarbituric acid reactive substances (TBARS) method according to Heath and Packer, where the reaction's optical density was measured at 532, and the non-specified absorbance of interfering compounds at 600 nm was subtracted [26]. The results were then expressed as nmol of MDA per g of fresh weight (nmol MDA/g).

*2.4. The In Vivo Application of a Selected Sanitation Mean*

2.4.1. The Preparation of the Bacterial Inoculum

The bacterial strains of *Salmonella enterica* subsp. *enterica* (ATCC 51741) and *Listeria monocytogenes* (ATCC 19111) used in this study were obtained by the Department of Nursing (Cyprus University of Technology, Limassol, Cyprus). Briefly, fresh inoculum for each bacterium (log 8 cfu/mL) was separately prepared after overnight (16–18 h) incubation at 37 °C in brain–heart infusion broth (BHI, HiMedia, Mumbai, India), as reported by Chrysargyris et al. [15].

2.4.2. The Procedure for Treatment Application

Based on the results derived from the preliminary screening, the two most promising treatments for EO and hydrosol applications that preserved fresh basil's visual quality (appearance) and marketability were selected for further investigation of their antimicrobial activity against *S. enterica* and *L. monocytogenes* inoculated on fresh basil (Figure 1). At the same time, the positive effects of treatments on other investigated parameters such as respiration rate and nutritional value (i.e., antioxidants, ascorbic acid content) were also taken into account for the selection of the most promising doses (combination of concentration and time). Thus, for the investigation of *O. dubium* EO and hydrosol antibacterial activity, the following treatments were used: (i) control (sterile distilled water), (ii) chlorine (i.e., sodium hypochlorite-NaOCl at 0.02% *v*/*v*), (iii) EO Dose A (0.01%—1 min), (iv) EO Dose B (0.001%—5 min), (v) hydrosol Dose A (0.1%—5 min) and (vi) hydrosol Dose B (0.01%—10 min).

Inoculation and treatment application were performed according to Singh et al. [27], with slight modifications. Briefly, fresh basil (fresh stems and leaves) was washed with 0.05% NaOCl, rinsed with sterile distilled water and afterwards air-dried under a laminar flow cabinet. Then, 25 g of fresh basil was aseptically placed in a sterile stomacher bag, where 2 mL of bacterial inoculum (concentration: 8 log cfu/mL) was evenly sprinkled on basil's surface. In order to allow for bacterial cell attachment on leaf surfaces, bags were left open for 1 h (under laminar flow cabinet) (Figure 1). Afterwards, an appropriate volume of treatment solution (1:10 *w/v* ratio) was added into the respective bags that were subsequently closed for the appropriate time of application for each treatment (1, 5 or 10 min). The solution was then discarded and bags were again closed and placed at 4 °C for storage (total duration six days). After one and six days of storage, microbiological analysis was performed as described below in order to determine the antimicrobial efficacy of the applied treatments. The storage temperature of 4 °C was selected based on fresh basil's appropriate storage conditions and/or previous reports in order to examine the effects of the treatments on pathogens' performances [28].

2.4.3. Microbiological Analysis

The efficacy of the assessed treatments was measured by estimating the surviving bacterial population as previously reported by Singh et al. [27]. Basil was homogenized with maximum recovery diluent (MRD) in a 1:10 *w/v* ratio, and serial decimal dilutions were prepared in MRD. Selective growing media for *S. enterica* (Xylose-lysine-deoxycholate agar-XLD agar) and *L. monocytogenes* (PALCAM agar) were used and 100 μL from each dilution was spread on their surfaces. For *S. enterica*, samples were incubated at 37 °C for 24 h, whilst for *L. monocytogenes* incubation took place at 37 °C for 48 h. After incubation, typical colonies on each medium were counted (XLD: pink colonies with a black center; PALCAM agar: grey–green with a black center and halo), and results were calculated as log cfu/g.

*2.5. Statistical Analysis*

The present study was set up as a Completely Randomized Design (CRD), consisting of four biological replications per treatment. One-way analysis of variance (one-way ANOVA) was performed in IBM SPSS (version 25.0), and treatment means were compared on each sampling day with Duncan's multiple range test (for $p = 0.05$). In order to compare the data on the initial and final day (day 0 and 6, respectively) for non-treated samples (control), the independent samples' *t*-tests were performed.

## 3. Results and Discussion

*3.1. Preliminary Screening*

3.1.1. The Effects on Weight Loss and Respiration Rate

Basil, like many other leafy vegetables, is a highly perishable commodity with a very short shelf life. Moisture loss (observed as weight loss) during the postharvest

storage of fresh commodities (including fresh herbs) is associated with the decay and deterioration of leaves. Weight losses are mainly attributed to an increased metabolism and the activation of processes such as respiration and transpiration. All these lead to leaf wilting, discoloration (i.e., browning and/or yellowing) and rapid senescing [29]. Increased weight loss was observed with 0.001%—1 min, 0.01%—10 min and 0.1%—10 min of *O. dubium* EO application (2.20, 2.37 and 3.09%, respectively) on the third day of storage compared to non-treated ones (control) (0.87%), while on the sixth day of storage an increase was also noted with 0.001%—1 min (up to 3.45%) (Figure 2A). It is worth mentioning that weight losses on leafy vegetables that are higher than 3% have been associated with adverse quality attributes and a shorter shelf life [30]. A previous study showed that rosemary EO (up to 0.2%) applied on fresh rosemary stored at 4 °C for 12 days did not present great weight losses [16]. This could have been the result of the characteristics of each plant, where rosemary is a xerophilic aromatic plant, which can absorb water under high humidity levels, compared to basil, which cannot. Moreover, in the present study, the concentrations of the EO used were lower than the ones used in the aforementioned study, which could partially explain the observed higher weight loss in our study. *O. dubium* EO has been previously applied on tomato and cucumber fruits, where increased weight losses were observed (higher value: 5.22%) [31]. In the present study, lower EO concentrations were used in combination with shorter times of application, and this could explain the lower losses observed. Increased weight loss was observed on the third day, when basil was treated with *O. dubium* hydrosol for 5 min (all concentrations), 0.01%—10 min and 0.1%—1 min (Figure 2B). Moreover, all applied hydrosol treatments (except 0.001%—10 min) resulted in an increase in water loss (i.e., increased weight loss) on the sixth day as opposed to non-treated (control) samples. Interestingly, from the observations in the present study, the application of *O. dubium* hydrosol as mainly a hydrophilic solution (with hydrophobic and hydrophilic components) could not prevent water loss or allow for the rehydration of basil's leaves.

