horticulturae

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The Effect of Salinity and Drought on the Essential Oil Yield and Quality of Various Plant Species of the Lamiaceae Family (*Mentha spicata* L., *Origanum dictamnus* L., *Origanum onites* L.)

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Abstract: *Mentha spicata* L., *Origanum dictamnus* L., and *Origanum onites* L. are aromatic plants that produce very important essential oils. They are considered model plants with beneficial health properties due to their antioxidant content. Enhancing the yield while maintaining the quality of essential oil is of significant commercial importance. Salinization and drought cause various effects on the yield and quality of the bioactive constituents in essential oil. By assessing the response of these plants and their secondary metabolites accumulation to different salt stress and irrigation levels, this study aims to gain insights into how plants adapt to and cope with salinity and drought. A pot experiment was conducted in the spring of 2020 to assess the effect of salinity and drought stress on the growth and essential oils content of the three aromatic plant species mentioned above. The soil mixture used was perlite and peat in a ratio of 1:1:6, while four salinity treatments (25, 50, 100, and 150 mM NaCl) and two levels of irrigation were applied (100% and 50%). Salinity significantly affects total chlorophyll concentration especially in higher concentrations (100 and 150 mM) in *M. spicata* plants, especially under 50% soil water irrigation. Under the same conditions, *M. spicata* contained the higher proline concentration, which was significantly greater than that in *O. dictamnus* and *O. onites*. Similar variations of malondialdehyde and hydrogen hyperoxide were revealed among the three species, with significantly higher values in *M. spicata* when subjected to both excess salinity and drought conditions. The major compounds identified in *M. spicata* were carvone, in *O. dictamnus* carvacrol, and *p*-cymene and in *O. onites* carvacrol. It is important to highlight that *O. onites* had the highest concentration of essential oil, and that the concentration increased with the increase of NaCl. This suggests that the presence of NaCl in the soil may have a stimulating effect on the production of essential oil in *O. onites*. However, it is plausible that the stress caused by NaCl triggers a physiological response in *O. onites*, leading to increased production of essential oil. This could be a protective mechanism to enhance the plant's resistance to the stressor. Overall, *O. onites* and *O. dictamnus* appeared to be more resistant to these stress conditions than *M. spicata*, since they maintained their growth and essential oil quality indicators at higher levels. These two species possess mechanisms that prevent or minimize lipid peroxidation, thus protecting their cell membranes and maintaining their ultrastructure integrity.

Keywords: proline; secondary metabolites; lipid peroxidation; essential oils; aromatic plants

Citation: Stefanakis, M.K.; Giannakoula, A.E.; Ouzounidou, G.; Papaioannou, C.; Lianopoulou, V.; Philotheou-Panou, E. The Effect of Salinity and Drought on the Essential Oil Yield and Quality of Various Plant Species of the Lamiaceae Family (*Mentha spicata* L., *Origanum dictamnus* L., *Origanum onites* L.). *Horticulturae* **2024**, *10*, 265. https://doi.org/10.3390/horticulturae10030265

Academic Editor: Othmane Merah

Received: 2 January 2024
Revised: 28 February 2024
Accepted: 28 February 2024
Published: 10 March 2024

1. Introduction

The cultivation of aromatic plants aims to exploit the parts that contain active compounds that are used in various ways, especially for medicinal purposes [1]. The essential oils (EOs) that act as antiparasitics or disinfectants display a broad range of antioxidant, antimicrobial, antiseptic, antifungal, antibacterial, cytotoxic/anticancer, antigenotoxic, and antiviral activities [1]. The morphological and chemical diversity of the genus *Origanum* is extraordinary. Out of 67 taxa, nine species thrive in Greece, including 18 hybrids, the majority of which are found throughout the Mediterranean region [2]. The classification of *Origanum* is quite intricate. It is difficult, for many species, to define sectional boundaries, making their morphological markers inefficient for differentiating them [3]. Genetic, environmental, physiological, and agronomic variables affect the volatile oils' chemical compositions [4,5]. Carvacrol is the main essential oil component in oregano herbs, responsible for the characteristic "oregano" scent. Other volatile compounds that dominate the essential oils of oregano species are thymol, *p*-cymene, and γ-terpinene, whereas in *O. onites* the dominant compound is borneol [1,6–9]. The genus *Mentha* includes more than 30 species of herbaceous perennial plants. *Mentha spicata* (*M. spicata*) spreads mainly in the temperate and subtemperate zones of the world. It is considered a rich source of essential oils, which are widely used in the pharmaceutical industry and in food production [10]. The major compounds found in the essential oil of *M. spicata* are carvone, limonene, and 1,8-cineole. These findings are consistent with literature data [1,11].

Changes in climatic conditions lead to increased biotic and abiotic stress for the plants. The productivity of aromatic plants was affected globally due to these stresses [12]. Plant growth and essential oil production are influenced by various environmental factors, drought and salinity stresses being two of them. Other factors such as geographical area of cultivation, cultivation cycle, cultivation conditions, harvest year, cultivars or varieties, and age, can also influence essential oil production and its composition [13].

Saline soil has a negative impact on plants, leading to physiological and metabolic disturbances. This affects various aspects of plant development, growth, yield, and the quality of essential oils, especially in aromatic plants. Salinity can hinder seed germination, reduce survival percentage, alter morphological characteristics, and decrease overall EO yield and its components [14]. The discovery of high-yielding genotypes of these plants is particularly promising, as numerous studies have documented the response of aromatic plants to salinity stress [15–18]. The tolerance to salinity depends on the interaction between salinity and other environmental factors (such as drought stress) [19]. Salt stress is a significant challenge for aromatic plants and can have various negative effects on their growth and productivity. One of the primary impacts of salt stress is a decrease in osmotic potential, leading to reduced availability of water for plants. This can result in water stress and negatively affect plant growth. In addition to water stress, salt stress also affects the physical structure of the soil. It diminishes water permeability and soil aeration, further hindering plant growth.

Drought stress significantly reduces plant yield and modifies the polyphenol content as well as the antioxidant capacities of plants [20]. Its impact on the amount of essential oils, antioxidant properties, and polyphenol content, however, is also a subject of debate. Despite reports of increased essential oil content and improved antioxidant activity during drought treatment, the scientific data were insufficient to make any broader conclusions, according to Kren et al. (2012) and Szabo et al. (2020) [21,22]. Thus, further studies to determine the optimal water supply for different species are paramount.

