*horticulturae*

MDPI

*Article*

# Enhancing Rosemary (*Rosmarinus officinalis*, L.) Growth and Volatile Oil Constituents Grown under Soil Salinity Stress by Some Amino Acids

**Ahmad H. Al-Fraihat [1,*], Sati Y. Al-Dalain [2], Ahmad A. Zatimeh [3] and Moawiya A. Haddad [4]**

[1] Department of Nutrition and Food Processing, Al-Huson University College, Al-Balqa Applied University, Al-Salt 19117, Jordan
[2] Department of Medical Support, Al-Karak University College, Al-Balqa Applied University, Al-Salt 19117, Jordan
[3] Department of Applied Science, Irbid University College, Al-Balqa Applied University, Al-Salt 19117, Jordan
[4] Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University, Al-Salt 19117, Jordan
* Correspondence: ahmad@bau.edu.jo

**Abstract:** The current study was carried out during the two consecutive winter seasons of 2019/2020 and 2020/2021 at the Experimental Farm of Ash-Shoubak University College, Jordan, as a pot experiment. This experiment was planned to evaluate the impact of various soil salinity levels (1.17, 3.34, 6.51, and 9.68 ds/m) and amino acid types (control, L-tryptophan acid at 100 ppm, glutamine acid at 200 ppm, and L-tryptophan acid + glutamine acid) as well as their combinations on growth, salt resistance index, and some of the chemical constituents of rosemary plants. The obtained results indicated that plant height and the total herb dry weight of rosemary, the salt resistance index (SRI), and the total chlorophyll in leaves were discernably reduced with increasing soil salinity levels compared with the control. However, salinity enhanced leaf proline content. Each amino acid or its mixture improved plant growth, chlorophyll content, and SRI parameters. The SRI percentage of *R. officinalis* enhanced to more than 100% under a soil salinity level of 1.17 ds/m, combined with amino acids at any type, when compared with the other combination treatments. Furthermore, GC/MS showed that the identified compounds ranged from 98.39% to 99.18% and the unidentified compounds from 0.82% to 1.61% from the volatile oil of rosemary plants. The major constituents of volatile oil samples were camphor (34.95% to 40.21%), D-verbenone (13.74% to 15.23%), and α-pinene (13.21% to 16.73%).

**Keywords:** rosemary; salinity; L-tryptophan; glutamine; growth; volatile oil

check for updates

**Citation:** Al-Fraihat, A.H.; Al-Dalain, S.Y.; Zatimeh, A.A.; Haddad, M.A. Enhancing Rosemary (*Rosmarinus officinalis*, L.) Growth and Volatile Oil Constituents Grown under Soil Salinity Stress by Some Amino Acids. *Horticulturae* **2023**, *9*, 252. https://doi.org/10.3390/horticulturae9020252

Academic Editor: Aušra Brazaitytė

Received: 9 December 2022
Revised: 9 February 2023
Accepted: 9 February 2023
Published: 13 February 2023

**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

Aromatic and medicinal plants are the sources of serious drugs that have been utilized for thousands of years [1]. Additionally, Al-Dalain et al., referes that [2], rosemary (*R. officinalis* L.) is an important species of the Lamiaceae (Labiatae) family, and it naturally originates in all areas of the Mediterranean Sea. Rosemary essential oil is utilized in numerous ways, such as in the flavor, fragrance, and medicinal industries [3,4]. Rosemary herb has been widely utilized in folk medicine as a natural preservative for its high antimicrobial and antioxidant properties, and it has been used as a spice [5]. There is a considerable interest in the industry in the essential oil of rosemary owing to its free radical scavenger, antioxidant, and antibacterial properties, which are related to phenolic diterpenes [6]. One of the most significant abiotic factors is salinity. FAO [7] reported that the most generally used definition of saline soil is one with an EC of 4 $dsm^{-1}$ or higher; soils with ECs above 15 $dsm^{-1}$ are considered strongly saline. The direct impact of salts on plant growth, according to [8–10], can be categorized into three main groups: a decline in the physical structure of the soil that affects soil aeration and water permeability; (ii) a fall in the osmotic

potential of the soil solution that decreases plant accessible water; and (iii) an increase in the concentration of particular ions that impede plant metabolism (mineral deficiencies and specifically in nutrient toxicity). However, [11] showed that, in comparison to controls, using salinity levels of 2000, 3000, and 4000 ppm significantly reduced rosemary growth traits, the salt resistance index, and volatile oil percentage as well as the total chlorophyll content (SPAD) and the total carbohydrate percentage. It is well known that higher plants use L-tryptophan as a physiological precursor to auxins. According to the research, L-tryptophan has a more favorable impact on plant development and yield than pure auxins [12]. Tryptophan is one of the most important plant amino acids. It could regulate ion transport, alter stomatal opening, act as an osmolyte, and detoxify the negative effects of heavy metals [13]. The effects of exogenous administration of salicylic acid and L-tryptophan on plant growth, development, and stress tolerance have been examined by several researchers. At the same time, the greatest results for plant height, branch count, and the fresh and dry weights of chamomile flowers came from the interaction between the highest salinity level (11.28 ds/m) and a high dose of amino acids. Sprayed chamomile plants with 375 ppm amino acids gave the highest values of chlorophyll a and b as well as carotenoid content under salinity stress conditions [14]. Additionally, [15] pointed out that the exogenous application of glutamine acid showed enhancement and significant rises in the growth parameter of wheat (*Triticum aestivum*) compared with control plants under different salinity levels. According to previous studies, soil salinity increases in agricultural areas, and an attempt should be made to improve the growth and productivity of aromatic plants in those areas. It was found that amino acids play a role in improving the growth of growing plants under conditions of salt stress. Therefore, the main aim of the present study is to evaluate the role of L-tryptophan and glutamine acids in counteracting the deleterious impact of soil salinity on the growth traits, salt resistance (%), volatile oil percentage, constituents, and total chlorophyll content of the rosemary plant.

## 2. Materials and Methods

The current study was conducted in the Experimental Farm of Ash-Shoubak University College, Ash-Shoubak, Jordan, throughout two consecutive winter seasons in 2019/2020 and 2020/2021. This study looked at the effects of foliar applications of different amino acid types (control sprayed with normal water, 100 ppm L-tryptophan acid (Trp.), 200 ppm glutamine acid (Gln.), and 100 ppm Trp. + 200 ppm Gln.) on the growth, dry herb weight, percentage of salt resistance, total chlorophyll content, proline content, volatile oil percentage, and constituents of rosemary plants grown under different soil salinity levels (1.17, 3.34, 6.51, and 9.68 ds/m). Foliar sprays of amino acid types were applied to rosemary plants four times: at 25, 40, 55, and 70 days following the planting date and after the first cut date. Each experimental unit received 4 letters of solution utilizing a spreading agent (super film at a rate of 1 mL/L). The tested amino acid concentrations were based on previous studies conducted on comparable plants of the Labiatae family grown under stress conditions.

