

Article

Effects of Irrigation, Peat-Alternative Substrate and Plant Habitus on the Morphological and Production Characteristics of Sicilian Rosemary (*Rosmarinus officinalis* L.) Biotypes Grown in Pot

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Abstract: Irrigation and growing substrate are considered as essential cultivation practices in order to obtain good productive and qualitative performance of potted rosemary plants. In pot growing, the chemical, physical and biological characteristics of the substrate must be stable over time in order to allow regular plant growth. However, the effects of cultivation techniques on the characteristics of potted rosemary are little known. Peat is traditionally used as the organic growing medium; however, despite numerous advantages, its use has determined a degradation of peatlands in the northern hemisphere and an increase in greenhouse gases in the atmosphere. The aim of the present study was to assess the effects of irrigation and peat-alternative substrates on the morphological, aesthetic and production characteristics of potted Sicilian rosemary biotypes with different habitus types. Two years, two different irrigation levels, three peat-alternative substrates and three types of rosemary plant habitus were tested in a split-split-split-plot design for a four-factor experiment. The results highlight that irrigation and substrate determined significant differences for all tested parameters. Rosemary plants demonstrated the best performances when irrigation was more frequent; vice versa, the greatest percent content in essential oil was obtained when irrigation events were less frequent. The chemical–physical characteristics of peat-alternative substrates changed with decreases in the peat content and increases in the compost content. The erect habitus biotype showed the best adaptation capacity to the various treatments. Our results suggest that irrigation and peat-alternative substrates significantly affect the growth of rosemary plants and should, therefore, be taken into consideration in order to improve the cultivation of this species in pots for ornamental purposes.

Keywords: aromatic species; alternative substrates; irrigation; plant habitus; sustainable cultivation



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1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is a xerophytic, evergreen shrub widely used for food and ornamental purposes, and is a long-time favourite for pot plants and private gardens [1–4]. Due to its biotechnical characteristics and hardiness in times of environmental stress [5,6], it is also used to protect against soil erosion and as a pioneer species during reforestation in fire-damaged areas [5,7].

Its wealth of bioactive compounds is also considered to be highly effective, also reported in the Pharmacopoeia [8,9].

Numerous phytochemical studies have demonstrated the presence of polyphenolic derivatives in the essential oil (EO) that provides the species with a number of pharmacological and medicinal properties, including antibacterial, anti-inflammatory, antioxidant, antitumor and antidiabetic actions [1,10–12]. Recent studies have demonstrated that the accumulation and composition of secondary metabolites in rosemary and, in general, in medicinal and aromatic plants, are highly influenced by genetic [8,13–16], environmental [17–22] and cultivation factors [23–27]. With regard to cultivation aspects, a number of studies have focused on the effects of some agronomic practices on growth, productivity and essential oil constituents of rosemary, in open-field conditions. Some authors [28] reported that the growth, quality and quantity of rosemary essential oil varied with organic and inorganic fertilizers. It was demonstrated [29] that combined application of vermicompost and chemical fertilizers helped to increase crop productivity and sustained the soil fertility. It was found [30] that the composition of rosemary oil could be altered by fertilization programs in regions of poor soil. It was observed [22] that the application of deficit irrigation affected the morphological and physiological characteristics of rosemary, while humidity influenced parameters related with plant–water relations. Singh [31] found that plant spacing, fertilizer and irrigation regimes affected herbage and oil yield but did not influence oil-content percentage of the species. Other authors [32] highlighted that growth media and regulators influenced significantly the vegetative propagation of rosemary. However, the effects of cultivation techniques on the morphological and production characteristics in pot-grown rosemary are, as yet, little known. Most studies focus on the influence of fertilization [33,34] and type of substrate [34–38] on plant growth and essential-oil production of rosemary. In particular, the choice of the growing medium seems to be crucial for cultivation of rosemary and, in general, of aromatic and medicinal plants due to significant effects on their vegetative and productive characteristics.