Overall, salt stress poses a significant obstacle to agricultural production. The aim of this study is to understand the adverse effects of salt stress in combination with drought in specific Lamiaceae species (*M. spicata*, *O. dictamnus*, *O. onites*), in order to develop strategies to mitigate its impact and improve plant productivity in these challenging environments. We chose these three species of the Lamiaceae family as they are endemic to the collection areas while these areas are at risk of salinity and drought due to the intensive use of fertilizers and climate change.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Origanum dictamus (dittany) plants were transferred from the primary material of the area of Messara (Coordinates; 34°57′57″ N, 24°50′26″ E). *Origanum onites* (oregano) plants were collected from the seed of a mother plantation in Agiasos of Lesvos, (Coordinates; 39°04′77″ N, 26°22′06″ E). *Mentha spicata* (spearmint) was transferred as rooted cuttings from plants of the Stylia area in Corinthia (Coordinates; 37°59′54″ N, 22°33′37″ E).

The experiment was established in spring of 2020 with the above plants of three-year growth set up in pots in a greenhouse. The soil substrate consisted of a mixture of soil, perlite, and peat in a ratio of 1:1:6. Fertilization was applied to the plants on 10 November 2020 with 8 units of N, 8 units of P, and 13 units of K in the following forms: 65 g NH_4NO_3, 60 g KH_2PO_4, and 70 g KNO_3. On 22 March 2021, four salinity treatments were chosen after preliminary experiments (25 mM NaCl, 50 mM NaCl, 100 mM NaCl, and 150 mM NaCl); per treatment, 1.462 g NaCl (*w*/*v*), 2.926 NaCl, 5.85 NaCl, and 8.78 g NaCl were dissolved and each pot was irrigated with 0.39 g NaCl per 0.5 l water. The experimental design was completely randomized with four replicates.

Every two days, the soil moisture was measured using a Theta probe ML2, and watering schedules were adjusted to keep the soil moisture levels at 50% of the water capacity. Water was applied at a 50% capacity to all plants with the exception of the control plants. A tape measure was used to measure the height of the plants. Harvesting was performed at 8 cm height using a sickle. The plants were then dried in an airy shed.

2.2. Chlorophyll Estimation

After sixty days from the beginning of the treatment with salt, a sample of a third of the top completely grown leaf was taken for chlorophyll analysis. Fresh leaf blade material (0.1 g) was put in 25 mL glass test tubes, and 15 mL of 96% (*v*/*v*) ethanol was poured into each tube in order to estimate the amount of chlorophylls. The plant material-filled tubes were kept in an incubator set at 79.8 °C for three to four hours, or until the samples had completely discolored. The measurement of chlorophyll a and b absorption was conducted at 665 and 649 nm, correspondingly. The total chlorophyll calculation method was applied according to Wintermans and Mots (1988) [23].

2.3. Determination of Proline

Each plot's fully grown leaf samples were sliced into small pieces. Roughly 0.3 g of these samples was weighed and then individually added to glass vials holding 10 mL of 80% (*v*/*v*) ethanol. After 30 min of heating at 60 °C, the extracts were filtered and diluted with 80% (*v*/*v*) ethanol to a volume of 20 milliliters. The acid ninhydrin reagent method was used to quantify the free Pro content in various plant extracts [24]. Then, 500 mM of thick H_2SO_4 was mixed with around one gram of ninhydrin. Next, two mM each of the aqueous alcohol extract and acid ninhydrin were put into test tubes. To reduce evaporation, glass marbles were placed over the test tubes, which were kept at 95 °C for 60 min. They were then allowed to cool to room temperature. Lastly, 4 mL of toluene was added to each sample replicate. After the solution layers were separated, the toluene layer was carefully decanted, put in glass corvettes, and its absorbance at 518 nm was measured.

2.4. Estimation of Lipid Peroxidation and Hydrogen Hyperoxide

At the end of the experimental period (see above), the amount of lipid peroxidation of Lamiaceae leaves was quantified, and the concentration of malondialdehyde (MDA) was determined through reaction with 2-thiobarbituric acid (TBA) [25]. To a sample of 0.1 g of fresh leaf blade tissue, 0.5 mL of 0.1% (*w*/*v*) trichloroacetic acid (TCA) was added to homogenize the samples. For ten minutes, the homogenate was centrifuged at 15,000× *g* and 4 °C. A mixture of 0.5 mL of the supernatant and 1.5 mL of 0.5% TBA diluted in 20% TCA was prepared. Incubation lasted 25 min at 95 °C. Further incubation in an ice bath brought the process to a halt. Following a 10 min centrifugation at 10,000× *g* and

4 °C, the absorbance of the supernatant was measured at 532 and 600 nm. The value at 532 nm was used as the substrate for the non-specific absorption value at 600 nm. Using the MDA extinction coefficient of 155 mM^{-1} cm^{-1} and Lambert–Beer's equation, the concentration of MDA was determined [26]. The findings are displayed as µmol MDA g^{-1} FW. Following the literature procedure, the H_2O_2 concentration of leaves was determined [27,28]. Then, 500 mg of fresh leaf material was homogenized in 3 mL of 1% TCA. Following centrifugation, 1.5 mL of 1 M KI and 10 mM K buffer (0.75 mL) were added to 0.75 mL of the filtrate, and the absorbance of each sample was measured at 390 nm.

2.5. Isolation of Essential Oils

For 10 days, the collected plant material was allowed to air dry at room temperature in the shade and darkness. In order to minimize hydrodistillation overheating artifacts, each sample was subjected to hydrodistillation three times for two hours using a modified Clevenger-type apparatus with a water-cooled oil receiver. Following normal protocols, the volatiles were trapped in 5 mL of gas chromatography-grade n-pentane [29]. They were then dried over anhydrous sodium sulfate and stored in closed, airtight Pyrex containers at −4 °C. The amount of essential oil is stated as mL per 100 g^{-1} d.w.