### *2.1. Plant Cultivation*

It was decided to purchase rosemary (*R. officinalis*, L.) seedlings from a private nursery. During both seasons, rosemary seedlings were planted on October 12th in 30 cm pots with a1/1, $v/v$ sand: clay. The physical and chemical properties of the used mixture are presented in Table 1. Under pot culture (8 kg soil mixture) circumstances, the response of rosemary plants to various salt levels was assessed. By dissolving the natural salt crust of sea water in distilled water and then adding the soil according to its weight, four levels of artificial soil salinity were created. All seedlings grew at a similar rate and were 20 cm long. Per pot, two seedlings were sown. The plot had roughly 48 pots, and three plants were randomly chosen from each replication to observe growth metrics, herb yield, and total rosemary chlorophyll content. Six plants were used to obtain the mean value of each

parameter (two plants from each replication). When necessary, all suggested agricultural methods for cultivating rosemary plants were followed.

**Table 1.** Physical and chemical properties of used experimental mixture soil.

| Physical Analysis | | | | | | | | | | Soil Texture | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Clay (%)** | | | **Silt (%)** | | | | **Sand (%)** | | | **Sandy** | | |
| 22.37 | | | 7.93 | | | | 69.70 | | | | | |
| Chemical analysis | | | | | | | | | | | | |
| pH | EC ($dsm^{-1}$) | Soluble cations (m.mol/L) | | | | | Soluble anions (m.mol/L) | | | Available (ppm) | | |
| | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $Zn^{++}$ | $Mo^{++}$ | $Cl^{-}$ | $HCO_3^{-}$ | $SO_4^{-}$ | N | P | K |
| **7.85** | 0.68 | 1.80 | 0.95 | 0.30 | 1.10 | 1.32 | 3.04 | 1.12 | 0.84 | 127 | 46 | 51 |

### *2.2. Experimental Design*

The statistical layout of this experiment was a split-plot design experiment between salinity level (four levels) as the main plot and acids (four amino types) as a subplot in randomized complete blocks design (RCBD) with three replicates. The combination treatments between soil salinity level and amino acid concentration consisted of 16 treatments.

### *2.3. Data Recorded*

#### 2.3.1. Growth Traits and Salt Resistance Index Percentage

Plant height (cm) and dry weight of herb/plant (g) were recorded after 85 days from the planting date and after 85 days from the first cut. Three plants were randomly chosen from each experimental unit in both seasons. After 85 days from the date of planting and first cut in both seasons, dry herb weight (which was dried in an oven at 45 °C) per plant was assessed. Moreover, the salt resistance index percentage (SRI%), as a real indicator for soil salinity tolerance was calculated by using the equation in [11] on rosemary: SRI (%) = mean herb weight per plant of the salt-treated plants/mean herb weight per plant of control one × 100.

#### 2.3.2. Total Chlorophyll Content (SPAD Unit)

After 90 days from the date of planting and in both seasons, fresh leaf samples from existing rosemary plants were examined for total chlorophyll content (SPAD unit), which was measured using a SPAD-502 m by following [16].

#### 2.3.3. Proline Content (mg/g as Dry Weight)

The method described in [17] was used to measure the free amino acid proline (mg/g as dry weight) in dried rosemary leaves during both seasons.

### *2.4. Volatile Oil Percentage*

According to [18], the volatile oil from dried rosemary plants was isolated for 3 h by hydro distillation after 120 days from planting, and the first cut to obtain the volatile oil percentage was as follows: essential oil percentage = amount of oil extracted (mL)/weight of plant sample (g) × 100.

#### Gas Chromatography-Mass Spectrometry (GC-MS) of Volatile Oil

Using a Hewlett Packard 5890 GC and a Hewlett Packard 5971 MS system operating in the EI mode at 70 eV, samples of rosemary volatile oil were examined using GC-MS. On an HP-5 capillary column (30 m 0.25 mm, film thickness 0.17 m), EO separation was carried out. It was set to hold at 60 °C for 3 min, increase it by 4 °C/min to 210 °C, hold it there for 15 min, increase it by 10 °C/min to 300 °C, and then hold it there for 15 min. For both columns, helium was the carrier gas, flowing at a constant rate of 1 mL/min.

Agilent Chemstation software was used to analyze the data, and the individual components were identified by comparing them to the pure compounds that were also coinjected, as well as by comparing the MS fragmentation patterns and retention indices to data found in libraries or other published sources (NIST/EPA/NIH 2008; HP1607 purchased from Agilent Technologies and Adams, 2011). Peak area normalization was utilized to derive the relative proportion percentages of the volatile oil elements [19].

### *2.5. Statistical Analysis*

Data from the current study were statically analyzed, and the differences between the means of the treatments (soil salinity levels and amino acid types) were considered significant when they were more than the least significant differences (LSD) at the 5% levels by utilizing the computer program of Statistics, version 9 [20].

## 3. Results

### *3.1. Plant Height (cm)*

The results presented in Table 2 indicate that rosemary plant height was dramatically reduced by high salt treatments (3.34, 6.51, and then 9.68 ds/m) compared to the lowest level of salinity (1.17 ds/m) in both cuttings during the two tested seasons. The height of rosemary plants often dropped as soil saline levels rose until they reached their lowest point at 9.68 ds/m. Additionally, in the majority of cases, Trp. and Gln. acids alone or in a mixture significantly boosted rosemary plant height throughout two seasons compared with the untreated plants.

The combination treatment with Trp. and Gln. acids at 100 and 200 ppm, respectively, considerably improved plant height in most situations when compared with the control and other levels under study. Additionally, except in the case of a soil salinity of 1.17 ds/m plus the Trp. + Gln. amino acids, the combination of salinity and the Trp. + Gln. amino acids reduced plant height compared with the control.

**Table 2.** Effect of soil salinity level, amino acid types, and their combination treatments on plant height (cm) of *R. officinalis* during the 2019/2020 and 2020/2021 seasons.