Peat is traditionally used as the organic growing medium in pot cultivation [38,39]. Peat is partially decayed organic matter, the result of the degradation of bog plants and bryophyte moss [40,41]. Although its use has many advantages—such as a reduction in pH and salinity, good hydraulic retention capacity, a decrease in pathogen load and weeds, and greater ease of handling and mixing—over time the continuous harvesting of peat for agricultural purposes has led to the degradation of peatlands in the northern hemisphere and an inevitable increase in greenhouse gases in the atmosphere [42]. As a consequence, many countries have begun to impose restrictions on the use of this material. Peatlands are home to a wide range of natural habitats that guarantee biological diversity and the survival of a number of species currently considered at risk. This important ecosystem not only plays a fundamental role in carbon-fixing and in storing natural water resources but also safeguards the historical and geochemical memory of our planet [43–45].

In recent times, peat has become increasingly expensive and difficult to obtain [46], leading to the search for alternative substrates [47]. The use of alternative substrates in pot plants presupposes, however, that a number of chemical–physical and hydraulic properties, such as bulk density, pH, electrical conductivity, cation-exchange capacity, hydraulic-retention capacity, organic-matter content and porosity, can be guaranteed [48–51]. A number of studies [37,47,52–56] have demonstrated that organic residues, such as municipal solid waste, sewage sludge and pruning residues (following an adequate composting process) can be used as alternative growing substrates to peat, with optimal results. Some authors [34] studied the effect of growing substrates on the EO content of rosemary, concluding that all growing substrates are suitable when fertilization and irrigation practices are well-controlled. It was demonstrated [35] that peat can be replaced with a mixture of compost, chicken manure and biochar in rosemary cultivation, while other authors [37] achieved better rooting and length/weight of rosemary roots when using vermicompost.

The main aim of this study was to assess the effects of irrigation, peat-alternative substrates and plant habitus on the morphological, aesthetic and production characteristics of Sicilian rosemary biotypes grown in pots.

2. Materials and Methods

2.1. Rosemary Experimental Field

Tests were carried out in the two years 2016 and 2017 in Sciacca (Italy) (37°30'33" N–13°05'20" E, 60 m a.s.l.), in an experimental field belonging to the Department of Agriculture, Food and Forest Sciences of the University of Palermo. Three Sicilian rosemary biotypes (code RSM) were gathered from a collection field of 10-year-old rosemary mother plants. Plant material was characterized taxonomically using analytical keys and by comparing it with exsiccata that had been previously prepared. The voucher specimen codes of the exsiccata (SAAF-S/0357) were deposited at the Department of Agricultural, Food and Forest Sciences Herbarium of University of Palermo (Italy). The plants were selected according to different growth habitus types: RSM_2 (SAAF-S/0357_a) presented an erect habitus, RSM_6 (SAAF-S/0357_b) a semi-erect habitus and RSM_5 (SAAF-S/0357_c) a prostrate habitus (Figure 1).

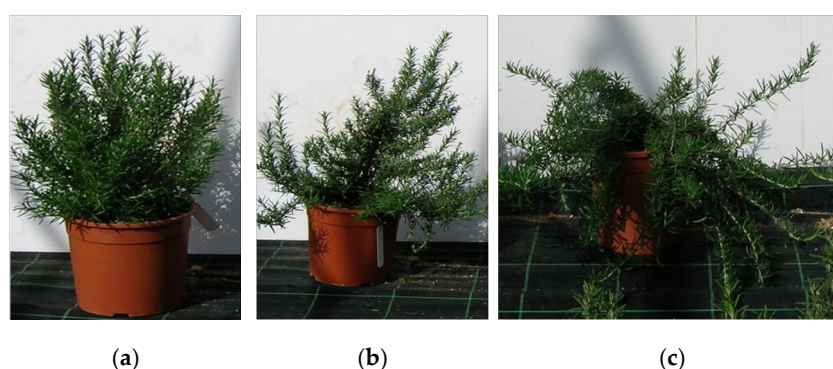


Figure 1. Types of plant habitus of rosemary biotypes in the study. (a) refers to erect habitus, (b) refers to semi-erect habitus, (c) refers to prostrate habitus.