2.6. GC-MS Analysis of Essential Oils

The GC-MS analysis of the extracted volatile oils was carried out using a Shimadzu GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu GCMS-QP 5050 mass-selective quadrupole mass spectrometer as the detector and the appropriate data system. The fused silica capillary column (Supelco, Merck, München, Germany, SBP-5, with 0.25 µm film thickness, 30 m × 0.25 mm i.d.) was directly linked to the ion source, and the GC was outfitted with a Grob-type split-splitless injector. A carrier gas of helium with a back pressure of 0.8 Atm was employed. The injector temperature was 250 °C and the oven temperature program was started at 50 °C for 5 min and then increased at a rate of 5 °C/min up to 150 °C, was retained at this temperature for 10 min and increased again at a rate of 5 °C/min up to 280 °C, where it remained for 20 min. The range of the scan was 30–700 m/z. With an ionization energy of 70 eV, a GS-MS detection electron ionization system was employed.

2.7. Identification and Quantification of Essential Oils Components

Based on the mass spectrometer's detection of the total number of fragments (total ion count) of the metabolites, the components were quantified. The chemical components were identified by analyzing their mass spectra using the NIST21, NIST107, and PMW_TOX2 mass spectra libraries, and by comparing each component's retention time (Rt) with those of commercially accessible compounds [30]. Additionally, components were identified by contrasting the data with those from the literature [31]. Retention indices were computed using the standard hydrocarbon retention durations (C_9–C_{25}) as a guide, based on the work of Van den Dool and Kratz (1963) [32]. Co-injection with standard substances was also performed when needed.

2.8. Statistical Analysis

The experimental layout included three species and four salt concentrations, and four replicates (plants) per treatment. Data were subjected to analysis of variance (ANOVA). For comparison of means, the Duncan multiple range test was used ($p \leq 0.05$) using the SPSS 24.0 statistical package (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Plant Growth

In dittany and oregano, a 100% watering regime and higher NaCl concentrations caused a trend toward an increase in plant height at the end of bloom that was roughly 17% higher than that of the control (Table 1). At the same conditions, no changes in spearmint

height were revealed. The application of 50% irrigation and high NaCl concentration induced similar results in plant height in all of the plants. The height of the plants did not differ between salinity treatments, but the dry weight of dittany and oregano increased significantly. The ratio of dry weight to fresh weight did not seem to be affected between salinity treatments.

Table 1. Effect of NaCl in height (cm) and dry weight/fresh weight of different Lamiaceae species *Mentha spicata* L., *Origanum dictamnus* L., and *Origanum onites* L. Each value is the mean of 4 replications ± standard error.

Treatnents	First Month of Treatment 100% Irrigation	At the End of Blooming 100% Irrigation	First Month of Treatment 50% Irrigation	At the End of Blooming 50% Irrigation	DW/FW 100% Irrigation	DW/FW 50% Irrigation
0 mM NaCl						
Spearmint	16.27 ± 1.72	32.00 ± 2.45	16.00 ± 1.30	31.00 ± 2.30	0.68 ± 0.07	0.64 ± 0.07
Dittany	17.20 ± 2.39	23.60 ± 1.14	17.00 ± 2.10	23.00 ± 1.40	0.17 ± 0.05	0.16 ± 0.02
Oregano	14.60 ± 1.82	25.10 ± 0.90	14.50 ± 1.82	24.60 ± 0.89	0.63 ± 0.03	0.70 ± 0.06
25 mM NaCl						
Spearmint	16.17 ± 1.47	33.67 ± 1.86	16.00 ± 1.40	33.00 ± 1.60	0.67 ± 0.02	0.60 ± 0.03
Dittany	15.20 ± 1.31	26.60 ± 3.91	15.00 ± 1.10	26.00 ± 2.10	0.21 ± 0.03	0.20 ± 0.04
Oregano	13.00 ± 0.71	27.80 ± 3.96	12.60 ± 0.60	26.90 ± 2.40	0.64 ± 0.06	0.67 ± 0.07
50 mM NaCl						
Spearmint	15.40 ± 1.79	31.67 ± 3.37	15.00 ± 1.50	32.50 ± 3.20	0.72 ± 0.07	0.67 ± 0.06
Dittany	17.20 ± 2.77	26.20 ± 1.92	17.00 ± 1.60	26.00 ± 1.92	0.22 ± 0.02	0.20 ± 0.03
Oregano	14.60 ± 2.30	27.60 ± 2.07	14.00 ± 1.30	26.40 ± 1.87	0.61 ± 0.09	0.57 ± 0.08
100 mM NaCl						
Spearmint	15.17 ± 0.75	31.17 ± 1.94	15.00 ± 0.50	31.50 ± 1.40	0.70 ± 0.11	0.68 ± 0.09
Dittany	19.60 ± 2.68	26.00 ± 2.60	19.00 ± 1.80	25.40 ± 2.30	0.23 ± 0.04	0.21 ± 0.03
Oregano	16.40 ± 2.05	27.60 ± 2.80	16.20 ± 2.50	26.60 ± 2.10	0.69 ± 0.02	0.60 ± 0.04
150 mM NaCl						
Spearmint	15.67 ± 1.21	32.17 ± 2.9	15.50 ± 1.10	32.00 ± 2.20	0.68 ± 0.17	0.66 ± 0.10
Dittany	17.80 ± 2.68	27.80 ± 2.24	16.00 ± 2.68	27.00 ± 2.35	0.20 ± 0.01	0.18 ± 0.02
Oregano	15.20 ± 2.05	28.80 ± 2.78	14.70 ± 1.50	27.40 ± 2.60	0.70 ± 0.05	0.63 ± 0.08

3.2. Secondary Metabolites

3.2.1. Proline and Chlorophyll Content

At the highest concentration (150 mM) of NaCl and simultaneous 50% irrigation, spearmint contained the highest proline concentrations, which were significantly greater compared to those of oregano and dittany species in the same conditions (five times higher than the control), ($p \leq 0.05$). A progressive increase in proline content was revealed in all studied species with increasing dose of NaCl. Spearmint seems to be more susceptible to salinity and drought as it showed a drastic increase even at 50 mM NaCl in both irrigation treatments (by four and four-and-a-half times, respectively). Salinity or drought affected the proline concentration less in dittany and oregano showing an increase two-and-a-half and three times higher under 50% irrigation and 150 mM NaCl, respectively (Figure 1).