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| Soil salinity levels (ds/m) | | | | | |
| 1.17 | | 50.23 | 52.79 | 48.68 | 48.08 |
| 3.34 | | 44.74 | 47.42 | 43.19 | 43.92 |
| 6.51 | | 38.93 | 39.08 | 37.13 | 38.08 |
| 9.68 | | 26.51 | 28.84 | 28.33 | 27.88 |
| LSD 5% | | 0.41 | 0.63 | 0.46 | 0.16 |
| Amino acid types | | | | | |
| Control | | 36.42 | 36.93 | 35.06 | 35.84 |
| Trp. | | 38.21 | 40.91 | 38.50 | 37.68 |
| Gln. | | 42.26 | 44.27 | 40.72 | 41.21 |
| (Trp. + Gln. *) | | 43.53 | 46.03 | 43.06 | 43.23 |
| LSD 5% | | 0.41 | 0.59 | 0.34 | 0.27 |
| The combination between soil salinity levels and amino acid types | | | | | |
| 1.17 | Control | 46.40 | 47.73 | 44.60 | 46.00 |
| | Trp. | 47.27 | 51.20 | 48.03 | 46.33 |
| | Gln. | 52.77 | 54.70 | 50.00 | 48.50 |
| | (Trp. + Gln.) | 54.47 | 57.53 | 52.10 | 51.50 |
| 3.34 | Control | 40.37 | 42.63 | 38.07 | 40.30 |
| | Trp. | 42.40 | 45.90 | 43.27 | 41.60 |
| | Gln. | 47.80 | 50.23 | 44.67 | 45.63 |

**Table 2.** *Cont.*

| Treatments | | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|---|
| | | | 1st Cut | 2nd Cut | 1st Cut | 2nd Cut |
| 6.51 | (Trp. + Gln.) | | 48.40 | 50.90 | 46.77 | 48.13 |
| | Control | | 35.20 | 32.77 | 33.33 | 34.30 |
| | | Trp. | 38.30 | 39.47 | 35.60 | 36.07 |
| | | Gln. | 40.40 | 41.30 | 38.80 | 40.40 |
| | (Trp. + Gln.) | | 41.83 | 42.80 | 40.77 | 41.53 |
| 9.68 | Control | | 23.70 | 24.57 | 24.23 | 22.77 |
| | | Trp. | 24.87 | 27.07 | 27.10 | 26.70 |
| | | Gln. | 28.07 | 30.83 | 29.40 | 30.30 |
| | (Trp. + Gln.) | | 29.40 | 32.90 | 32.60 | 31.73 |
| | LSD 5% | | 0.82 | 1.19 | 0.74 | 0.49 |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

### *3.2. Total Dry Weight per Plant (g)*

According to Table 3, applying soil salinity treatments reduced *R. officinalis* herb dry weight per plant compared with the lowest level of salinity in the two cuts over both seasons. Utilizing 3.34, 6.51, and 9.68 ds/m revealed the significance of this drop. Simultaneously, as soil salt levels increased, the dry weight of the herb per plant declined, reaching its lowest value when employing 9.68 ds/m. Furthermore, in all cuts and both seasons, L-tryptophan, glutamine, and L-tryptophan + glutamine significantly increased the herb dry weight of the rosemary plant (g) compared with the control. Additionally, L-tryptophan and glutamine were combined at 100 and 200 ppm, respectively, to produce the maximum dry weight of herb per plant values. In comparison with rosemary plants grown under soil salinity levels alone during the two succeeding seasons, spraying the plants with L-tryptophan + glutamine at 100 and 200 ppm, respectively, resulted in the maximum herb dry weight per plant (g).

**Table 3.** Effect of soil salinity level, amino acid types, and their combination treatments on total dry weight (g) of *R. officinalis* during the 2019/2020 and 2020/2021 seasons.

| Treatments | | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|---|
| | | | 1st Cut | 2nd Cut | 1st Cut | 2nd Cut |
| Soil salinity levels (ds/m) | | | | | | |
| 1.17 | | | 38.68 | 38.75 | 36.76 | 37.31 |
| 4.34 | | | 32.66 | 33.22 | 31.77 | 30.95 |
| 6.51 | | | 28.76 | 28.16 | 27.87 | 26.39 |
| 9.68 | | | 24.43 | 26.77 | 24.13 | 25.82 |
| | LSD 5% | | 0.38 | 0.42 | 0.41 | 0.37 |
| Amino acid types | | | | | | |
| | Control | | 26.19 | 26.91 | 25.08 | 24.68 |
| Trp. | | | 29.59 | 30.34 | 27.66 | 27.94 |
| Gln. | | | 33.49 | 33.55 | 32.52 | 33.04 |
| | (Trp. + Gln. *) | | 35.26 | 36.09 | 35.26 | 34.80 |
| | LSD 5% | | 0.35 | 0.32 | 0.36 | 0.37 |
| The combination between soil salinity levels and amino acid types | | | | | | |
| **1.17** | **Control** | | 34.70 | 34.80 | 32.43 | 33.63 |
| | | **Trp.** | 38.40 | 37.07 | 35.23 | 36.43 |
| | | **Gln.** | 40.13 | 40.70 | 38.70 | 39.17 |
| | **(Trp. + Gln.)** | | 41.50 | 42.43 | 40.67 | 40.00 |

**Table 3.** *Cont.*

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| **3.34** | **Control** | 26.70 | 27.67 | 24.93 | 23.57 |
| | **Trp.** | 29.83 | 31.40 | 29.03 | 28.13 |
| | **Gln.** | 36.00 | 34.30 | 34.70 | 35.30 |
| | **(Trp. + Gln.)** | 38.10 | 39.50 | 38.40 | 36.80 |
| **6.51** | **Control** | 22.17 | 22.83 | 21.90 | 21.13 |
| | **Trp.** | 26.97 | 26.83 | 23.53 | 22.43 |
| | **Gln.** | 31.90 | 30.83 | 31.73 | 30.07 |
| | **(Trp. + Gln.)** | 34.00 | 32.13 | 34.30 | 31.93 |
| **9.68** | **Control** | 21.20 | 22.33 | 21.07 | 20.40 |
| | **Trp.** | 23.17 | 26.07 | 22.83 | 24.77 |
| | **Gln.** | 25.93 | 28.37 | 24.93 | 27.63 |
| | **(Trp. + Gln.)** | 27.43 | 30.30 | 27.67 | 30.47 |
| **LSD 5%** | | **0.71** | **0.70** | **0.74** | **0.73** |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

### *3.3. Salt Resistance Index Percentage (SRI%)*

The data Table 4 show that rosemary's salt resistance index percentage dramatically changed in response to various soil salinity levels. At the 3.34, 6.51, and 9.68 ds/m levels of soil salinity, SRI% was significantly lower than the lowest level of salinity (1.17 ds/m) in both seasons. Using different amino acid types in both cuts and both seasons dramatically raised the rosemary salt resistance index percentage compared with the control. Additionally, the usage of L-tryptophan, glutamine, and L-tryptophan + glutamine raised the salt resistance index (%), in turn. Generally, the treatment of amino acids (L-tryptophan + glutamine), along with the majority of salinity levels, resulted in an increase in the salt resistance index (%) of *R. officinalis* compared with the lowest level of salinity (1.17 ds/m) or those of the used salinity levels in both cuts over the two tested seasons.