Each accession was propagated using stem cuttings from the apical plant parts 6 cm in length. The cuttings were treated with 1-Naphthaleneacetic acid (0.50%) and then rooted in an open cold frame. The rooted cuttings were transplanted into 18-cm pots on 20 May 2016 and 15 May 2017. Four types of substrate were used with varying percentages of peat (70, 50, 40 and 20%), compost (20, 30 and 50%) and perlite (constant at 30%). The substrate was fortified with a slow-release N–P–K fertilizer + microelements (15–19–15 + 2 + 5) at a rate of 3 gr L⁻¹ prior to transplanting.

A drip irrigation system was used for water delivery. Irrigation management consisted of integrating 100% field capacity every 4 days or 2 days. A split-split-split plot experimental design was used for a 4-factor experiment with 3 replicates. The main plot was year (Y) with two treatment levels: Y₁ (2007) and Y₂ (2008). The sub-plot factor was irrigation (I) with two treatment levels: I₁ (integration 100% field capacity every 4 days) and I₂ (integration 100% field capacity every 2 days). The sub-sub-plot factor was the substrate (S) with four treatment levels: S₁ (50% peat, 20% compost, 30% perlite); S₂ (40% peat, 30% compost, 30% perlite); S₃ (30% peat, 40% compost, 30% perlite) and S₄ (70% peat, 30% perlite) as the control. The sub-sub-sub-plot factor was plant habitus (H) with three treatment levels: H₁ (erect habitus); H₂ (semi-erect habitus) and H₃ (prostrate habitus).

2.2. Morphological, Aesthetic and Production Characteristics of the Plants

For all of the rosemary treatments in the experiment, the main morphological and aesthetic characteristics were determined approx. 90 days from transplanting in order to attribute an ornamental value to the plant: plant height, plant diameter, height-to-diameter ratio, number of primary and secondary branches per plant, branch length and width, number of leaves per cm of branch and general appearance of the plant. Flowering stage was assessed only when 50% of the plant had flowers and was determined using a visible value scale between 1 (few flowers) and 3 (abundant flowers). The fresh-matter weight

of plant parts was also determined. The plant material was subsequently dried in an oven at 65 °C for 48 h until it reached a constant weight; the plant dry-matter weight was then calculated. Essential oil content was obtained by hydrodistillation of air-dried plant material (50–100 g) for 3 h in accordance with international guidelines [57].

2.3. Chemical-Physical Properties of Compost

At the beginning of plant growth, the main chemical–physical analyses were carried out on all of the substrates used: pH, electrical conductivity, bulk density, total porosity, air capacity at pF1 and available water capacity. In particular, the compost used was supplied by the company SIRTEC Sistemi Ambientali SRL (Alcamo, Italy). The composting process used by the company included treatment of the preselected organic waste (organic matter from recycled municipal waste or organic residues from agro-industrial processing).

2.4. Weather Data

Data on rainfall and temperature were collected from a meteorological station belonging to Agro-Meteorological Information Service of the Sicilian Government [58]: the station was situated close to the experimental field. The station was equipped with an MTX datalogger (model WST1800) and various climate sensors. More specifically, a temperature sensor MTX (model TAM platinum PT100 thermo-resistance with anti-radiation screen) and a rainfall sensor MTX (model PPR with a tipping bucket rain gauge) provided data on average daily air temperatures (°C), total daily rainfall frequency (d mm > 1) (%) and rainy days per year (d mm > 1) (%).

2.5. Statistical Analysis

Statistical analyses were carried out using MINITAB 19 for Windows. The data were compared using analysis of variance. The difference between means of values was carried out using the Tukey test.

3. Results

3.1. Temperature Trends

Maximum and minimum temperature trends for the experimental site over the two years 2016/2017 are shown in Figure 2.