The highest chlorophyll contents were found in oregano that was well irrigated without salinity treatment. Salinity significantly affects total chlorophyll concentration in higher concentrations (100 and 150 mM) in spearmint plants, especially under 50% soil water irrigation (Figure 2). In addition, salinity and drought negatively affect the total chlorophyll's concentration in dittany and oregano species but to a lower degree (Figure 2). At the higher concentrations (150 mM) of NaCl and 50% irrigation, spearmint contained the lowest chlorophyll concentrations, which were significantly lower than those of oregano and dittany species ($p \leq 0.05$).

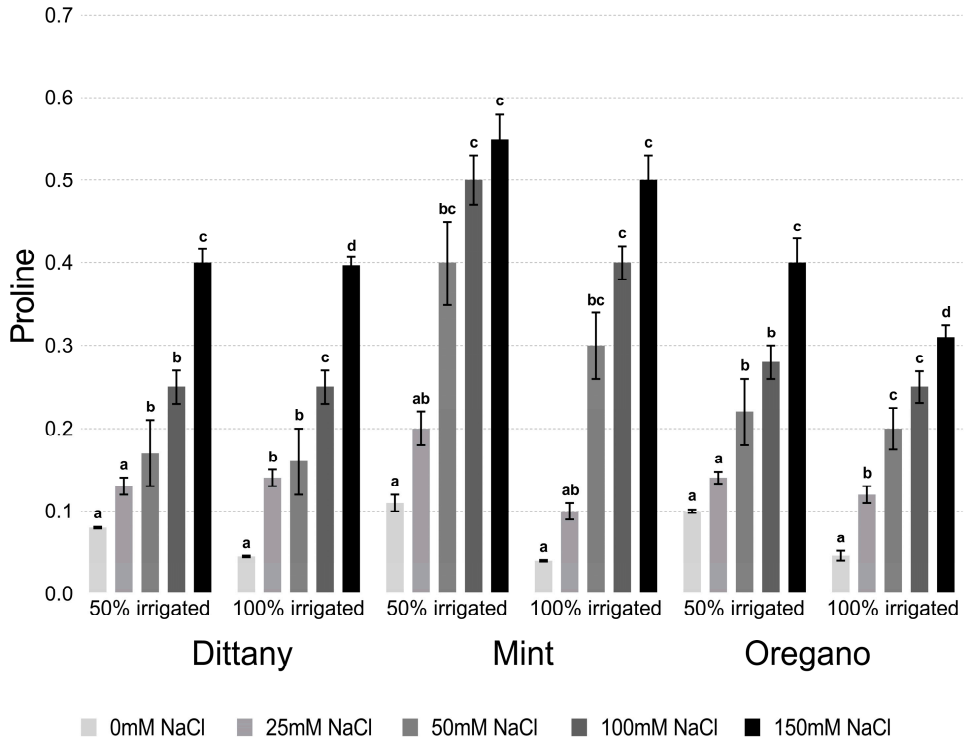

Figure 1. Effect of NaCl on proline (mg g^{-1} FW) content in leaves of different Lamiaceae species *Origanum dictamnus* L., *Mentha spicata* L., and *Origanum onites* L. Each value is the mean of 4 replications ± standard error. Means not sharing the same letter are significantly different at $p \leq 0.05$.

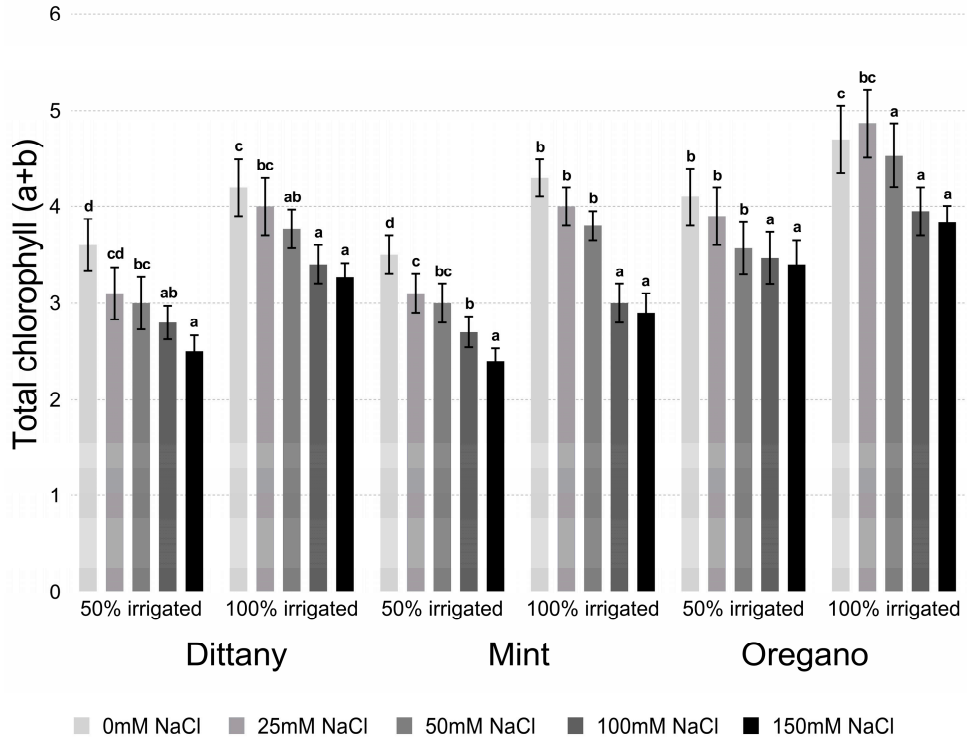

Figure 2. Effect of NaCl on total chlorophyll (a + b) (mg g^{-1} FW) content in leaves of different Lamiaceae species *Origanum dictamnus* L., *Mentha spicata* L., and *Origanum onites* L. Each value is the mean of 4 replications ± standard error. Means not sharing the same letter are significantly different at $p \leq 0.05$.

3.2.2. Lipid Peroxidation and Hydrogen Peroxide Assays

Salinity conditions combined with drought significantly increased the concentration of MDA in all species of the Lamiaceae family, as observed in Figure 3. Concentrations of MDA in salinity treatments, especially in higher concentrations of spearmint plants, were about ten times higher than those in the control plants (Figure 3). Moreover, it was noticed that MDA negatively contributed to spearmint salinity tolerance.