**Table 4.** Effect of soil salinity level, amino acid types, and their combination treatments on salt resistance index (%) of *R. officinalis* during the 2019/2020 and 2020/2021 seasons.

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| Soil salinity levels (ds/m) | | | | | |
| 1.17 | | 111.48 | 111.36 | 113.34 | 110.95 |
| 3.34 | | 94.11 | 95.46 | 97.94 | 92.04 |
| 6.51 | | 82.88 | 80.93 | 85.92 | 78.49 |
| 9.68 | | 70.41 | 76.93 | 74.38 | 76.78 |
| LSD 5% | | 1.03 | 0.98 | 1.33 | 0.78 |
| Amino acid types | | | | | |
| Control | | 75.48 | 77.33 | 77.34 | 73.40 |
| | Trp. | 85.28 | 87.19 | 85.28 | 83.10 |
| | Gln. | 96.52 | 96.43 | 100.25 | 98.26 |
| (Trp. + Gln. *) | | 101.61 | 103.72 | 108.71 | 103.50 |
| LSD 5% | | 1.01 | 0.96 | 1.12 | 1.13 |
| The combination between soil salinity levels and amino acid types | | | | | |
| 1.17 | Control | 100.0 | 100.00 | 100.00 | 100.00 |
| | Trp. | 110.66 | 106.52 | 108.64 | 108.35 |
| | Gln. | 115.66 | 116.98 | 119.32 | 116.48 |
| | (Trp. + Gln.) | 119.66 | 121.95 | 125.39 | 118.95 |

**Table 4.** *Cont.*

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| 3.34 | Control | 76.94 | 79.52 | 76.87 | 70.09 |
| | Trp. | 85.97 | 90.23 | 89.51 | 83.65 |
| | Gln. | 103.75 | 98.58 | 106.99 | 104.98 |
| | (Trp. + Gln.) | 109.79 | 113.51 | 118.40 | 109.43 |
| 6.51 | Control | 63.88 | 65.61 | 67.53 | 62.85 |
| | Trp. | 77.71 | 77.12 | 72.56 | 66.71 |
| | Gln. | 91.93 | 88.63 | 97.84 | 89.40 |
| | (Trp. + Gln.) | 97.98 | 92.35 | 105.76 | 94.98 |
| 9.68 | Control | 61.09 | 64.20 | 64.95 | 60.67 |
| | Trp. | 66.77 | 74.91 | 70.40 | 73.67 |
| | Gln. | 74.74 | 81.53 | 76.87 | 82.16 |
| | (Trp. + Gln.) | 79.06 | 87.09 | 85.30 | 90.64 |
| | LSD 5% | 2.03 | 1.93 | 2.34 | 2.11 |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

### *3.4. Total Chlorophyll Content (SPAD)*

The decrease in the total chlorophyll content was significant during the first and second cuts in both seasons (Table 5). Generally, the total chlorophyll (SPAD unit) decreased as the soil salinity levels increased up to 9.68 ds/m. Additionally, the decrease in this regard was about 17.34% and 22.48% and about 18.61% and 19.35% in the first and second cuts during the first and second seasons, respectively. Likewise, all amino acid types significantly increased the total chlorophyll content of rosemary leaves compared with the control in both cuts in the two seasons. Moreover, the total chlorophyll content (SPAD unit) significantly increased with the 100 ppm of L-tryptophan + glutamine type compared with the control in both cuts through the two seasons (Table 5). The rosemary plants were sprayed with L-tryptophan + glutamine at 100 and 200 ppm, respectively, and exposed to soil salinity levels of 1.17 and 3.34 ds/m, although there was a noticeable rise in this regard. Such outcomes were consistent over both seasons.

**Table 5.** Effect of soil salinity level, amino acid types, and their combination treatments on total chlorophyll content (SPAD) of *R. officinalis* during the 2019/2020 and 2020/2021 seasons.

| Treatments | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|
| | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| Soil salinity levels ds/m) | | | | |
| 1.17 | 47.50 | 47.83 | 46.17 | 46.08 |
| 3.34 | 43.25 | 42.08 | 42.67 | 44.92 |
| 6.51 | 41.83 | 39.83 | 39.50 | 38.67 |
| 9.68 | 39.17 | 37.08 | 37.58 | 37.08 |
| LSD 5% | 0.67 | 0.64 | 0.76 | 0.51 |
| Amino acid types | | | | |
| Control | 39.08 | 37.58 | 38.42 | 38.58 |
| Trp. | 40.58 | 39.67 | 38.58 | 39.00 |
| Gln. | 45.25 | 44.08 | 43.50 | 44.33 |
| (Trp. + Gln. *) | 46.83 | 45.50 | 45.42 | 44.83 |
| LSD 5% | 0.49 | 0.51 | 0.58 | 0.59 |
| The combination between soil salinity levels and amino acid types | | | | |

**Table 5.** *Cont.*

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| 1.17 | Control | 44.33 | 43.33 | 42.67 | 43.00 |
| | Trp. | 45.67 | 46.67 | 44.67 | 43.33 |
| | Gln. | 48.33 | 50.67 | 47.67 | 49.33 |
| | (Trp. + Gln.) | 51.67 | 50.67 | 49.67 | 48.67 |
| 3.34 | Control | 39.33 | 38.33 | 39.67 | 41.33 |
| | Trp. | 40.67 | 39.33 | 39.00 | 42.33 |
| | Gln. | 45.33 | 44.33 | 45.33 | 48.33 |
| | (Trp. + Gln.) | 47.67 | 46.33 | 46.67 | 47.67 |
| 6.51 | Control | 37.33 | 35.00 | 36.67 | 35.33 |
| | Trp. | 39.33 | 37.00 | 36.33 | 34.67 |
| | Gln. | 45.00 | 42.67 | 41.33 | 41.33 |
| | (Trp. + Gln.) | 45.67 | 44.67 | 43.67 | 43.33 |
| 9.68 | Control | 35.33 | 33.67 | 34.67 | 34.67 |
| | Trp. | 36.67 | 35.67 | 34.33 | 35.67 |
| | Gln. | 42.33 | 38.67 | 39.67 | 38.33 |
| | (Trp. + Gln.) | 42.33 | 40.33 | 41.67 | 39.67 |
| LSD 5% | | 1.08 | 1.09 | 1.26 | 1.14 |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

### *3.5. Proline Content (mg/g as Dry Weight)*

The data from Table 6 indicate that rosemary leaves had more proline by dry weight in the two cuttings during both seasons when soil salinity treatments were applied at a higher level, of 9.68 ppm, compared with the controls. By using the soil salinity levels of 3.34, 6.51, and 9.68 ds/m, there was an increase in proline content, and there was a decrease in this connection when using the level of 1.17 ds/m. When foliar amino acid types were used on the controls in the two cuts during both seasons, proline content rose. However, when compared with the control and the other substances under research, L-tryptophan + glutamine at 100 and 200 ppm, respectively, produced the highest values for this constant, followed by glutamine at 200 ppm. In addition, the treatment of L-tryptophan + glutamine and combined with those at a salinity level of 9.68 ds/m, when compared with those of salinity alone (3.34 and 6.51 ds/m) or those of the other ones of a combination including amino acid types and soil salinity in both seasons, led to an increase in proline content in rosemary leaves.