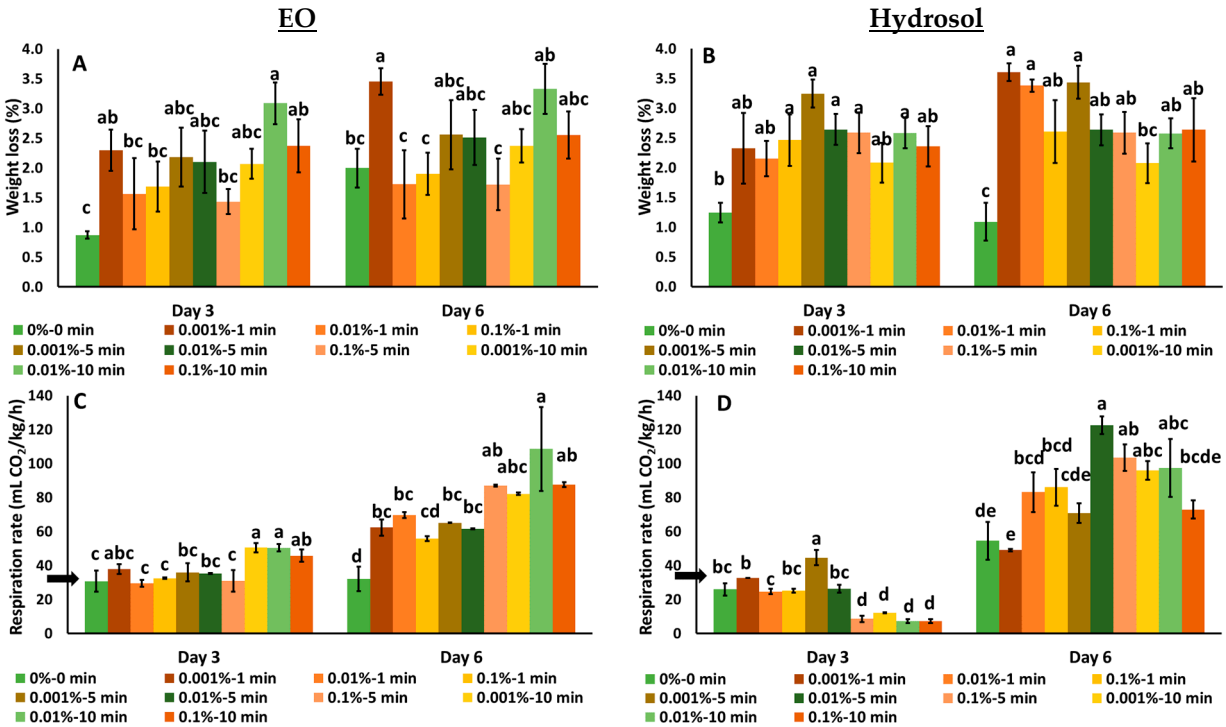



**Figure 2.** Effects of preliminary screening application of *O. dubium* EO (**A**,**C**) and hydrosol (**B**,**D**) on fresh basil's weight loss and respiration rate after six days of storage at 4 °C. Data shown are presented as the mean ± standard error (four biological replications per treatment). The values for day 0 refer to the control (non-treated, 0.00%) and are presented with an arrow. For each day, significant differences between treatments ($p < 0.05$) are indicated with different Latin letters.

During storage of leafy greens, an increase in respiration rate (observed by increase $CO_2$ production) could be observed. This increase is associated for the most part with the storage temperature and evidently results in the acceleration of plant metabolism, the degradation of leaf green pigments, and senescing [12,29]. Basil's respiration rate was found to increase on the third day of storage with 10 min EO application (all concentrations) compared to the control; 0.01%—1 min, 0.1%—1 min and 0.1%—5 min (Figure 2C). In addition, all EO treatments (except 0.1%—10 min) were found to increase the product's respiration rate on the last day of storage (day 6). The application of rosemary EO (dipping in 1:1500 and 1:500 *v/v* for 10 min) resulted in no significant increases in fresh rosemary's respiration even after 12 days of storage at 4 °C. However, these observations are due to the slow metabolism of rosemary (compared to basil) [16]. The respiration rate of fresh basil was found to increase on the third day with 0.001%—5 min *O. dubium* hydrosol, while a decrease was observed with 0.1%—5 min and 10 min (all concentrations) on the same day (Figure 2D). At the end of storage (day 6), the application of *O. dubium* hydrosol 0.01%—5 min, 0.1%—5 min, 0.01%—10 min and 0.001%—10 min increased basil's respiration rate (122.50, 103.50, 97.42 and 96.01 mL $CO_2$/kg/h). When marjoram hydrosol was applied to shredded carrots, no significant differences were observed [32]. On the same study, marjoram EO resulted in an increase in the carrots' respiration rates [32]. It is worth mentioning that previous studies indicated that the applied treatments (i.e., EOs) on fresh produce could lead to the disruption of the plant cell wall and the disturbance of gas exchanges (i.e., increases in respiration) [29,31]. Moreover, increased respiration rates have been linked with a decrease in the chlorophyll content of leafy greens [33].

#### 3.1.2. The Effects on Sensory Attributes

Basil's sweet, pleasant and distinct aroma is one of the main organoleptic characteristics that attracts consumers' attention when purchasing culinary herbs. However, the aroma of basil as well as of other herbs tends to fade during the end of the postharvest storage [16,34]. The aroma of fresh basil was reported as pleasant but less basil-like with the application of *O. dubium* EO 0.1%—5 min and 0.001%—10 min compared to the control on the third day of storage. On the last day of storage (day 6), all applied EO treatments were found to result in a less pleasant/acceptable aroma (especially 0.1%—5 min and 0.1%—10 min) (Figure 3A). A previous report mentions that high *O. dubium* EO concentrations negatively affected the organoleptic characteristics such as aroma on tomato and cucumber fruits [31]. Decreased aroma scores were also reported on the third day with treatments of *O. dubium* hydrosol 0.1%—5 min and 0.001%—10 min. In addition, basil treated with EO 0.01%—10 min showed a lower score on the aroma scale compared to 0.001%—1 min and the control on the last day, indicating a less basil-like aroma (day 6) (Figure 3B). It has been previously mentioned that the application of natural products such as EOs and hydrosols with strong and intense aromas could negatively affect the aroma of the product which they are applied to [13,31]. In addition, during the storage of fresh herbs and aromatic plants, a less aromatic end product might be found due to the dehydration of leaves (i.e., increases in water loss), and the possible disruption of glandular trichomes on herb leaves could evidently result in EOs release [1,34]. All those statements could explain the less basil-like aroma observed in the current study. As previously stated, the "perfect combination" of a natural product (i.e., EO) and fresh produce should be considered, and the applied product should complement and/or preserve the aroma of fresh commodities without negative interferences [35].

Like many other leafy vegetables and herbs, basil's intense green color tends to fate during storage and especially near the end of its shelf life due to the degradation of the green leaf pigments (i.e., chlorophylls) [2]. During the last day of storage, basil treated with *O. dubium* EO 0.1%—5 min and 0.1%—10 min presented decreased appearance scores, indicating less green-colored leaves (Figures 3C and S1). On the other hand, *O. dubium* hydrosol did not result in any significant differences on fresh basil's appearance throughout storage (Figures 3D and S1). As observed in the present study, the use of *O. dubium* EO at high concentrations presented phytotoxic effects (i.e., browning, especially with a

longer time of application, i.e., 10 min). In contrast, the use of *O. dubium* hydrosol did not negatively affect the color of basil, and this might be attributed to the antioxidant activity of the hydrosol and the less strong activity compared to the EO. This is due to the nature of the hydrosol that contains compounds that can also be found in their respective EOs that present antioxidant activities and protect from the oxidation and degradation of leaf chlorophylls [31,36].