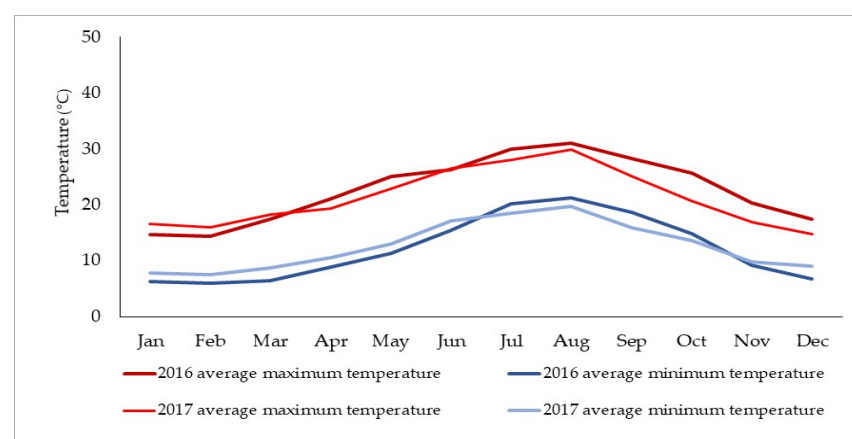


Figure 2. Maximum and minimum air temperature trends during the test period.

Average air temperatures over the approx. 90 days of testing in both years increased between May and August. Maximum air temperatures were on average higher in 2016 than 2017 in May, July and August. In June, average maximum temperatures were similar in both years. Regarding minimum air temperatures, average values were highest in July and August in 2016, whilst in May and June in 2017.

3.2. Chemical–Physical Properties of Substrates

Average values for electrical conductivity and pH (Figure 3) of the peat-alternative substrates increased as the percentage content of the compost in the mix increased in the 4 test substrates.

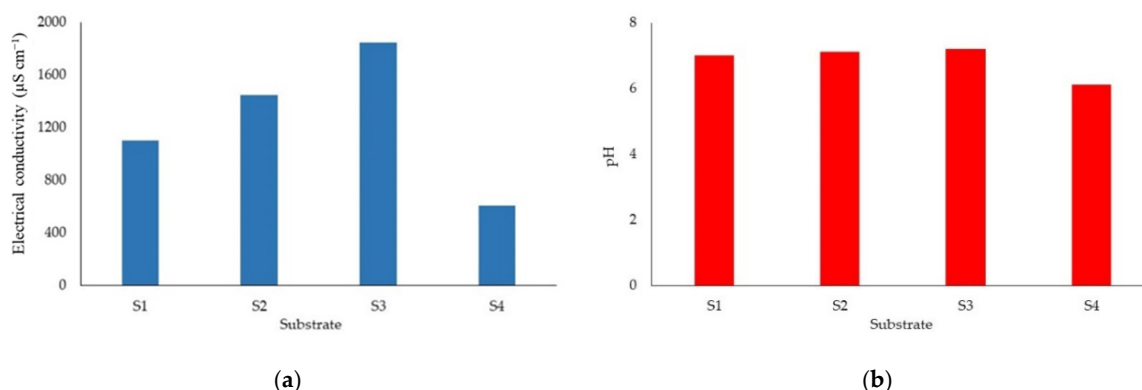


Figure 3. Chemical properties of substrates. Graph (a) refers to electrical conductivity values, while graph (b) refers to pH values of substrates.

Furthermore, an increase in average electrical conductivity and pH was recorded following a decrease in the peat content in the substrates.

From a physical point of view, it was noted that the gradual substitution of the peat in the mix also determined an increase in bulk density and a slight decrease in the air capacity at pF1 and in porosity. Regarding available water content, the substrates with a greater compost content produced higher average values compared to the control substrate (Table 1).

Table 1. Physical properties of substrates. Average values are shown.

| Parameter | Growing Substrate | | | |
|-------------------------------------|-------------------|----------------|----------------|----------------|
| | S ₁ | S ₂ | S ₃ | S ₄ |
| Bulk density (g cm^{-3}) | 0.23 | 0.25 | 0.33 | 0.17 |
| Total porosity (%) | 89.59 | 88.88 | 86.07 | 91.86 |
| Air capacity at pF1 (%) | 41.16 | 43.42 | 32.22 | 46.44 |
| Available water (%) | 20.65 | 19.81 | 24.33 | 18.32 |

3.3. Effects of Year, Substrate, Irrigation and Plant Habitus on Rosemary Plants

Data on the morphological, aesthetic and production characteristics of the rosemary plants, under the influence of the main factors, in years 2016 and 2017, are shown in Tables 2 and 3 and in Figures 4–6.