**Table 6.** Effect of soil salinity level, amino acid types, and their combination treatments on proline content in leaves (mg/g as dry weight) of *R. officinalis* during the 2019/2020 and 2020/2021 seasons.

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| Soil salinity levels (ds/m) | | | | | |
| | 1.17 | 3.64 | 3.73 | 3.58 | 3.58 |
| | 3.34 | 4.18 | 4.18 | 3.92 | 4.13 |
| | 6.51 | 4.50 | 4.67 | 4.74 | 4.66 |
| | 9.68 | 6.35 | 6.35 | 7.25 | 7.19 |
| LSD 5% | | 0.07 | 0.08 | 0.11 | 0.12 |
| Amino acid types | | | | | |
| Control | | 3.75 | 3.56 | 4.12 | 3.94 |
| | Trp. | 4.17 | 4.33 | 4.60 | 4.70 |
| | Gln. | 5.27 | 5.41 | 5.29 | 5.32 |

**Table 6.** *Cont.*

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | 1st Cut | 2nd Cut | 1st Cut | 2nd Cut |
| (Trp. + Gln. *) | | 5.50 | 5.63 | 5.48 | 5.61 |
| LSD 5% | | 0.09 | 0.07 | 0.08 | 0.09 |
| The combination between soil salinity levels and amino acid types | | | | | |
| 1.17 | Control | 3.27 | 3.13 | 3.23 | 3.07 |
| | Trp. | 3.37 | 3.50 | 3.53 | 3.33 |
| | Gln. | 3.83 | 4.03 | 3.73 | 3.80 |
| | (Trp. + Gln.) | 4.13 | 4.27 | 3.83 | 4.13 |
| 3.34 | Control | 3.53 | 3.30 | 3.47 | 3.33 |
| | Trp. | 3.37 | 3.83 | 3.80 | 4.03 |
| | Gln. | 4.87 | 4.73 | 4.13 | 4.57 |
| | (Trp. + Gln.) | 7.97 | 4.83 | 4.27 | 4.60 |
| 6.51 | Control | 3.73 | 3.57 | 3.73 | 3.70 |
| | Trp. | 3.73 | 4.17 | 4.30 | 4.43 |
| | Gln. | 5.20 | 5.33 | 5.33 | 5.13 |
| | (Trp. + Gln.) | 5.33 | 5.60 | 5.60 | 5.37 |
| 9.68 | Control | 4.47 | 4.23 | 6.03 | 5.67 |
| | Trp. | 6.20 | 5.80 | 6.77 | 7.00 |
| | Gln. | 7.17 | 7.53 | 7.97 | 7.77 |
| | (Trp. + Gln.) | 7.57 | 7.83 | 8.23 | 8.33 |
| LSD 5% | | 0.17 | 0.15 | 0.18 | 0.19 |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

### 3.6. Volatile Oil Percentage

As shown in Table 7, owing to soil salinity stress, the percentage of volatile oil in rosemary herb increased in the two cuts during both seasons compared with the lowest level of salinity (1.17 ds/m). With the salinity level, this decline was notable in the first and second cuts in the two seasons. In general, as the soil salt levels rose to 3.34, 6.51, and 9.68 ds/m, the volatile oil percentage increased. In both cuts during the first and second seasons, the volatile oil percent was steadily raised by utilizing L-tryptophan, glutamine, and L-tryptophan + glutamine. Additionally, compared with the other subjects under research, the administration of L-tryptophan + glutamine (100 and 200 ppm, respectively) produced the highest rise in this regard. In terms of combinations, it became obvious that the rosemary plant's highest volatile oil percentage values were reached by combining 1.17 or 3.34 ds/m of soil salinity with 100 ppm and 200 ppm of L-tryptophan + glutamine in both seasons, with a noticeable difference between them in most cases.

**Table 7.** Effect of soil salinity level, amino acid types, and their combination treatments on volatile oil percentage in herb of *R. officinalis* during 2019/2020 and 2020/2021 seasons.

| Treatments | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|
| | 1st Cut | 2nd Cut | 1st Cut | 2nd Cut |
| Soil salinity levels (ds/m) | | | | |
| 1.17 | 1.08 | 1.12 | 1.12 | 1.14 |
| 3.34 | 1.21 | 1.21 | 1.19 | 1.19 |
| 6.51 | 1.23 | 1.25 | 1.22 | 1.22 |
| 9.68 | 1.15 | 1.13 | 1.15 | 1.16 |
| LSD 5% | 0.006 | 0.006 | 0.009 | 0.007 |
| Amino acid types | | | | |

**Table 7.** *Cont.*

| Treatments | | The First Season (2019/2020) | | The Second Season (2020/2021) | |
|---|---|---|---|---|---|
| | | **1st Cut** | **2nd Cut** | **1st Cut** | **2nd Cut** |
| Control | | 1.13 | 1.13 | 1.12 | 1.13 |
| | Trp. | 1.16 | 1.16 | 1.15 | 1.16 |
| | Gln. | 1.19 | 1.19 | 1.19 | 1.20 |
| (Trp. + Gln. *) | | 1.19 | 1.22 | 1.21 | 1.22 |
| LSD 5% | | 0.008 | 0.006 | 0.006 | 0.007 |
| The combination between soil salinity levels and amino acid types | | | | | |
| 1.17 | Control | 1.04 | 1.08 | 1.08 | 1.09 |
| | Trp. | 1.08 | 1.11 | 1.11 | 1.13 |
| | Gln. | 1.10 | 1.15 | 1.14 | 1.16 |
| | (Trp. + Gln.) | 1.11 | 1.16 | 1.14 | 1.19 |
| 3.34 | Control | 1.16 | 1.16 | 1.17 | 1.14 |
| | Trp. | 1.22 | 1.19 | 1.16 | 1.18 |
| | Gln. | 1.22 | 1.23 | 1.19 | 1.22 |
| | (Trp. + Gln.) | 1.24 | 1.26 | 1.23 | 1.23 |
| 6.51 | Control | 1.20 | 1.21 | 1.18 | 1.18 |
| | Trp. | 1.20 | 1.23 | 1.19 | 1.20 |
| | Gln. | 1.24 | 1.26 | 1.23 | 1.24 |
| | (Trp. + Gln.) | 1.28 | 1.29 | 1.26 | 1.26 |
| 9.68 | Control | 1.12 | 1.08 | 1.07 | 1.10 |
| | Trp. | 1.16 | 1.11 | 1.13 | 1.13 |
| | Gln. | 1.19 | 1.14 | 1.19 | 1.19 |
| | (Trp. + Gln.) | 1.30 | 1.18 | 1.20 | 1.21 |
| LSD 5% | | 0.015 | 0.012 | 0.014 | 0.015 |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