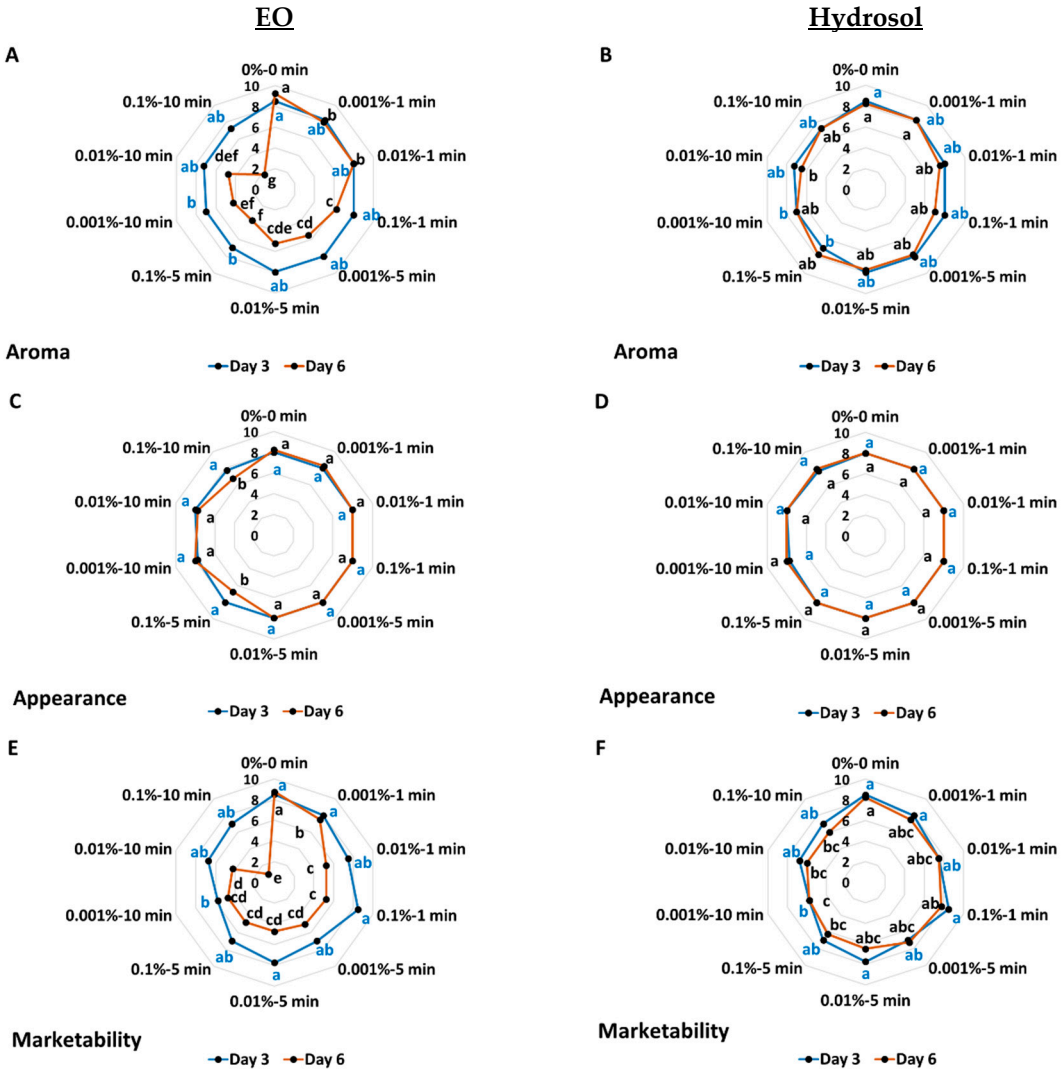



**Figure 3.** Effects of preliminary screening application of *O. dubium* EO (**A**,**C**,**E**) and hydrosol (**B**,**D**,**F**) on fresh basil's sensory attributes (aroma, appearance and marketability) after six days of storage at 4 °C. For each day, significant differences between treatments ($p < 0.05$) are indicated with different Latin letters.

A less marketable product was found on the third day of storage when treated with 0.001%—10 min of *O. dubium* EO, while all applied treatments resulted in a less marketable product at the end of storage (sixth day) (Figure 3E). A less marketable product was reported with *O. dubium* hydrosol 0.001%—10 min compared to 0.1%—1 min, 0.001%—1 min, 0.01%—5 min and the control on the third day. On the last day of storage, products treated with 0.1%—5 min and 10 min (all concentrations) were marked with lower scores (i.e., lower marketability) compared to the non-treated ones (Figure 3F). A previous study showed that the application of sideritis and bay leaf hydrosols resulted in more acceptable lettuce compared to when thyme, sage and rosemary hydrosols were used [13]. This shows that hydrosols with more intense and distinct aromas interfere with a product's aroma,

resulting in less acceptable products. This was also observed in our study, where the EO and hydrosol from *O. dubium* presented strong aromas, and at the same time longer times of application resulted in less acceptable products as judged by the panelists.

#### 3.1.3. The Effects on Quality Parameters

##### Effects on Color

Changes in the vibrant green color of basil might occur during storage due to the degradation of leaves' green pigments (i.e., chlorophylls). Treatment with *O. dubium* EO 0.01%—1 min showed a higher L* value (48.38) on day 6 compared to 0.1%—1 min and the control (44.89 and 44.48, respectively), suggesting a more vibrant green color in leaves (Table 2 and Figure S1). The application of *O. dubium* EO 0.1%—1 min, 0.001%—5 min, 0.001%—10 min and 0.1%—10 min resulted in decreased h values at the end of storage (i.e., less green color). An increase in CI values was observed with 0.01%—1 min, 0.001%—5 min, 0.1%—5 min and 10 min (all concentrations) of *O. dubium* EO on day 6, indicating a less dark green color. It has been previously shown that the use of EOs could prevent the development of browning and/or yellowing on leaves due to their antioxidant activity [37]. Notably, *O. dubium* EO has shown great in vitro antioxidant activity in the past [38]. Fresh basil's color was also affected by the application of *O. dubium* hydrosol, as shown in Table 2 and Figure S1. The application of *O. dubium* hydrosol at 0.001%—10 min resulted in a higher b* value than 0.001%—1 min at the end of storage (less intense green color), whilst 0.01%—10 min lead to a higher h value than that of 0.001%—10 min on the same day (i.e., a more green color) (Table 2). A decreased C value was found with 0.001%—1 min *O. dubium* hydrosol application compared to that of 0.001%—10 min, whereas treatment with hydrosol 0.001%—10 min presented a higher CI value (−12.71), as opposed to 0.001%—1 min, 0.01%—10 min, 0.01%—5 min and the control (−14.28, −14.41, −14.61, and −14.30, respectively), suggesting a less dark green color of fresh basil leaves (Table 2). As with the EOs from aromatic plants, hydrosols consist of compounds (hydrophobic and hydrophilic) with great antioxidant activity, suggesting that they also could prevent the degradation of chlorophylls and the preservation of basil's green color [10,13].

##### The Effects on Pigments, Phenols, Antioxidants, Flavonoids and Ascorbic Acid Content

As previously mentioned, at the end of the storage of leafy greens and herbs, yellowing and/or browning might occur due to the oxidation and degradation of leaves' green-colored pigments (i.e., chlorophylls) and the appearance of yellow ones (i.e., carotenoids) [39]. The pigments (chlorophylls and carotenoids) of fresh basil leaves were found to decrease on the sixth day when treated with *O. dubium* EO 0.001%—10 min compared to 0.01%—5 min (Table 3). This indicates that the antioxidant activity of the EO might have faded after six days and was not able to prevent chlorophylls' oxidation [3,31]. The application of *O. dubium* hydrosol 0.001%—5 min and 0.01%—10 min resulted in the higher chlorophyll and carotenoid contents of fresh basil compared to 0.01%—5 min and 0.001%—1 min treatments (Table 3). As such, it seems that increased hydrosol concentration and the time of application preserved the basil's pigments, whilst a decrease was found with EO application at the end of storage. This might be attributed to the volatile nature of EOs that evaporate after some time; thus, the fade in their antioxidant activity. Moreover, in some cases, increased respiration was also observed, suggesting an increase in metabolism, which could have also resulted in chlorophyll degradation [40]. This observation was also mentioned by Curutchet et al. on spearmint and peppermint stored at 0 °C [3].

**Table 2.** Effects of *O. dubium* EO and hydrosol application on basil color parameters: L*, a*, b*, hue (h), chroma value (C) and color index (CI), stored at 4 °C for 6 days.