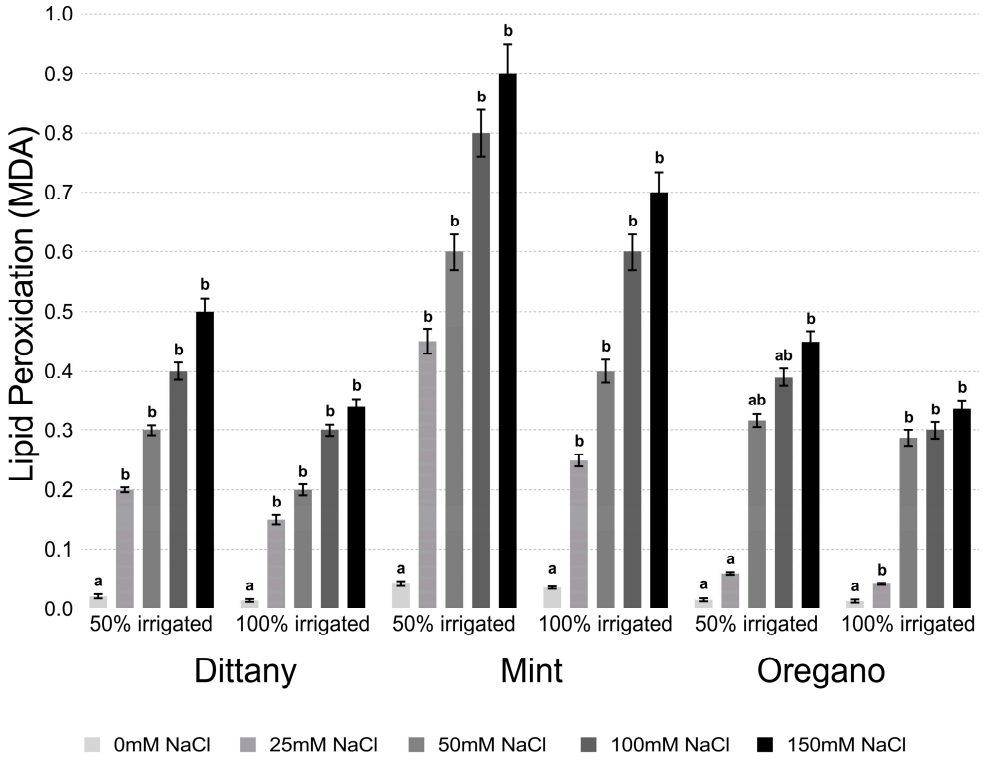

Figure 3. Effect of NaCl on MDA (μmol g^{-1} FW) content in leaves of different Lamiaceae species *Origanum dictamnus* L., *Mentha spicata* L., and *Origanum onites* L. Each value is the mean of 4 replications ± standard error. Means not sharing the same letter are significantly different at $p \leq 0.05$.

A similar trend to that of MDA was observed for H_2O_2 in all species of the Lamiaceae family. The H_2O_2 values in spearmint plants after salinity and 50% irrigation treatments were up to two times higher than those in the dittany and oregano species (Figure 4). This study demonstrated that both MDA and H_2O_2 increased in Lamiaceae plants but, in spearmint plants, after simultaneous salinity treatment and drought stress, the increase was about two times higher than in oregano plants.

3.3. Essential Oil Content and Main Constituents

Table 2 presents the content of the volatile fractions of the plants analyzed. It is obvious that oregano had the highest concentration of essential oil, which increased with the increase of NaCl in the soil substrate (by 25% of the control at 150 mM NaCl). In dittany, exposure to 25 mM NaCl caused a significant reduction of oil production by 45% ($p < 0.05$); then, increasing the salinity resulted in an augmentation of the essential oil that was always lower than the control by almost 19% at the higher salt level. In spearmint, it seems that the essential oil level was less affected by the presence of NaCl.

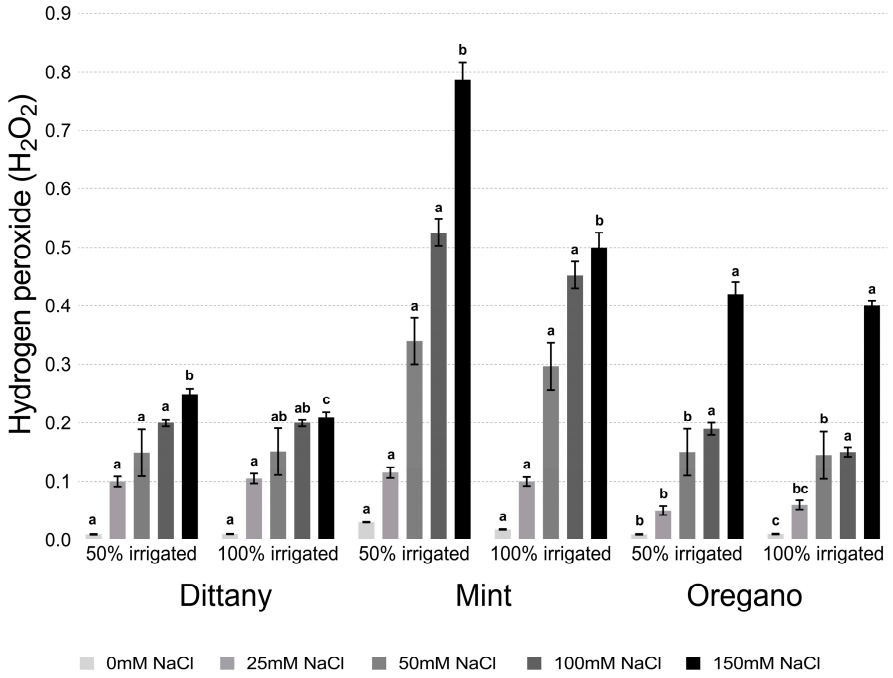

Figure 4. Effect of NaCl on H_2O_2 (μmol g^{-1} FW) content in leaves of different Lamiaceae species *Origanum dictamnus* L., *Mentha spicata* L., and *Origanum onites* L. Each value is the mean of 4 replications ± standard error. Means not sharing the same letter are significantly different at $p \leq 0.05$.

Table 2. Content of essential oils of the examined plant species under salt stress (mL 100 g^{-1} D.W.). Mean values ($n = 4 \pm$ se) not sharing the same letter are significantly different at $p \leq 0.05$.