Volatile Oil Constituents Identified by CG/MS

The data presented in Table 8 revealed that the soil salinity or/and foliar spraying with different types of amino acids as well as their interactions had an impact on the volatile oil compounds. The profile of the rosemary volatile oil exposed to L-tryptophan, glutamine, and L-tryptophan + glutamine under 1.17 and 9.68 ds/m could be determined by conducting a CG/MS chemical analysis. Additionally, the GC/MS results highlighted that the identified compounds ranged from 98.39% to 99.18% as well as the unidentified compounds ranged from 0.82% to 1.61% from the separated compounds. In total, 18 substances were found, and the analytical data were summarized. Camphor, α-pinene, d-verbenone, eucalyptol, and verbenone were the major components, and they recorded 34.95–40.21%, 13.21–16.73%, 13.74–15.23%, 9.07–12.47% and 5.79–7.12% as the highest values in this study, respectively. Camphor obtained the highest value (40.21%) from the interaction treatment of 9.68ds/m combined with the L-tryptophan + glutamine acids but the lowest value (34.95%) from 9.68 ds/m combined with the L-tryptophan treatment. Furthermore, the maximum value for eucalyptol (12.47%) was observed from 9.68 ds/m combined with the L-tryptophan treatment, while the minimum value (9.07%) was shown by 1.17 ds/m combined with the glutamine treatment.

**Table 8.** Main constituents of volatile oil under soil salinity (1.17 and 9.68 ds/m) and amino acid type treatments on *R. officinalis* during the 2020/2021 season.

| Compound | Soil Salinity (ds/m) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1.17 (Control) | | | | 9.68 | | | |
| | Control | Trp. * | Gln. | (Trp. + Gln.) | Control | Trp. * | Gln. | (Trp. + Gln.) |
| γ-Terpinene | 0.98 | 0.75 | 1.01 | 0.86 | 0.77 | 0.68 | 0.71 | 0.73 |
| α-Pinene | 14.88 | 15.12 | 15.01 | 16.62 | 15.22 | 16.73 | 14.62 | 13.24 |
| β-Pinene | 3.51 | 2.42 | 2.88 | 3.47 | 2.94 | 1.88 | 4.01 | 3.41 |
| β-Myrcene | 0.35 | 0.42 | 0.61 | 0.43 | 0.51 | 0.48 | 0.57 | 0.39 |
| Camphene | 4.25 | 4.32 | 4.05 | 3.86 | 3.52 | 4.71 | 3.52 | 4.36 |
| Linalool | 0.89 | 1.01 | 0.90 | 1.03 | 0.89 | 0.76 | 0.95 | 0.88 |
| Terpinolene | 0.76 | 0.75 | 0.81 | 0.64 | 0.79 | 0.81 | 0.73 | 0.71 |
| Pinocarvone | 0.01 | 0.05 | 0.04 | 0.01 | 0.07 | 0.12 | 0.13 | 0.08 |
| ρ-cymene | 1.13 | 1.09 | 1.01 | 1.07 | 1.07 | 1.11 | 1.01 | 1.06 |
| Thuja2,4-Diene | 0.41 | 0.41 | 0.43 | 0.51 | 0.53 | 0.48 | 0.43 | 0.51 |
| Camphor | 36.52 | 38.25 | 40.15 | 37.12 | 37.11 | 34.95 | 39.01 | 40.21 |
| Eucalyptol | 11.21 | 10.03 | 9.07 | 9.48 | 11.02 | 12.47 | 10.95 | 10.55 |
| δ-2-Carene | 0.72 | 0.68 | 0.72 | 0.78 | 0.81 | 0.73 | 0.81 | 0.91 |
| Thymol | 0.43 | 0.41 | 0.39 | 0.47 | 0.51 | 0.47 | 0.46 | 0.37 |
| Verbenone | 6.87 | 6.54 | 6.57 | 7.12 | 6.97 | 6.05 | 5.79 | 5.97 |
| α-Humulene | 0.21 | 0.24 | 0.28 | 0.19 | 0.31 | 0.34 | 0.37 | 0.27 |
| Bornyl acetate | 0.91 | 1.07 | 0.89 | 0.43 | 0.98 | 0.97 | 0.96 | 0.75 |
| D-Verbenone | 15.14 | 15.23 | 14.28 | 15.02 | 13.74 | 14.65 | 14.09 | 13.99 |
| Identified compounds | 99.18 | 98.70 | 98.89 | 99.11 | 98.30 | 98.39 | 99.12 | 98.39 |
| Unidentified compounds | 0.82 | 1.30 | 1.11 | 0.89 | 1.70 | 1.61 | 0.88 | 1.61 |

* Trp. = L-tryptophan acid at 100 ppm and Gln. = glutamine acid at 200 ppm.

## 4. Discussion

### *4.1. Effect of Salinity Stress*

The decreases in plant height, the dry weight of herbs, and the salt resistance index were noticed when rosemary plants were exposed to soil salinity stress compared with those un-stressed by salinity in both seasons, as presented in the study results. Additionally, the same trend was recorded for the total chlorophyll content and the volatile oil percentage, especially with the highest level under the study of salinity (9.68 ds/m). Salt in the soil solution also slowed the plant's ability to absorb water, which resulted in slower development and growth. This was salinity's osmotic effect. Additionally, excessive soil salts that entered the transpiration stream can eventually harm the cells in the leaves that were transpiring, which could further reduce photosynthesis and growth [21]. Along with proline's beneficial effects on salt tolerance at the organism level, salt tolerance also significantly improved at the cellular level. The plant's secondary carbon-based compound levels and the proportional allocation of available carbon to the individual compounds, such as terpenoids and phenolics, were partly under genetic and environmental conditions. The intraspecific variation in the carbon-based secondary compound concentrations is therefore a serious factor that needs to be considered when evaluating plant resistance to any stress (such as soil salinity). For instance, in vitro research on brown mustard (*Brassica juncea*) showed that stressed calli accumulated more free proline than unstressed calli [22]. In addition, [23], on sage; [24], on *Catharanthus roseus*; [25], on sweet basil; [26], on rosemary; [27], on cluster bean; [28], on sweet basil; and [29], on peppermint and spearmint, have also reported similar results.