| | Time (min) | Concentration | L* | a* | b* | h | C | CI |
|---|---|---|---|---|---|---|---|---|
| Day 0 | 0 | 0.00% | 45.75 ± 0.51 | −18.15 ± 0.30 | 25.77 ± 0.39 | 125.16 ± 0.23 | 31.52 ± 0.48 | −15.41 ± 0.28 |
| EO | 0 | 0.00% | 44.48 ± 1.10 $^{b}$ | −18.23 ± 0.95 | 25.47 ± 1.54 | 125.67 ± 0.36 $^{a}$ | 31.32 ± 1.80 | −16.21 ± 0.55 $^{b}$ |
| | 1 | 0.001% | 45.83 ± 0.84 $^{ab}$ | −17.42 ± 0.62 | 25.39 ± 0.60 | 124.42 ± 0.60 $^{ab}$ | 30.80 ± 0.80 | −14.96 ± 0.09 $^{ab}$ |
| | | 0.01% | 48.38 ± 0.53 $^{a}$ | −18.58 ± 0.18 | 27.32 ± 0.39 | 124.24 ± 0.32 $^{ab}$ | 33.04 ± 0.39 | −14.08 ± 0.31 $^{a}$ |
| | | 0.10% | 44.89 ± 1.24 $^{b}$ | −17.65 ± 0.89 | 26.16 ± 1.50 | 124.06 ± 0.53 $^{b}$ | 31.56 ± 1.72 | −15.12 ± 0.44 $^{ab}$ |
| | 5 | 0.001% | 46.17 ± 0.81 $^{ab}$ | −17.73 ± 0.34 | 26.17 ± 0.80 | 124.16 ± 0.47 $^{b}$ | 31.62 ± 0.83 | −14.74 ± 0.49 $^{a}$ |
| | | 0.01% | 46.20 ± 0.93 $^{ab}$ | −16.99 ± 0.33 | 24.64 ± 0.39 | 124.59 ± 0.45 $^{ab}$ | 29.93 ± 0.45 | −14.98 ± 0.52 $^{ab}$ |
| | | 0.10% | 46.68 ± 0.69 $^{ab}$ | −17.05 ± 0.89 | 24.70 ± 1.49 | 124.68 ± 0.39 $^{ab}$ | 30.02 ± 1.72 | −14.85 ± 0.35 $^{a}$ |
| | 10 | 0.001% | 46.34 ± 1.25 $^{ab}$ | −17.47 ± 0.54 | 26.02 ± 0.99 | 123.93 ± 0.54 $^{b}$ | 31.35 ± 1.09 | −14.60 ± 0.61 $^{a}$ |
| | | 0.01% | 47.59 ± 0.75 $^{ab}$ | −18.71 ± 0.39 | 27.08 ± 0.80 | 124.69 ± 0.36 $^{ab}$ | 32.92 ± 0.86 | −14.58 ± 0.42 $^{a}$ |
| | | 0.10% | 46.27 ± 1.04 $^{ab}$ | −17.43 ± 0.54 | 27.57 ± 0.45 | 122.26 ± 0.40 $^{c}$ | 32.62 ± 0.66 | −13.72 ± 0.24 $^{a}$ |
| Hydrosol | 0 | 0.00% | 47.57 ± 1.38 | −18.58 ± 0.73 | 27.53 ± 1.34 $^{ab}$ | 124.07 ± 0.46 $^{ab}$ | 33.22 ± 1.50 $^{ab}$ | −14.30 ± 0.58 $^{b}$ |
| | 1 | 0.001% | 47.52 ± 0.62 | −17.73 ± 0.51 | 26.23 ± 1.05 $^{b}$ | 124.011 ± 0.54 $^{ab}$ | 31.67 ± 1.13 $^{b}$ | −14.28 ± 0.41 $^{b}$ |
| | | 0.01% | 47.73 ± 0.68 | −18.04 ± 0.26 | 27.17 ± 0.54 $^{ab}$ | 123.59 ± 0.34 $^{ab}$ | 32.62 ± 0.57 $^{ab}$ | −13.94 ± 0.29 $^{ab}$ |
| | | 0.10% | 48.18 ± 1.37 | −18.96 ± 0.78 | 29.29 ± 2.02 $^{ab}$ | 123.12 ± 0.69 $^{ab}$ | 34.91 ± 2.12 $^{ab}$ | −13.65 ± 0.66 $^{ab}$ |
| | 5 | 0.001% | 47.67 ± 0.71 | −18.80 ± 0.55 | 28.03 ± 0.90 $^{ab}$ | 123.86 ± 0.40 $^{ab}$ | 33.76 ± 1.03 $^{ab}$ | −14.10 ± 0.31 $^{ab}$ |
| | | 0.01% | 47.16 ± 0.78 | −18.38 ± 0.24 | 26.83 ± 0.77 $^{ab}$ | 124.48 ± 0.55 $^{a}$ | 32.53 ± 0.74 $^{ab}$ | −14.61 ± 0.50 $^{b}$ |
| | | 0.10% | 48.42 ± 0.89 | −18.65 ± 0.63 | 28.30 ± 1.16 $^{ab}$ | 123.43 ± 0.51 $^{ab}$ | 33.90 ± 1.29 $^{ab}$ | −13.68 ± 0.47 $^{ab}$ |
| | 10 | 0.001% | 50.40 ± 0.72 | −19.45 ± 0.48 | 30.40 ± 0.82 $^{a}$ | 122.61 ± 0.32 $^{b}$ | 36.09 ± 0.93 $^{a}$ | −12.71 ± 0.26 $^{a}$ |
| | | 0.01% | 47.98 ± 1.00 | −19.26 ± 0.49 | 27.96 ± 0.87 $^{ab}$ | 124.58 ± 0.25 $^{a}$ | 33.95 ± 0.99 $^{ab}$ | −14.41 ± 0.42 $^{b}$ |
| | | 0.10% | 49.02 ± 1.19 | −19.05 ± 0.91 | 28.59 ± 1.58 $^{ab}$ | 123.74 ± 0.27 $^{ab}$ | 34.36 ± 1.81 $^{ab}$ | −13.68 ± 0.45 $^{ab}$ |

Values are presented as the mean ± standard error (four biological replicates per treatment). The values for day 0 refer to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are indicated with different Latin letters for each treatment (EO and hydrosol, separately) in each column.

Basil, as well as other herbs and aromatic plants, is considered a good source of antioxidants (i.e., polyphenols, flavonoids, ascorbic acid, carotenoids, etc.) [1,34]. Herbs could be considered to be commodities with high nutritional value and health benefits. However, during their storage, losses in antioxidants and other compounds might occur, negatively affecting their nutritional value. Moreover, fresh commodities senescing is highly associated with increased metabolism and the ignition of plant defense mechanisms (i.e., the production of antioxidants and antioxidant enzymes' activity) in order to fight the occurring oxidative stress [29]. A decrease in the phenolic content of basil leaves was observed with *O. dubium* EO 0.1%—1 min, whereas 0.01%—10 min resulted in a higher polyphenol content when compared with the control (Table 4). Basil's phenolic content decreased with the application of hydrosol 0.001%—1 min compared to 0.01%—10 min, 0.1%—1 min, 0.01%—1 min and the control (Table 4). The application of EOs has been shown to initiate plant defense mechanisms (i.e., the production of antioxidants) on the applied product [41]. For instance, the application of thyme EO (at concentrations of 0.05, 0.1 and 0.15%) was found to increase lettuce' s antioxidant's capacity and flavonoids [41]. In another study, the application of *O. dubium* EO 0.5% for 20 min on tomato and cucumber fruits resulted in a decrease in antioxidants' capacity [31]. This observation suggests that high EO concentrations and longer times of application cause higher stress, so that eventually antioxidant capacity was found in lower levels. On the other hand, increased antioxidants' activities were observed with 0.01%—1 min, 0.1%—5 min, 0.01%—10 min *O. dubium* EO (DPPH: 8.97, 9.18, and 9.29 mg trolox/g, respectively), whereas lower antioxidant activities were found with 0.001%—5 min (DPPH: 6.38 mg trolox/g). Positive stress (also called eustress) caused by various physical, biological or chemical stressful factors could result in the activation of signaling pathways that would lead to an increased content of bioactive compounds and commodities of higher quality [42]. The application

of thyme EO encapsulated in chitosan nanoparticles was found to decrease the oxidative stress (i.e., the production of hydrogen peroxide and malondialdehyde) on fresh basil and extended the product's shelf life [1]. This supports the antioxidant activity of the EOs and the protective effects against oxidative stress that will lead to early senescing. The application of 0.01%—10 min *O. dubium* EO resulted in increased antioxidant capacity (FRAP: 12.85 mg trolox/g) compared to 0.1%—10 min, 0.01%—5 min, 0.1%—1 min and control (non-treated) (FRAP: 9.87, 9.73, 8.40, and 10.32 mg trolox/g, respectively) (Table 4). On the other hand, Viacava et al. reported an increase in lettuce antioxidant capacity with encapsulated thyme EO immediately after application but not through storage (up to 12 days) [41]. These observations comply with the volatile nature of the EOs and the fact that their activity fades due to their volatile nature. A decrease in basil's antioxidant capacity was observed with hydrosol 0.001%—1 min, 0.1%—5 min and 0.001%—10 min (DPPH: 4.64, 4.91, and 5.45, mg trolox/g, respectively). The application of *O. dubium* hydrosol 0.001%—1 min was found to decrease the antioxidant activity of basil compared to 0.01%—10 min, 0.1%—10 min, 0.1%—1 min, 0.01%—1 min and the control (Table 4). The hydrosols from aromatic plants could contain compounds that are also found in their respective EOs [10]. At the same time, they present great antioxidant activity. As a result, this could have led to the preservation and/or enhancement of basil's antioxidant levels. Previous studies mentioned that EOs and hydrosols, when applied to fresh produce, could increase the phenolic and antioxidant activity of these products, whilst igniting the resistance against postharvest pathogens and diseases [9,43]. In addition, the applied means (EOs, hydrosols, etc.) could be interpreted as abiotic stressors by fresh produce and could ignite defense mechanisms such as an increase in antioxidants [44].