No significant differences were found for the factor year regarding all of the parameters in the study except for branch width. However, the factor irrigation produced significant effects for all of the parameters tested. Looking more closely at the effect of the two levels of irrigation on the morphological and production parameters, seemingly contrasting results were found. The highest average values for primary branching, flowering stage, number of leaves per cm/branch and percent content of EO were found under irrigation level I₁, whereas highest average values for height, diameter, height-to-diameter ratio, general appearance of the plants, fresh and dry weight, number of secondary branches, and length and width of branches were found under irrigation level I₂.

The plants demonstrated the best morphological, aesthetic and production performances when irrigation was more frequent. Vice versa, the greatest percent content in EO was obtained when irrigation events were less frequent (Table 3, Figure 4).

The factor substrate determined significant differences for all of the morphological and production parameters, with the exception of plant height-to-diameter ratio and flowering. More specifically, greatest average plant heights (29.46 cm) were recorded when the plants were cultivated using the control substrate, whereas the lowest average values were observed in the other substrates. Likewise, the highest average plant diameters (48.71 cm) and general appearance scores (6.10) were recorded for the control substrate. The greatest number of primary (15.29) and secondary (19.41) branches was obtained in rosemary plants grown in the control substrate; the smallest number of primary and secondary branches was found with substrates S₁ and S₃, respectively. The greatest average values relating to branch length and width were found for substrate S₃, whilst the lowest average values were obtained with the control substrate. Regarding production parameters, the greatest average fresh weights and dry weights were obtained using the control substrate, confirming trends observed for most of the morphological parameters. The percent content of EO ranged between 0.68% (S₁ and S₃) and 0.66% (S₂ and S₄); the difference between the different substrates in terms of average percentages in EO was, therefore, minimal (Table 3, Figure 5).

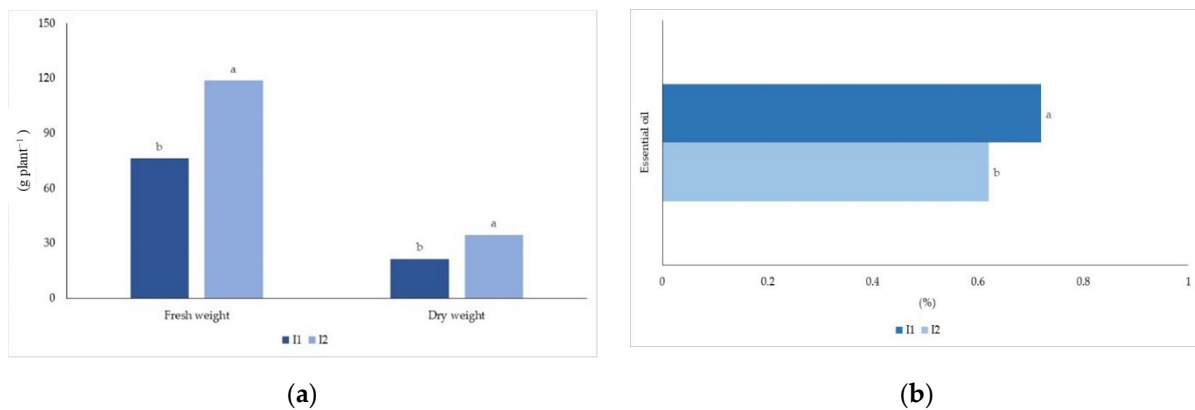


Figure 4. Effect of irrigation on production characteristics of rosemary biotypes. Graph (a) refers to effect of irrigation on fresh and dry weight, while graph (b) refers to effect of irrigation on essential oil. Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