### *4.2. Effect of Amino Acids*

The results pointed out that by using L-tryptophan or/and glutamine amino acids significantly enhanced rosemary growth and the total chlorophyll content, as well as the total proline, the volatile oil percentage, and some volatile oil components. The amino acids under study produced an increase in rosemary's salt resistance index percentage

compared with the control. As glutamate finally appears in glutamine (Gln), protein, and glutathione, it is clear that glutamate is more quickly absorbed by the cells than it is digested [30]. Furthermore, some of the functional L-tryptophan's activities have been demonstrated, even if its physiological impacts on plants have not yet been fully clarified. Trp. administration on an exogenous basis raises auxin levels in plant tissues [31]. In Arabidopsis, IAA is similarly generated from tryptophan via indole-3-acetaldoxime [32]. Moreover, [14] showed that spraying chamomile plants with amino acids dramatically raised the levels of chlorophylls a and b. [33] sprayed roselle plants with L-tryptophan at a concentration of 100 ppm, which improved growth traits (plant height and total dry weight), produced a larger yield, and improved the chemical components of roselle sepals (carbohydrate contents and the total chlorophyll content (a + b) in leaves).

### *4.3. Effect of Combination between Amino Acids and Salinity Stress*

Biostimulant compounds with a single amino acid or a mix of amino acids have been shown to help plant growth and quality, especially when the environment is not good (under stress conditions). Additionally, amino acids are well-known biostimulants that promote plant development and considerably lessen the harm brought on by abiotic stressors [34]. Moreover, the exogenous administration of amino acids can significantly increase growth and yield while reducing the negative effects of salt ingestion [35]. In general, holding water was used to simulate drought stress 5 days following L-tryptophan treatment. Additionally, the findings imply that L-tryptophan topically applied may help maize resist the effects of drought [36]. Along the same lines, [15] pointed out that the exogenous application of glutamine amino acid showed enhancement and significant rises in the growth parameter of wheat compared with control plants under different salinity levels. Additionally, [37] revealed that high sodium chloride levels (6000 mg/L) cause poor development, cell yellowing, and, occasionally, plant damage. In contrast to high quantities (15 mg/L), which do not limit cell growth and division, moderate amounts of phenylalanine (5 and 10 mg/L) boosted the production of chlorophyll and several secondary metabolites.

## 5. Conclusions

Regarding the data obtained and discussed, spraying amino acids (L-tryptophan or/and glutamine acids at 100 and 200 ppm, respectively) can be recommended for the cultivation of rosemary (*R. officinalis* L.) in soil suffering from soil salinity, to increase plant tolerance to soil salinity. The results obtained in the possibility of growing rosemary plants were invested in the conditions of soil containing high salinity rates. The productivity of many medicinal and aromatic plants of the same plant family and other families can be improved under a high level of soil salinity.

**Author Contributions:** Conceptualization, A.H.A.-F., S.Y.A.-D. and A.A.Z.; methodology, A.H.A.-F., S.Y.A.-D. and A.A.Z.; validation, M.A.H.; formal analysis, M.A.H.; investigation, A.H.A.-F., S.Y.A.-D. and A.A.Z.; resources, A.H.A.-F., S.Y.A.-D. and A.A.Z.; data curation, A.H.A.-F.; writing—original draft preparation, A.H.A.-F.; writing—M.A.H.; visualization, S.Y.A.-D.; supervision, A.H.A.-F., M.A.H.; project administration, A.H.A.-F.; funding acquisition, S.Y.A.-D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Informed Consent Statement:** A written informed consent was obtained from all people help in research prior to the publication of the study.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no potential conflict of interest.