Flavonoids consist of an abundant polyphenolic phytochemical family that can be found in medicinal and aromatic plants as well many other plant-based foods. Many studies have shown the variety of health benefits that these compounds possess including antioxidant, antimicrobial, anti-inflammatory, anticancer and antidepressant properties, among others [45]. Total flavonoids were found to be increased with 0.01%—10 min of *O. dubium* EO (20.46 mg rutin/g) compared to all applied treatments (Table 4). Furthermore, EO applications at 0.1%—5 min, 0.001%—5 min and 0.01%—10 min presented higher flavonoid contents (15.20, 15.10 and 20.46 mg rutin, respectively) compared to 0.1%—1 min (9.40 g rutin/g). The flavonoid content of basil was decreased with the application of hydrosol 0.1%—5 min and 0.001%—1 min after 6 days of storage at 4 °C compared to 0.1%—1 min, 0.1%—10 min, 0.01%—1 min and non-treated samples (control) (Table 4). It is worth mentioning that the flavonoid content of fresh herbs could vary during processing and storage [3]. This could possibly be attributed to the ignition of plant defense mechanisms with longer times of EO application due to the possible stress caused by the postharvest application and or handling during processing [29]. This phenomenon is mainly a response to stress (biotic and/or abiotic) which results in increased polyphenols [46]. Even though stress is caused, the increase in polyphenols (including flavonoids) is considered to be a good thing, as the nutritional value of fresh produce is increased as long as other sensory characteristics are not negatively affected.

Ascorbic acid (i.e., vitamin C) is an antioxidant compound present in basil and other leafy green herbs. This compound is a water-soluble vitamin with great antioxidant activity, whilst highly sensitive to light and heat, especially during the storage of leafy vegetables [47]. The decrease in AA content during the storage of leafy greens at their optimum temperature (0–2 °C) is associated with stress caused by mishandling, harvesting and eventually causes senescing [41]. Fresh basil's AA content increased with *O. dubium* EO 0.01%—10 min and 0.1%—10 min on the last day of storage (day 6) (6.30 and 5.82 mg AA/100 g) as opposed to the control; 0.001%—10 min, 0.1%—1 min and 0.1%—5 min (4.89, 4.81, 4.27, and 4.24 mg AA/100 g, respectively) (Table 4). The application of *O. dubium* hydrosol was found to increase basil's AA content (except at 0.001%—5 min, 0.01%—5 min, and 0.01%—1 min) (Table 4). The increase in AA content during the EO and hydrosol application might be attributed to the ignition of plant defense mechanisms (i.e., the production of

antioxidants such as AA) against the abiotic stress caused by the postharvest applications as well as the antioxidant activity of *O. dubium* EO [38]. The increase in AA could be perceived as an increase in the nutritional value of basil. It has been previously shown that the application of rosemary EO at 0.07% and 0.2% resulted in a significant decrease in rosemary's AA content [16]. The use of different EOs, their concentration and time of application, as well as the different herb investigated, could explain the difference between the two studies.

**Table 3.** Effects of *O. dubium* EO and hydrosol application on fresh basil's pigments (chlorophyll a—Chl a, chlorophyll b—Chl b, total chlorophyll—Tot chl and total carotenoids—Tot car), stored at 4 °C for 6 days.

| | Time (min) | Concentration | Chl a (mg/g) | Chl b (mg/g) | Tot Chl (mg/g) | Tot Car (mg/g) |
|---|---|---|---|---|---|---|
| Day 0 | 0 | 0.00% | 0.38 ± 0.06 | 0.14 ± 0.02 | 0.51 ± 0.08 | 0.09 ± 0.01 |
| EO | 0 | 0.00% | 0.72 ± 0.08 $^{ab*}$ | 0.31 ± 0.07 $^{ab}$ | 1.03 ± 0.14 $^{ab*}$ | 0.15 ± 0.01 $^{ab*}$ |
| | 1 | 0.001% | 0.70 ± 0.03 $^{ab}$ | 0.27 ± 0.02 $^{ab}$ | 0.98 ± 0.06 $^{ab}$ | 0.15 ± 0.01 $^{ab}$ |
| | | 0.01% | 0.61 ± 0.08 $^{ab}$ | 0.22 ± 0.04 $^{ab}$ | 0.83 ± 0.12 $^{ab}$ | 0.13 ± 0.02 $^{ab}$ |
| | | 0.10% | 0.66 ± 0.11 $^{ab}$ | 0.26 ± 0.07 $^{ab}$ | 0.92 ± 0.18 $^{ab}$ | 0.14 ± 0.02 $^{ab}$ |
| | 5 | 0.001% | 0.77 ± 0.04 $^{ab}$ | 0.32 ± 0.03 $^{ab}$ | 1.09 ± 0.07 $^{ab}$ | 0.15 ± 0.00 $^{ab}$ |
| | | 0.01% | 0.81 ± 0.03 $^{a}$ | 0.34 ± 0.02 $^{a}$ | 1.15 ± 0.05 $^{a}$ | 0.17 ± 0.01 $^{a}$ |
| | | 0.10% | 0.62 ± 0.02 $^{ab}$ | 0.22 ± 0.01 $^{ab}$ | 0.84 ± 0.03 $^{ab}$ | 0.14 ± 0.00 $^{ab}$ |
| | 10 | 0.001% | 0.54 ± 0.06 $^{b}$ | 0.19 ± 0.03 $^{b}$ | 0.73 ± 0.09 $^{b}$ | 0.12 ± 0.01 $^{b}$ |
| | | 0.01% | 0.71 ± 0.06 $^{ab}$ | 0.26 ± 0.03 $^{ab}$ | 0.97 ± 0.09 $^{ab}$ | 0.15 ± 0.01 $^{ab}$ |
| | | 0.10% | 0.64 ± 0.12 $^{ab}$ | 0.24 ± 0.05 $^{ab}$ | 0.87 ± 0.017 $^{ab}$ | 0.14 ± 0.02 $^{ab}$ |
| Hydrosol | 0 | 0.00% | 0.59 ± 0.08 $^{abc}$ | 0.22 ± 0.05 $^{abc}$ | 0.81 ± 0.12 $^{abc}$ | 0.13 ± 0.01 $^{abc}$ |
| | 1 | 0.001% | 0.37 ± 0.10 $^{c}$ | 0.13 ± 0.03 $^{c}$ | 0.50 ± 0.13 $^{c}$ | 0.09 ± 0.02 $^{c}$ |
| | | 0.01% | 0.73 ± 0.09 $^{ab}$ | 0.28 ± 0.06 $^{ab}$ | 1.01 ± 0.14 $^{ab}$ | 0.16 ± 0.02 $^{a}$ |
| | | 0.10% | 0.53 ± 0.06 $^{abc}$ | 0.18 ± 0.02 $^{abc}$ | 0.71 ± 0.08 $^{abc}$ | 0.12 ± 0.01 $^{abc}$ |
| | 5 | 0.001% | 0.77 ± 0.03 $^{a}$ | 0.31 ± 0.03 $^{a}$ | 1.07 ± 0.06 $^{a}$ | 0.17 ± 0.01 $^{a}$ |
| | | 0.01% | 0.51 ± 0.05 $^{bc}$ | 0.16 ± 0.01 $^{bc}$ | 0.67 ± 0.06 $^{bc}$ | 0.12 ± 0.01 $^{bc}$ |
| | | 0.10% | 0.54 ± 0.04 $^{abc}$ | 0.19 ± 0.02 $^{abc}$ | 0.73 ± 0.06 $^{abc}$ | 0.11 ± 0.01 $^{bc}$ |
| | 10 | 0.001% | 0.72 ± 0.10 $^{ab}$ | 0.29 ± 0.06 $^{ab}$ | 1.01 ± 0.16 $^{ab}$ | 0.15 ± 0.02 $^{ab}$ |
| | | 0.01% | 0.76 ± 0.06 $^{a}$ | 0.30 ± 0.05 $^{a}$ | 1.06 ± 0.11 $^{a}$ | 0.16 ± 0.01 $^{a}$ |
| | | 0.10% | 0.59 ± 0.09 $^{abc}$ | 0.21 ± 0.04 $^{abc}$ | 0.80 ± 0.13 $^{abc}$ | 0.13 ± 0.02 $^{abc}$ |

Values are presented as the mean ± standard error (four biological replicates per treatment). The values for day 0 refer to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are indicated with different Latin letters for each treatment (EO and hydrosol, separately) in each column. An asterisk (*) indicates significant differences between the initial (day 0) and last day of storage of the control (non-treated, 0.00%).