Treatments	*M. spicata*	*O. dictamnus*	*O. onites*
0 mM NaCl	3.24 ± 0.08 b	3.61 ± 0.18 d	4.86 ± 0.09 b
25 mM NaCl	3.60 ± 0.05 c	2.00 ± 0.06 a	4.53 ± 0.08 a
50 mM NaCl	2.94 ± 0.07 a	2.11 ± 0.03 a	5.54 ± 0.15 c
100 mM NaCl	3.06 ± 0.13 ab	2.60 ± 0.12 b	6.57 ± 0.11 e
150 mM NaCl	3.15 ± 0.11 ab	2.93 ± 0.13 c	6.07 ± 0.16 d
p-value	<0.001	<0.001	<0.001

The composition of essential oils was not affected in terms of the main constituents in spearmint and dittany, while in oregano, at 25 and 50 mM NaCl treatments there was a variation in the ratio of carvacrol to its precursors. Moreover, in the treatments with 25 and 50 mM NaCl, there is a clear differentiation in the ratio of carvacrol to precursors of 0.68 and 0.65, respectively. The highest salinity procedures, such as 100 and 150 mM NaCl with a ratio of carvacrol to precursors of 0.99 and 0.98, respectively, are the same as those of the control (1.03) (Tables 3–5). Since, in salinity interventions, the plants preceded the growth stages, this difference cannot be due to a more advanced stage of ripening.

Table 3. Composition of the essential oils of *Mentha spicata* L.

Compounds	tR [1]	RI [2]	RI [3]	0 mM NaCl	25 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl	*p*-Value
Limonene	8.402	1026	1029	21.69 ± 0.01 a	23.22 ± 0.05 b	21.81 ± 0.23 a	23.16 ± 0.10 b	22.88 ± 0.25 b	<0.001
1,8-Cineole	8.463	1029	1031	9.81 ± 0.03 a	9.54 ± 0.05 a	11.46 ± 0.18 b	10.40 ± 0.08 b	10.34 ± 0.11 b	<0.001
Carvone	11.912	1242	1243	53.11 ± 0.12 a	51.32 ± 0.02 a	52.70 ± 0.07 a	52.60 ± 0.18 a	50.91 ± 0.15 a	>0.05
β-Caryophyllene	14.661	1418	1419	1.81 ± 0.02 e	1.36 0.01 c	0.87 ± 0.01 a	1.11 ± 0.04 b	1.61 ± 0.04 d	<0.001
% Total				85.80	85.44	86.84	87.26	85.29	

[1] tR: Retention time (min); [2] RI: Retention Indices from experimental using a SBP-5 column using a homologous series of n-alkanes (C_9–C_{25}); [3] RI: Retention indices according to literature. Alphabetic characters (a–e) in the table demonstrate the significant differences among the various NaCl treatments, as they resulted from the post hoc analyses, based on Tukey's Honest Significant Difference (HSD) test.

Table 4. Composition of the essential oils of *Origanum dictamnus* L.

Compounds	tR [1]	RI [2]	RI [3]	0 mM NaCl	25 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl	*p*-Value
α-Thujene	6.493	930	932	1.24 ± 0.02 e	1.05 ± 0.01 b	0.77 ± 0.01 a	1.14 ± 0.00 c	1.18 ± 0.01 d	<0.001
α-Terpinene	8.185	1012	1014	2.05 ± 0.00 b	1.91 ± 0.01 a	1.91 ± 0.03 a	2.05 ± 0.01 b	2.07 ± 0.00 b	<0.001
p-Cymene	8.314	1022	1024	34.81 ± 0.07 b	43.25 ± 0.04 c	33.31 ± 0.62 a	35.16 ± 0.10 b	33.57 ± 0.29 a	<0.001
γ-Terpinene	8.924	1052	1054	13.54 ± 0.07 c	10.16 ± 0.03 a	13.37 ± 0.10 c	12.41 ± 0.04 b	12.50 ± 0.12 b	<0.001
Linalool	9.602	1092	1095	2.02 ± 0.04 ab	3.05 ± 0.03 d	1.95 ± 0.05 a	2.07 ± 0.01 b	2.34 ± 0.02 c	<0.001
Thymol	12.514	1290	1290	0.12 ± 0.01 a	0.15 ± 0.01 b	0.16 ± 0.01 b	0.12 ± 0.00 a	0.16 ± 0.00 b	<0.001
Carvacrol	12.669	1299	1298	34.77 ± 0.05 b	25.25 ± 0.27 a	36.13 ± 0.37 c	34.41 ± 0.20 b	35.84 ± 0.15 c	<0.001
% Total				88.55	84.82	87.60	87.36	87.65	

[1] tR: Retention time (min); [2] RI: Retention Indices from experimental using a SBP-5 column using a homologous series of n-alkanes (C_9–C_{25}); [3] RI: Retention indices according to literature. Alphabetic characters (a–e) in the table demonstrate the significant differences among the various NaCl treatments, as they resulted from the post hoc analyses, based on Tukey's Honest Significant Difference (HSD) test.

Table 5. Composition of the essential oils of *Origanum onites* L.