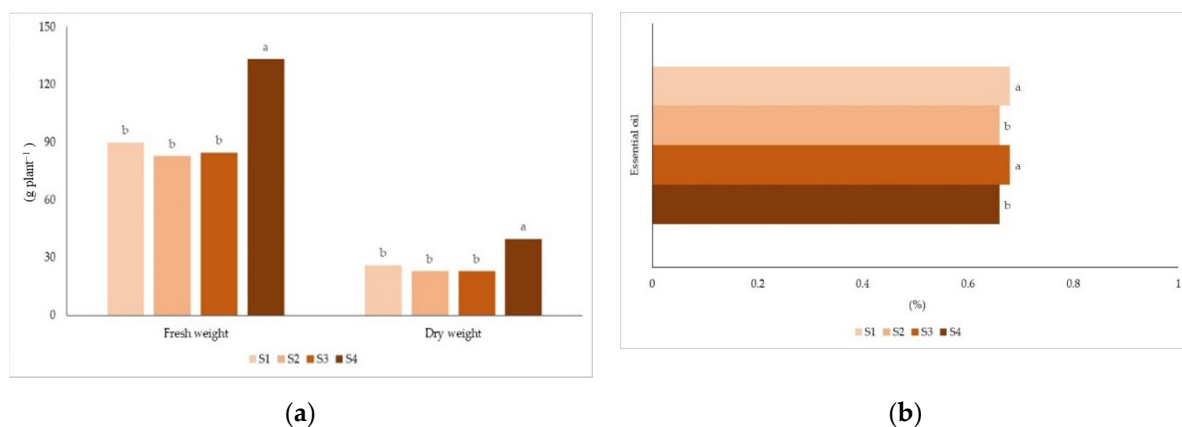


Figure 5. Effect of substrate on production characteristics of rosemary biotypes. Graph (a) refers to effect of substrate on fresh and dry weight, while graph (b) refers to effect of substrate on essential oil. Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

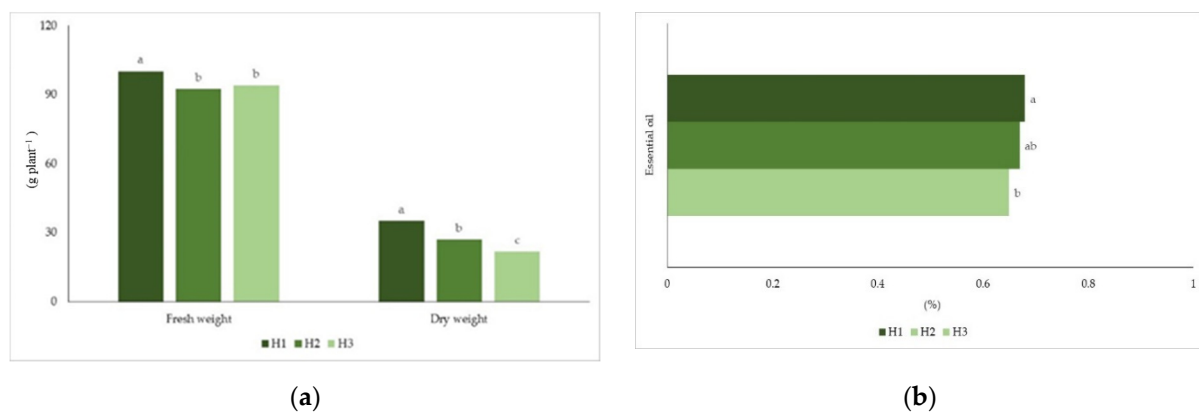


Figure 6. Effect of plant habitus on production characteristics of rosemary biotypes. Graph (a) refers to effect of plant habitus on fresh and dry weight, while graph (b) refers to effect of plant habitus on essential oil. Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test.

The factor plant habitus had a significant effect on all of the parameters in the study. More specifically, biotype RSM_2, with erect growth habitus, differed from the others in terms of greater height (39.29 cm), greater height-to-diameter ratio (1.29), greater number of primary branches, better general appearance, more abundant flowering, greater fresh (106.08 g) and dry (35.05 g) weight and greater EO percent content (0.68%). The biotype RSM_6 with semi-erect growth habitus, showed greater diameter (50.73 cm) and greater branch length (33.12 cm) and width (3.80 cm) than other biotypes. The EO percent content was intermediate between the other two biotypes. Biotype RSM_5 with prostrate habitus showed the highest number of secondary branches but also obtained the lowest EO percentage (0.65%) of the three rosemary biotypes (Table 3, Figure 6).