### The Effects on Damage Indexes

The nutritional value of fresh commodities is reduced during senescing caused by stress factors [48]. During the storage of fresh commodities, the accumulation of free radicals, reactive oxygen species (i.e., singlet oxygen-$^1O_2^*$, superoxide radical ion-$O_2^{\bullet -}$, hydrogen peroxide-$H_2O_2$, hydroxyl radical-$HO^{\bullet}$) and reactive nitrogen species (i.e., nitric dioxide-$NO_2^{\bullet}$, nitric oxide-$NO^{\bullet}$, peroxinitrite- $ONOO^-$) has been reported [49]. This phenomenon is an indication of stress which results in increased metabolism and the rapid deterioration of quality attributes [29]. Increased levels of $H_2O_2$ were observed with a 0.01%—5 min application of EO compared to all other applied treatments (except 0.001%—5 min) (Table 5).

On the other hand, the application of *O. dubium* hydrosol at 0.1%—5 min, 0.001%—10 min and 0.1%—10 min (0.33, 0.33 and 0.32 μmol $H_2O_2$/g, respectively) was found to decrease the production of $H_2O_2$ at the end of storage (day 6) compared to the control (non-treated) (0.42 μmol $H_2O_2$/g). In a previous study, rosemary EO at 0.2% applied for 10 min was found to decrease the $H_2O_2$ production of fresh rosemary even after 12 days of storage at 4 °C [16]. The results of the present study are in accordance with the previous study, suggesting the antioxidant activity of the EOs (especially at higher concentrations, i.e., 0.1% and 0.2%) and their protective effects against oxidative processes. Similarly, the antioxidant activity of *O. dubium* hydrosol could have contributed to the lower $H_2O_2$ levels found in the present study. The application of *O. dubium* EO at 0.1%—5 min and 10 min (all concentrations: 0.001%, 0.01% and 0.1%) were found to decrease the MDA production of basil (5.86, 5.88 and 5.39 nmol MDA/g, respectively) at the end of storage (day 6), as opposed to the control (7.68 nmol MDA/g) (Table 5). A decrease in MDA levels was also observed with a hydrosol application for 1 min (all concentrations); 0.001%—5 min, 0.01%—10 min and 0.1%—10 min compared to the control and 0.01%—5 min. MDA is a secondary derivative from the peroxidation of plant cell wall phospholipids indicating the further process of oxidative stress. This decrease could be attributed to the protective (antioxidant) activity of both EO and hydrosol applied in our study.

**Table 4.** Effects of *O. dubium* EO and hydrosol application on fresh basil's phenols, antioxidants (DPPH, FRAP), total flavonoids and ascorbic acid (AA) content, stored at 4 °C for 6 days.

| | Time (min) | Concentration | Phenols (mg GEA/g) | DPPH (mg trolox/g) | FRAP (mg trolox/g) | Flavonoids (mg rutin/g) | AA (mg AA/100 g) |
|---|---|---|---|---|---|---|---|
| Day 0 | 0 | 0.00% | 3.92 ± 0.23 | 8.42 ± 0.24 | 11.26 ± 0.51 | 13.21 ± 0.59 | 5.52 ± 0.20 |
| EO | 0 | 0.00% | 5.67 ± 0.32 $^{bc*}$ | 7.64 ± 0.24 $^{bcd}$ | 10.32 ± 0.31 $^{bcde}$ | 11.17 ± 1.00 $^{bc}$ | 4.89 ± 0.18 $^{cde}$ |
| | 1 | 0.001% | 5.65 ± 0.23 $^{bc}$ | 8.30 ± 0.05 $^{abc}$ | 11.39 ± 0.42 $^{abcd}$ | 11.97 ± 0.06 $^{bc}$ | 5.75 ± 0.21 $^{abc}$ |
| | | 0.01% | 6.60 ± 0.23 $^{b}$ | 8.97 ± 0.21 $^{a}$ | 11.62 ± 0.86 $^{abcd}$ | 13.01 ± 1.06 $^{bc}$ | 5.39 ± 0.11 $^{bcd}$ |
| | | 0.10% | 4.53 ± 0.21 $^{d}$ | 6.70 ± 0.20 $^{de}$ | 8.40 ± 0.10 $^{e}$ | 9.40 ± 0.28 $^{c}$ | 4.27 ± 0.19 $^{e}$ |
| | 5 | 0.001% | 5.96 ± 0.22 $^{cd}$ | 6.38 ± 0.37 $^{e}$ | 12.17 ± 1.71 $^{abc}$ | 15.10 ± 2.46 $^{b}$ | 5.47 ± 0.39 $^{abcd}$ |
| | | 0.01% | 5.52 ± 0.20 $^{bcd}$ | 7.36 ± 0.26 $^{cd}$ | 9.73 ± 0.02 $^{de}$ | 13.39 ± 2.07 $^{bc}$ | 5.04 ± 0.22 $^{bcde}$ |
| | | 0.10% | 6.20 ± 0.66 $^{b}$ | 9.29 ± 0.34 $^{a}$ | 12.26 ± 0.73 $^{ab}$ | 15.20 ± 1.04 $^{b}$ | 4.24 ± 0.16 $^{e}$ |
| | 10 | 0.001% | 6.09 ± 0.12 $^{b}$ | 8.63 ± 0.43 $^{ab}$ | 12.10 ± 0.41 $^{abc}$ | 12.46 ± 1.58 $^{bc}$ | 4.81 ± 0.35 $^{de}$ |
| | | 0.01% | 8.51 ± 0.57 $^{a}$ | 9.18 ± 0.52 $^{a}$ | 12.85 ± 0.29 $^{a}$ | 20.46 ± 3.16 $^{a}$ | 6.27 ± 0.43 $^{a}$ |
| | | 0.10% | 5.85 ± 0.16 $^{bc}$ | 7.65 ± 0.24 $^{bcd}$ | 9.87 ± 0.38 $^{cde}$ | 10.90 ± 0.76 $^{bc}$ | 5.82 ± 0.27 $^{ab}$ |
| Hydrosol | 0 | 0.00% | 5.83 ± 0.61 $^{ab}$ | 7.92 ± 1.07 $^{a}$ | 10.75 ± 1.24 $^{abc}$ | 13.78 ± 1.03 $^{a}$ | 5.11 ± 0.09 $^{c}$ |
| | 1 | 0.001% | 3.73 ± 0.45 $^{c}$ | 4.64 ± 0.38 $^{c}$ | 8.18 ± 0.32 $^{d}$ | 9.85 ± 1.03 $^{ab}$ | 6.59 ± 0.31 $^{ab}$ |
| | | 0.01% | 6.60 ± 0.63 $^{a}$ | 8.51 ± 0.13 $^{a}$ | 11.85 ± 0.72 $^{a}$ | 13.48 ± 0.99 $^{a}$ | 6.40 ± 0.45 $^{abc}$ |
| | | 0.10% | 6.36 ± 0.26 $^{a}$ | 8.46 ± 0.60 $^{a}$ | 11.60 ± 1.00 $^{a}$ | 12.91 ± 1.89 $^{a}$ | 7.10 ± 0.39 $^{ab}$ |
| | 5 | 0.001% | 5.38 ± 0.61 $^{abc}$ | 6.91 ± 1.04 $^{ab}$ | 9.14 ± 1.40 $^{abcd}$ | 11.68 ± 0.86 $^{ab}$ | 5.83 ± 0.33 $^{bc}$ |
| | | 0.01% | 5.24 ± 0.18 $^{abc}$ | 6.90 ± 0.37 $^{ab}$ | 9.14 ± 0.62 $^{abcd}$ | 10.22 ± 0.77 $^{ab}$ | 5.85 ± 0.26 $^{bc}$ |
| | | 0.10% | 4.47 ± 0.76 $^{bc}$ | 4.91 ± 0.19 $^{bc}$ | 8.11 ± 1.07 $^{bcd}$ | 8.01 ± 0.70 $^{b}$ | 7.41 ± 0.53 $^{a}$ |
| | 10 | 0.001% | 4.27 ± 0.49 $^{bc}$ | 5.45 ± 0.68 $^{bc}$ | 7.68 ± 1.01 $^{cd}$ | 8.18 ± 1.49 $^{b}$ | 7.16 ± 0.47 $^{ab}$ |
| | | 0.01% | 5.90 ± 0.49 $^{ab}$ | 7.92 ± 0.52 $^{a}$ | 10.40 ± 0.87 $^{abc}$ | 11.57 ± 1.28 $^{ab}$ | 7.58 ± 0.73 $^{a}$ |
| | | 0.10% | 5.28 ± 0.71 $^{abc}$ | 7.83 ± 1.02 $^{a}$ | 11.50 ± 1.70 $^{a}$ | 13.44 ± 2.04 $^{a}$ | 6.52 ± 0.38 $^{ab}$ |

Values are presented as the mean ± standard error (four biological replicates per treatment). The values for day 0 refer to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are indicated with different Latin letters for each treatment (EO and hydrosol, separately) in each column. An asterisk (*) indicates significant differences between the initial (day 0) and last day of storage of the control (non-treated, 0.00%).