## References


1. Clark, A.M. Natural products as a resource for new drugs. *Pharm. Res.* **1996**, *13*, 1133–1141. [CrossRef]
2. Al-Dalain, S.; Al-Fraihat, A.H.; Al Kassabeh, E. Effect of aromatic plant essential oils on oxidative stability of sunflower oil during heating and storage. *Pak. J. Nutr.* **2011**, *10*, 864–870.
3. Mizrahi, I.; Juarez, M.; Bandoni, A. The essential oil of *Rosmarinus officinalis* growing in Argentina. *J. Essent. Oil Res.* **1991**, *3*, 11–15. [CrossRef]
4. Lee, C.-J.; Chen, L.-G.; Chang, T.-L.; Ke, W.-M.; Lo, Y.-F.; Wang, C.-C. The correlation between skin-care effects and phytochemical contents in Lamiaceae plants. *Food Chem.* **2011**, *124*, 833–841. [CrossRef]
5. Benincá, J.P.; Dalmarco, J.B.; Pizzolatti, M.G.; Fröde, T.S. Analysis of the anti-inflammatory properties of *Rosmarinus officinalis* L. in mice. *Food Chem.* **2011**, *124*, 468–475. [CrossRef]
6. Al-Fraihat, A.H.; Al-dalain, S.Y.; Al-Rawashdeh, Z.B.; Abu-Darwish, M.S.; Al-Tabbal, J.A. Effect of organic and biofertilizers on growth, herb yield and volatile oil of marjoram plant grown in Ajloun region, Jordan. *J. Med. Plants Res.* **2011**, *5*, 2822–2833.
7. Shaw, D.J. World Food Summit, 1996. In *World Food Security*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 347–360.
8. Yeo, A. Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Bot.* **1998**, *49*, 915–929. [CrossRef]
9. Grattan, S.; Grieve, C. Salinity–mineral nutrient relations in horticultural crops. *Sci. Hortic.* **1998**, *78*, 127–157. [CrossRef]
10. Carvajal, M.; Martinez, V.; Cerda, A. Influence of magnesium and salinity on tomato plants grown in hydroponic culture. *J. Plant Nutr.* **1999**, *22*, 177–190. [CrossRef]
11. Abdelkader, M.; Hassan, H.; Elboraie, E. Using proline treatments to promote growth and productivity of *Rosmarinus officinalis* L. plant grown under soil salinity conditions. *Middle East J. Appl. Sci.* **2019**, *9*, 700–710.
12. Zahir, Z.; Malik, M.; Arshad, M. Improving crop yields by the application of an auxin precursor L-tryptophan. *Pak. J. Biol. Sci.* **2000**, *3*, 133–135.
13. Rai, V. Role of amino acids in plant responses to stresses. *Biol. Plant.* **2002**, *45*, 481–487. [CrossRef]
14. Omer, E.; Said-Al-Ahl, H.; El-Gendy, A.; Shaban, K.A.; Hussein, M. Effect of amino acids application on production, volatile oil and chemical composition of chamomile cultivated in saline soil at sinai. *J. Appl. Sci. Res.* **2013**, *9*, 3006–3021.
15. El-Kassas, H.; Abdalla, K.S.; Ahmed, S. Enhancing salt tolerance of wheat plant (*triticum aestivum* L.) by application of proline, ascorbic acid, arginine, glutamine and glutathione. *J. Environ. Sci. Ain Shams* **2020**, *36*, 43–80. [CrossRef]
16. Markwell, J.; Osterman, J.C.; Mitchell, J.L. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynth. Res.* **1995**, *46*, 467–472. [CrossRef] [PubMed]
17. Bates, L.S.; Waldren, R.P.; Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
18. Guenther, G. The essential oils VIII. Robert ED Nastrand Comp. *Inc. Tor. N. Y. Lond.* **1961**, *1*, 569.
19. Mannu, A.; Melito, S.; Petretto, G.L.; Manconi, P.; Pintore, G.M.; Chessa, M. Geographical variation of the chemical composition in essential oils extracted from Sardinian Salvia verbenaca. *Nat. Prod. Res.* **2021**, *36*, 367–370. [CrossRef]
20. Boyd, C.; Petersen, S.; Gilbert, W.; Rodgers, R.; Fuhlendorf, S.; Larsen, R.; Wolfe, D.; Jensen, K.; Gonzales, P.; Nenneman, M. Analytical Software. 2009. Statistics 9. Tallahassee, Florida, USA. In *Evaluation of Methods Used to Improve Grasslands as Ring-Necked Pheasant (Phasianus colchicus) Brood Habitat*; South Dakota State University ProQuest Dissertations Publishing: Brookings, SD, USA, 2018; Volume 72, p. 82.
21. Munns, R.; James, R.A.; Läuchli, A. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* **2006**, *57*, 1025–1043. [CrossRef]
22. Liu, J.-H.; Inoue, H.; Moriguchi, T. Salt stress-mediated changes in free polyamine titers and expression of genes responsible for polyamine biosynthesis of apple in vitro shoots. *Environ. Exp. Bot.* **2008**, *62*, 28–35. [CrossRef]
23. Hendawy, S.; Khalid, K.A. Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. *J. Appl. Sci. Res* **2005**, *1*, 147–155.
24. Jaleel, C.A.; Sankar, B.; Sridharan, R.; Panneerselvam, R. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in Catharanthus roseus. *Turk. J. Biol.* **2008**, *32*, 79–83.
25. Said-Al Ahl, H.; Mahmoud, A.A. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. *Ozean J. Appl. Sci.* **2010**, *3*, 97–111.
26. Helaly, M.; Farouk, S.; Arafa, S.A.; Amhimmid, N. Inducing salinity tolerance of rosemary (*Rosmarinus officinalis* L.) plants by a chitosan or zeolite application. *Asian J. Adv. Agric. Res.* **2018**, *5*, 1–20. [CrossRef]
27. Nassar, A.S.; Meawad, A.; Abdelkader, M. Effect of salinity and lithoit on growth, yield components and chemical constituents of cluster bean (*Cyamopsis tetragonoloba*, Taub.). *Zagazig J. Agric. Res.* **2018**, *45*, 1913–1924. [CrossRef]
28. Ibrahim, A.M.; Awad, A.; Gendy, A.; Abdelkader, M. Effect of proline foliar spray on growth and productivity of sweet basil (*Ocimum basilicum*, L.) plant under salinity stress conditions. *Zagazig J. Agric. Res.* **2019**, *46*, 1877–1889. [CrossRef]
29. Aktsoglou, D.-C.; Kasampalis, D.S.; Sarrou, E.; Tsouvaltzis, P.; Chatzopoulou, P.; Martens, S.; Siomos, A.S. Improvement of the quality in hydroponically grown fresh aromatic herbs by inducing mild salinity stress is species-specific. *Folia Hortic.* **2021**, *33*, 265–274. [CrossRef]
30. El-Ghamry, A.M.; Abd El-Hai, K.M.; Ghoneem, K.M. Amino and humic acids promote growth, yield and disease resistance of faba bean cultivated in clayey soil. *Aust. J. Basic Appl. Sci* **2009**, *3*, 731–739.

31. Hanci, F. The Effect of L-Tryptophan and Melatonin on Seed Germination of Some Cool Season Vegetable Species under Salinity Stress. *Düzce Üniversitesi Bilim Ve Teknol. Derg.* **2019**, *7*, 1879–1891. [CrossRef]
32. Sugawara, S.; Hishiyama, S.; Jikumaru, Y.; Hanada, A.; Nishimura, T.; Koshiba, T.; Zhao, Y.; Kamiya, Y.; Kasahara, H. Biochemical analyses of indole-3-acetaldoxime-dependent auxin biosynthesis in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5430–5435. [CrossRef]
33. Gendy, A.S.; Nosir, W.S. Improving productivity and chemical constituents of Roselle plant (*Hibiscus sabdariffa* L.) as affected by phenylalanine, L-tryptophan and peptone acids foliar application. *Middle East J. Agric.* **2016**, *5*, 701–708.
34. Kowalczyk, K.; Zielony, T.; Gajewski, M. Effect of Aminoplant and Asahi on yield and quality of lettuce grown on rockwool. In Proceedings of the Conference of Biostimulators in Modern Agriculture, Warsaw, Poland, 7–8 February 2008; pp. 7–8.
35. Butt, M.; Ayyub, C.; Amjad, M.; Ahmad, R. Proline application enhances the growth of chili by improving physiological and biochemical attributes under salt stress. *Pak. J. Agric. Sci.* **2016**, *53*, 43–49.
36. Rao, S.; Qayyum, A.; Razzaq, A.; Ahmad, M.; Mahmood, I.; Sher, A. Role of foliar application of salicylic acid and l-tryptophan in drought tolerance of maize. *J. Anim. Plant Sci.* **2012**, *22*, 768–772.
37. A EL-Shennawy, O.; G El-Torky, M.; E El-Mokadem, H.; I Abass, B. Effect of NaCl and Phenylalanine on the Production of some Secondary Metabolites in In Vitro Cultures of Mentha longifolia. *Alex. Sci. Exch. J.* **2017**, *38*, 577–587.


**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.