Compounds	tR [1]	RI [2]	RI [3]	0 mM NaCl	25 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl	*p*-Value
α-Thujene	6.493	930	932	1.20 ± 0.01 b	1.72 ± 0.02 d	2.45 ± 0.03 e	1.00 ± 0.01 a	1.29 ± 0.02 c	<0.001
α-Pinene	6.621	937	939	0.78 ± 0.01 b	1.16 ± 0.00 d	1.62 ± 0.02 e	0.67 ± 0.00 a	0.85 ± 0.00 c	<0.001
Myrcene	7.695	986	990	1.28 ± 0.00 b	1.72 ± 0.01 c	2.47 ± 0.05 d	1.16 ± 0.01 a	1.34 ± 0.00 b	<0.001
α-Terpinene	8.176	1012	1014	3.18 ± 0.02 a	4.80 ± 0.02 c	5.24 ± 0.02 d	3.43 ± 0.01 b	3.41 ± 0.02 b	<0.001
p-Cymene	8.314	1022	1024	3.45 ± 0.01 a	4.73 ± 0.01 d	5.88 ± 0.01 e	3.51 ± 0.01 b	3.66 ± 0.01 c	<0.001
Limonene	8.400	1026	1029	1.12 ± 0.00 a	1.50 ± 0.00 d	1.96 ± 0.02 e	1.16 ± 0.01 b	1.25 ± 0.02 c	<0.001
γ-Terpinene	8.924	1052	1054	8.65 ± 0.01 a	12.55 ± 0.08 d	13.96 ± 0.03 e	9.59 ± 0.00 c	8.95 ± 0.02 b	<0.001
cis-Linalool oxide	9.439	1172	1174	0.78 ± 0.00 a	1.11 ± 0.01 d	1.03 ± 0.02 c	0.82 ± 0.01 b	0.82 ± 0.01 b	<0.001
Linalool	9.602	1092	1095	11.71 ± 0.02 d	10.77 ± 0.07 a	11.40 ± 0.07 c	10.96 ± 0.08 b	11.25 ± 0.03 c	<0.001
Borneol	10.728	1162	1165	1.52 ± 0.00	1.36 ± 0.00	1.08 ± 0.00	1.59 ± 0.00	1.45 ± 0.00	–
Terpinen-4-ol	10.891	1172	1174	6.52 ± 0.00 b	8.53 ± 0.53 d	4.56 ± 0.01 a	7.62 ± 0.01 c	6.81 ± 0.02 b	<0.001
α-Terpineol	11.097	1188	1188	3.93 ± 0.01 b	3.91 ± 0.02 b	2.97 ± 0.00 a	4.16 ± 0.02 c	3.95 ± 0.05 b	<0.001
Thymol	12.514	1290	1290	0.32 ± 0.03 c	0.20 ± 0.01 a	0.17 ± 0.00 a	0.27 ± 0.01 bc	0.26 ± 0.03 b	<0.001
Carvacrol	12.669	1299	1298	45.41 ± 0.15 d	36.46 ± 0.35 b	35.44 ± 0.24 a	45.56 ± 0.10 d	44.50 ± 0.03 c	<0.001
% Total				89.85	90.50	90.24	91.49	89.80	
Ratio of carvacrol to precursors				1.03	0.68	0.65	0.99	0.98	
Monoterpene Hydrocarbons				19.66	28.18	33.58	20.52	20.75	
Sum of precursors				44.12	53.86	54.62	45.67	45.03	

[1] tR: Retention time (min); [2] RI: Retention Indices from experimental using a SBP-5 column using a homologous series of n-alkanes (C_9–C_{25}); [3] RI: Retention indices according to literature. Alphabetic characters (a–e) in the table demonstrate the significant differences among the various NaCl treatments, as they resulted from the post hoc analyses, based on Tukey's Honest Significant Difference (HSD) test.

4. Discussion

To mitigate the adverse effects of drought and salinity on aromatic plants and improve their productivity, it is crucial to understand the physiological and biochemical responses of aromatic plants under these conditions. Generally, the addition of salts to the soil solution limits the ability of the plant to absorb water, resulting in delayed growth and development [33]. The growth, yield, and quality of aromatic Lamiaceae plants can be adversely affected by salinity [34].

We focused on three species of aromatic plants in order to investigate their tolerance to saline and drought conditions because they are widely recognized for their commercial importance. By studying the physiological and biochemical responses of these aromatic species to salt stress, we aimed to gain a better knowledge of their tolerance mechanisms. This observation can be valuable for breeding and selecting salt-tolerant cultivars, as well as for implementing effective strategies to mitigate the negative effects of salt stress on the cultivation of aromatic plants.

Among the three aromatic species, dittany was revealed to be more resistant to salt and drought stress compared to oregano and spearmint, as there was a significant increase in its height and the ratio DW/FW (Table 1). The increase in salinity accelerated the entry of plants into flowering, especially for oregano. The vegetative growth of plants lasts throughout spring for spearmint, whereas a maximum growth at the end of April and early

May was observed for oregano. As a result, the growth and the height of the three aromatic plants under salinity treatments, especially in the higher concentrations and a simultaneous 50% of irrigation, was poor.

Salt stress is known to have detrimental effects on plant growth, development, and antioxidant capacity. One of the reasons for the reduced plant growth under salt stress is the accumulation of excess salts around the root zone, which interferes with water uptake. The increase in salinity accelerated the entry of plants into flowering, especially for island oregano. Similar results that show the negative relationship between the salinity stress and the plant growth parameters were also reported previously [35], although the response was different in different genotypes. Drought stress leads to turgor loss, trim down in photoassimilation, and metabolites that are required for cell division. As a consequence, impaired mitosis and cell elongation and expansion result in reduced growth [13,35,36]. Likewise, we observed a significant and uniform reduction in all measured production parameters (plant height, canopy diameter, leaf area, fresh root weight, and biomass production) under drought stress, across all the tested species.

The concentration of certain ions increases under salt stress, which can have inhibitory effects on plant metabolism. This specific ion toxicity can disrupt plant functions and lead to mineral nutrient imbalances and deficiencies [13,35].

The decreased chlorophyll content under water stress and salinity might be a result of the ion uptake disturbances with concomitant reduced photosynthesis and respiration of the aromatic plants. Among the three species, spearmint revealed that it lost a remarkable portion of the photosynthetic pigment resulting in growth inhibition.

Plant grown under salt and water stress may exhibit significantly higher levels of certain secondary plant metabolites compared to controls cultivated in normal conditions [13]. Additionally, those stresses adversely affect the content of total carbohydrates, fatty acids, and proteins, while increasing the level of amino acids, particularly proline. The distinctive cyclic structure of proline's side chain gives proline an exceptional conformational rigidity compared to other amino acids. It is essential in the primary metabolism and is the most extensively studied osmolyte due to its great importance for stress tolerance [18]. For osmotic adjustment, proline contributes to the stabilization of subcellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions. The rapid breakdown of proline upon stress relief may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress, as well as repairing stress-induced damage [37].

Proline is considered to be a protective osmolyte associated with the degree of stress tolerance; its accumulation indicates that the plants are under stress [38]. There is evidence that the synthesis and accumulation of proteins is related to the resistance of plants to water stress [39,40]. However, a correlation between proline accumulation and stress tolerance is not always apparent [41], and other authors have suggested that it is an indicator of some type of damage [42]. The influence of drought and salt stress on secondary compound accumulation is highly debatable and a lot of contradictory results are reported [24,26,34,43,44]. In this experiment, we observed an augmentation of proline biosynthesis in the more sensitive spearmint compared to the more resistant dittany and oregano. Similar results in proline content have also been observed as a result of water stress in oregano plants [45].