**Table 5.** Effects of *O. dubium* EO and hydrosol application on fresh basil's $H_2O_2$ and MDA production, stored at 4 °C for 6 days.

| | Time (min) | Concentration | $H_2O_2$ (μmol/g) | MDA (nmol/g) |
|---|---|---|---|---|
| Day 0 | 0 | 0.00% | 0.26 ± 0.02 | 4.00 ± 0.10 |
| EO | 0 | 0.00% | 0.37 ± 0.04 $^{b}$ | 7.04 ± 0.24 $^{ab*}$ |
| | 1 | 0.001% | 0.39 ± 0.01 $^{b}$ | 6.73 ± 0.49 $^{abc}$ |
| | | 0.01% | 0.40 ± 0.03 $^{b}$ | 7.68 ± 0.44 $^{a}$ |
| | | 0.10% | 0.39 ± 0.02 $^{b}$ | 6.69 ± 0.34 $^{abc}$ |
| | 5 | 0.001% | 0.43 ± 0.01 $^{ab}$ | 6.84 ± 0.40 $^{ab}$ |
| | | 0.01% | 0.50 ± 0.05 $^{a}$ | 6.41 ± 0.37 $^{abc}$ |
| | | 0.10% | 0.37 ± 0.01 $^{b}$ | 5.86 ± 0.33 $^{bc}$ |
| | 10 | 0.001% | 0.39 ± 0.03 $^{b}$ | 5.88 ± 0.36 $^{bc}$ |
| | | 0.01% | 0.39 ± 0.01 $^{b}$ | 5.75 ± 0.42 $^{bc}$ |
| | | 0.10% | 0.37 ± 0.02 $^{b}$ | 5.39 ± 0.64 $^{c}$ |
| Hydrosol | 0 | 0.00% | 0.42 ± 0.01 $^{a*}$ | 6.65 ± 0.15 $^{a*}$ |
| | 1 | 0.001% | 0.35 ± 0.01 $^{ab}$ | 4.19 ± 0.55 $^{e}$ |
| | | 0.01% | 0.38 ± 0.05 $^{ab}$ | 4.77 ± 0.35 $^{de}$ |
| | | 0.10% | 0.32 ± 0.01 $^{b}$ | 4.81 ± 0.21 $^{de}$ |
| | 5 | 0.001% | 0.38 ± 0.02 $^{ab}$ | 5.75 ± 0.07 $^{bc}$ |
| | | 0.01% | 0.38 ± 0.01 $^{ab}$ | 6.73 ± 0.13 $^{a}$ |
| | | 0.10% | 0.33 ± 0.01 $^{b}$ | 6.09 ± 0.10 $^{ab}$ |
| | 10 | 0.001% | 0.33 ± 0.01 $^{b}$ | 6.05 ± 0.31 $^{ab}$ |
| | | 0.01% | 0.38 ± 0.05 $^{ab}$ | 5.05 ± 0.12 $^{cd}$ |
| | | 0.10% | 0.32 ± 0.01 $^{b}$ | 4.49 ± 0.08 $^{de}$ |

Values are presented as the mean ± standard error (four biological replicates per treatment). The values for day 0 refer to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are indicated with different Latin letters for each treatment (EO and hydrosol, separately) in each column. An asterisk (*) indicates significant differences between the initial (day 0) and last day of storage of the control (non-treated, 0.00%).

From the preliminary screening, the two most promising doses (i.e., combinations of concentration and time) for each tested product (EO and hydrosol) were selected for further investigations of their antibacterial activity against *S. enterica* and *L. monocytogens* on fresh basil. The selection was based on the doses' abilities to preserve fresh basil's visual quality (appearance) and marketability as well as their positive effects on other investigated parameters such as respiration rate and nutritional value (i.e., antioxidant capacity and ascorbic acid content). It must be mentioned that the selected EO doses (dose A: 0.01%—1 min and dose B: 0.001%—5 min) and hydrosol doses (dose A: 0.1%—5 min and dose B: 0.01%—10 min) preserved the visual quality of fresh basil. However, the two EO doses were found to increase basil's respiration rate at the end of storage and presented a decrease in the product's marketability scoring from 8 to 6, which still refers to a marketable product of medium quality according to the marketability evaluation scale (where 5: marketable with few defects, i.e., wilting, decolorization (medium quality). Moreover, the selected hydrosol doses were found to result in slightly increased weight loss and respiration at the end of storage. However, this increase was less than 3% (this percentage indicates adverse quality attributes) [30]. At the same time, hydrosol dose A resulted in decreased phenols, antioxidant activity (DPPH) and flavonoids, indicating less stress (supported by lower $H_2O_2$ levels) due to its antioxidant properties [9,10].

### 3.2. In Vivo Antibacterial Activity

The contamination of leafy greens and herbs with foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica*, *Listeria monocytogenes*, *Shigella* spp. and *Staphylococcus aureus* can occur at any point of the food chain (cultivation, harvest, postharvest processing and packaging) [50]. One of the main crucial postharvest processes in an attempt to eliminate pathogens and lower a product's microbial load is decontamination with chlorinated water [7]. However, an unsuccessful washing could result in the survival and proliferation of pathogens on leaf surfaces that evidently will reach consumers [51]. There have been previous reports that the use of chlorinated water as a fresh produce disinfectant can leave harmful residues on the final products of harmful compounds such as trihalomethanes and haloketones, which have been characterized as potentially carcinogenic [6,7].

As alternative decontamination means, the use of natural products (i.e., EOs, hydrosols, plant extracts and natural compounds) with antimicrobial activity has gained great interest in the scientific community as well as the food industry [8,52]. The great antimicrobial and antioxidant activity of *O. dubium* EO and hydrosol in vitro and in vivo have been previously reported [31,38]. During the present study, it was found that all applied treatments decreased the population of *S. enterica* after one day post-application, whereas after six days of storage all applied treatments (except hydrosol, both doses) resulted in lower *S. enterica* numbers (up to 1.68 log decrease with EO dose A- 0.01%—1 min) (Table 6). Hydrosols from rosemary and thyme were found to decrease *E. coli* O157:H7 and *Salmonella enterica* subsp. *enterica* serovar Typhymurium inoculated on apples [53]. In the same study, other hydrosols (black cumin, sage and bay leaf) were also investigated, presenting different antibacterial activities [53]. This complies with our study and suggests that the antimicrobial activity of hydrosols depends on their plant origin, concentration and time of application, among other parameters.

**Table 6.** Effects of *O. dubium* EO and hydrosol in vivo application against *S. enterica* and *L. monocytogenes* inoculated on fresh basil stored at 4 °C for 6 days.

| | Concentration | *S. enterica* (log cfu/mL) | *L. monocytogenes* (log cfu/mL) |
|---|---|---|---|
| Day 1 | Control | 6.29 ± 0.09 $^{a}$ | 6.09 ± 0.06 $^{a}$ |
| | Chlorine | 4.37 ± 0.12 $^{c}$ | 5.11 ± 0.10 $^{bc}$ |
| | EO dose A | 4.53 ± 0.11 $^{c}$ | 5.35 ± 0.08 $^{b}$ |
| | EO dose B | 4.72 ± 0.33 $^{bc}$ | 4.88 ± 0.12 $^{c}$ |
| | Hydrosol dose A | 5.24 ± 0.15 $^{b}$ | 5.28 ± 0.05 $^{b}$ |
| | Hydrosol dose B | 4.68 ± 0.31 $^{bc}$ | 5.01 ± 0.03 $^{c}$ |
| Day 6 | Control | 6.13 ± 0.02 $^{a}$ | 6.04 ± 0.05 $^{a}$ |
| | Chlorine | 4.94 ± 0.23 $^{bc}$ | 4.36 ± 0.09 $^{d}$ |
| | EO dose A | 4.45 ± 0.16 $^{c}$ | 4.35 ± 0.06 $^{d}$ |
| | EO dose B | 5.18 ± 0.30 $^{b}$ | 4.46 ± 0.11 $^{d}$ |
| | Hydrosol dose A | 5.77 ± 0.04 $^{a}$ | 4.88 ± 0.05 $^{c}$ |
| | Hydrosol dose B | 5.72 ± 0.15 $^{a}$ | 5.39 ± 0.06 $^{b}$ |

Values are presented as the mean ± standard error (four biological replicates per treatment). Significant differences ($p < 0.05$) between the treatments are indicated with different Latin letters on each day. Control (0.00%, sterile $dH_2O$); Chlorine (0.02%); EO dose A (0.01% for 1 min); EO dose B (0.001% for 5 min); hydrosol dose A (0.1% for 5 min); and hydrosol dose B (0.01% for 10 min).

A previous study by de Medeiros Barbosa et al. reported great antimicrobial activity (up to a 5 log reduction) of *Origanum vulgare* and *Salvia rosmarinus* EOs against *Salmonella enterica* subsp. *enterica* serovar Enteritidis, *L. monocytogenes* and *E. coli* on chard and iceberg lettuce [54]. Another study mentioned a 3.2 log reduction with oregano EO application

on spinach and lettuce leaves inoculated with *Salmonella enterica* subsp. *enterica* serovar Newport, with EO concentrations ranging between 0.1 and 0.5% *v/v* [14]. It is worth mentioning that the great antimicrobial activity of oregano EO is attributed to its main compound (found in high levels), carvacrol [14,54]. Carvacrol is a is a monoterpenoid phenol and it is found in the EOs of many species of the Lamiaceae family including *Origanum*, *Thymus*, *Thymbra* and *Satureja* [55]. Moreover, this compound is identified by the European Commission and the Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS) to use in food (as an additive) [56]. Notably, the mechanisms behind the antimicrobial activity of carvacrol are not completely described. However, some studies reported the interaction of carvacrol with the phospholipid-linked acyl chains of the bacterial cell membrane, leading to the disruption and leakage of intercellular components [57].

The survival and proliferation of *L. monocytogenes* on fresh produce surfaces (including leafy greens and herbs) depends on the properties of the surface, like the presence of stomata and niches (i.e., crevices, spaces and wounds) [58]. Moreover, the existing surrounding microflora, nutrient and moisture availability also play a crucial role in the establishment and survival of foodborne pathogens on leafy vegetables [58]. During the first day of storage, *L. monocytogenes* was found in lower numbers on basil treated with *O. dubium* EO dose B (0.001%—5 min) (4.88 log cfu/mL) and hydrosol dose B (0.01%—10 min) (5.01 log cfu/mL) compared to the non-treated ones (control) (6.09 log cfu/mL) (Table 6). The antibacterial activity of the investigated hydrosol might be attributed to its composition (hydrosols contain some common compounds found in their respective EOs) in combination with its low pH value (in our case pH: 4.14). It is well known that the exposure of bacterial cells to pH values below their optimum could affect their survival (i.e., decrease in enzymatic activity) [59]. However, due to the low pH of hydrosols, the degradation of some compounds might occur and result in lower antimicrobial activity [9]. On the last day of storage (day 6), all applied treatments were found to decrease *L. monocytogenes* numbers, and the decrease was greater with *O. dubium* EO (both doses) and chlorine treatments (up to 1.69 log decrease with EO dose A, followed by chlorine—a 1.67 log reduction and EO dose B—a 1.58 log reduction). As it seems from the results of this study, the EO from Cypriot oregano was able to significantly decrease *L. monocytogenes* proliferation on fresh basil after one and six days of application. This might be the result of bacterial injury caused by the EO that eventually leads to bacterial death [60].

Many previous studies showed that the variation of the antimicrobial activity among EOs and hydrosols from aromatic plants against foodborne pathogens depends on their composition, concentration and application (dipping/vapor and time of application), as well as bacterial characteristics (i.e., Gram stain, cell wall and membrane composition, cell shape, strain and serotype) [58,61]. One of the main antibacterial mechanisms of action for EOs and hydrosols is the disruption of the cell wall caused by damages to the lipid bilayer [62]. This phenomenon evidently results in crucial changes on the cell wall permeability and the leakage of intracellular components. Other mechanisms include the interruption of ion and proton pumps, the inhibition of cell metabolism and the obtrusion of enzymes' activity [8,52,62]. In addition, it is well known that the action of EOs against Gram-positive bacteria (i.e., *L. monocytogenes*) is greater than the one against Gram-negative bacteria (i.e., *S. enterica*), mainly due to the outer cell wall membrane [61]. This supports the findings in our study. In addition, previous in vitro studies showed the great antibacterial activity of *O. dubium* EO against Gram-positive bacteria (i.e., *S. aureus*, *Micrococcus luteus*, and *Bacillus cereus*) compared to Gram-negative ones (i.e., *E. coli*, *Proteus mirabilis*, *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, *Pseudomonas talassi*, and *S.* Enteritidis) [36,38].

From the present study, it was evident that the EO and hydrosol from *O. dubium* were able to control the growth of *S. enterica* and *L. monocytogenes* during the six days of storage of fresh basil. However, as shown from previous studies and the present study increasing the concentration and time of application might present great antimicrobial activity, but this will probably negatively affect the quality of fresh commodities (especially of leafy

greens) [31,53]. In addition, a recent study reported the cytotoxicity signs of *O. dubium* EO at 0.001–0.05% in normal and tumorigenic human mesenchymal stem cells (hMSC-telo1) [63]. It is worth mentioning that the ethanolic extracts of *O. dubium* significantly reduced the viability of ovarian cancer cells (SkoV3) in vitro (1000 μg/mL for 72 h) [64]. In order to avoid these and the possibility of phyto- and cytotoxicity effects, caution should be taken when using natural products such as EOs and hydrosols with high biological activities. Even lower concentrations of *O. dubium* EO should be further assessed, while cytotoxicity tests on the hydrosol need to be assessed.

## 4. Conclusions

With the present study, the effects of Cypriot oregano (*O. dubium*) EO and hydrosol were evaluated for their efficacy to preserve fresh basil's quality characteristics and the elimination of foodborne pathogens. It was found that the application of oregano EO at high concentrations for longer times resulted in a less acceptable/marketable commodity, whilst hydrosol application presented a more acceptable and marketable commodity. An increase in AA content and antioxidants was observed with EO application, whereas hydrosol application resulted in a decrease in basil's polyphenols and antioxidant capacity. The increase in antioxidant capacity and AA content could also be reported as an increase in the nutritional value of basil. A significant reduction in the two investigated foodborne pathogens (*S. enterica* and *L. monocytogenes*) inoculated on fresh basil was observed especially against *L. monocytogenes* (EO: up to 1.21 log reduction and hydrosol: up to 1.08 log reduction). In conclusion, the application of *O. dubium* EO and hydrosol are promising as alternative sanitizing agents of the washing water during the postharvest processing of fresh basil despite that some slightly negative observations that have been reported (that did not result in severe senescing at the end of storage). However, there is essential need for further investigation regarding the application of these natural products and on other fresh produce (via dipping or vapor alone and/or in combination with other processes such as modified atmosphere) by keeping in mind the possible phytotoxic and cytotoxic effects as a dose–response effect.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10020159/s1, Figure S1: Effects of the preliminary screening of *O. dubium* EO and hydrosol application (different concentrations and times of application) on fresh basil stored at 4 °C for six days.

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