The findings of this study highlight the importance of lipid peroxidation and TBARs content as indicators of salt stress response in aromatic plants. Understanding the biochemical role of MDA is important in assessing the extent of lipid peroxidation and its impact on cellular structures. The increase in MDA levels indicates higher levels of lipid peroxidation and suggests the presence of cellular membrane damage. This can have detrimental effects on various cellular processes and overall plant performance. The contrasting responses observed in spearmint (sensitive), oregano, and dittany (tolerant) species provide insights into the mechanisms underlying salt and drought tolerance and susceptibility. Our results indicated that spearmint is more susceptible to salinity and drought-induced

lipid peroxidation, leading to membrane damage and ultrastructure changes. On the other hand, the tolerant oregano and dittany have mechanisms that prevent or minimize lipid peroxidation, thus protecting their cell membranes and maintaining their ultrastructure integrity. Similar results were reported by Krause et al. [46], who found that salt treatment caused an increase in lipid peroxidation, as revealed by high TBA content in sensitive barley genotypes, but no significant effect was shown in the most salt-tolerant genotypes. According to Chiappero et al. [47], drought effects were ameliorated by the reduction of MDA (malonyldialdehyde) levels, thus avoiding the accumulation of ROS and increasing antioxidant enzyme activities and the antioxidant level, specifically in relation to the total phenolic compounds. In the current experiment, stress treatments induced the enhancement of hydrogen peroxide, especially in spearmint plants compared to oregano plants. On the contrary, this enhancement of H_2O_2 indicates the prevalence of oxidative stress and this may be one of the possible mechanisms by which the toxicity of salinity and drought could be manifested in the plant tissues [24,48]. Nevertheless, H_2O_2 accumulation is another ROS that is implicated in enhanced lipid peroxidation and membrane damage, causing cell death [49,50].

Regarding the quantitative and qualitative composition of spearmint plants, the essential oil was not affected when referring to carbon as the most predominant component in all treatments. This result is in accordance with a previous study performed by Ounoki [51] and reveals that the essential oil composition of spearmint was unaffected by the treatments applied to the plant material [51]. In dittany plants, no statistically significant difference was observed in the quantitative and qualitative composition of dew essential oil. Salinity did not seem to affect the concentration of carvacrol, which is the main component. It should be noted that the quantities of *p*-cymene and γ-terpinene in the treatment with 25 mM NaCl seem to increase, whereas carvacrol decreases compared to controls.

In pennyroyal plant grown under water stress, a 40% increase in EO content was also shown [52], with comparable responses being observed for other Lamiaceae species grown under water stress, such as *Lavandula angustifolia* [12], *Ocimum basilicum* [12], and *Thymus vulgaris* [46,53]. However, in a one-year field trial, the researchers [19] observed that the Lamiaceae subjected to drought stress did not present variations in the amount of EOs, whereas the EO content actually decreased in *Lavandula latifolia* and *Salvia sclarea*. Other authors have also indicated negative effects on EO content due to water stress in *Mentha arvensis* [47,51,54] *Hyssopus officinalis* [55], *Rosmarinus officinalis* [56], *Salvia officinalis* [57], and *Thymus vulgaris* [58].

Finally, in oregano plants the quantitative and qualitative composition of the dew essential oil was affected after salinity treatments and drought. The percentage of the main component carvacrol did not significantly differ in treatments with both 100 and 150 mM NaCl. On the other hand, the essential oil extracted by plants treated with 25 and 50 mM NaCl were almost 10% poorer in carvacrol, with a decrease in the amount of monoterpenoids at the same time (Table 5). The monoterpene alcohol carvacrol is a compound with the characteristic flavor of oregano also found in other Lamiaceae species. This phenolic monoterpene, among others, is widely used for its pharmaceutical, cosmetic-, and food-related properties. γ-terpinene and *p*-cymene are considered the main precursors of carvacrol [51]. It has been suggested that the induction of EO yield under drought stress may be because when plants grow under stress conditions, they only allocate low amounts of carbohydrates from photosynthesis to plant development, and instead use these for the synthesis of secondary and reserve metabolites, thus generating a balance between growth and defense [12,24,46]. The increase in EO in this case could be working as a mechanism by which to dissipate "extra" energy [12,28]. Indeed, in favorable conditions, most of the plant's energy is directed towards primary metabolism and only a small portion to the secondary metabolism [46].

Further research in this area can contribute to the development of improved aromatic varieties with enhanced salt and drought tolerance.

5. Conclusions

Comparing the studied species, spearmint is more susceptible to salinity and drought, which induces lipid peroxidation, leading to membrane damage and ultrastructure changes. In contrast, oregano and dittany have developed mechanisms for preventing or minimizing lipid peroxidation. This indicates their ability to withstand salinity and drought stress without significant cell membrane damage or ultrastructure changes. These mechanisms may involve the activation of antioxidant enzymes or the accumulation of specific molecules that protect against oxidative stress.

Dittany plants have a strong ability to tolerate high levels of salinity in the soil, as indicated by the increase in essential oil yield. However, the concentration of carvacrol remained constant, regardless of the salinity levels.

Future work in the field should be undertaken to discern the roles and mechanisms affected by proline and other secondary metabolites, to reduce oxidative stress, and to study the response of chemotype of aromatics plants under salt and drought conditions.

Author Contributions: M.K.S., A.E.G. and V.L. contributed to the experimental part, literature search, and drafted the paper. G.O. and C.P. contributed to data analysis. E.P.-P. contributed to the collection, identification, and cultivation of the plant material and to the critical reading of the manuscript. M.K.S. supervised the phytochemical experiments and contributed to a critical reading of the manuscript. E.P.-P., A.E.G. and V.L. designed the study and supervised the experiments. G.O., C.P. and M.K.S. contributed to drafting and critically reading the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable for studies not involving humans or animals.

Informed Consent Statement: Not applicable for studies not involving humans or animals.

Data Availability Statement: The original contributions presented in the study are included in the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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