Considering the interaction between the main factors (Tables 2 and 3), analysis of the variance showed that the interaction of the factor year with the other factors did not determine significant effects on any of the morphological and production parameters. The irrigation-by-plant habitus interaction determined significant differences for plant diameter and the number of secondary branches; more specifically, the highest average diameter was obtained with the I₂-by-H₂ interaction, whilst the lowest average value was found with I₁-by-H₁ interaction, as showed in Supplementary Table S1.

The substrate-by-plant habitus interaction had significant effects on a series of characteristics, such as plant diameter, fresh and dry weight, the development of secondary branching, branch length and width, and EO content. With specific reference to the treatments, the greatest plant diameter was recorded with the interaction between the control substrate and the semi-erect habitus biotype. The lowest value was recorded with the interaction between S₃ (which contained the highest compost content) and H₃. The greatest number of secondary branches was observed with the interaction between the control substrate and the prostrate habitus biotype, whilst the smallest number was found with the interaction between the control substrate and the erect habitus biotype. The highest average values for branch length and width were obtained with the S₃-by-H₂ interaction. Regarding production parameters, the interaction between the control substrate and the prostrate habitus biotypes produced the highest average value for fresh weight.

Considering dry weight, highest averages were found when the control substrate interacted with the erect habitus biotype, whereas the lowest average values were produced with the interaction between substrate S₃ and the biotype with prostrate habitus. A closer look at EO content showed that the S₁-by-H₁ interaction produced the highest EO percentage (0.73%). The lowest average EO percentage (0.64%) was recorded with the S₄-by-H₁ and S₂-by-H₃ interactions, as showed in Supplementary Materials (Table S1).

Table 2. Morphological and aesthetic characteristics of rosemary plants in response to year, irrigation, peat-alternative substrate and plant habitus.

| Treatment | Plant Height (cm) | Plant Diameter (cm) | Height-to-Diameter Ratio | No. Primary Branches (per Plant) | No. Secondary Branches (per Plant) | Plant Branch Length (cm) | Plant Branch Width (cm) | No. Leaves cm Branch ⁻¹ | General Appearance of Plant | Flowering |
|-----------------------------|-------------------|---------------------|--------------------------|----------------------------------|------------------------------------|--------------------------|-------------------------|------------------------------------|-----------------------------|-----------|
| Year (Y) | | | | | | | | | | |
| Y1 | 26.18 a | 39.60 a | 0.73 a | 14.51 a | 14.22 a | 29.01 a | 3.43 a | 8.55 a | 5.40 a | 0.95 a |
| Y2 | 25.27 b | 38.66 b | 0.74 a | 14.25 a | 14.01 a | 28.53 a | 3.32 b | 8.67 b | 5.26 a | 0.94 a |
| Irrigation (I) | | | | | | | | | | |
| IW ₁ | 24.10 b | 36.68 b | 0.71 b | 15.39 a | 10.61 b | 27.96 b | 3.29 b | 8.89 a | 5.17 b | 1.58 a |
| IW ₂ | 27.83 a | 41.58 a | 0.76 a | 15.37 b | 17.63 a | 29.58 a | 3.46 a | 8.33 b | 5.50 a | 0.31 b |
| Substrate (S) | | | | | | | | | | |
| S ₁ | 25.35 b | 37.92 b | 0.72 a | 13.36 b | 12.92 b | 28.70 b | 3.32 b | 9.07 a | 5.21 b | 0.83 a |
| S ₂ | 24.59 b | 34.78 b | 0.77 a | 15.51 a | 12.43 b | 29.67 b | 3.47 ab | 8.81 ab | 4.99 b | 0.91 a |
| S ₃ | 24.46 b | 35.09 b | 0.74 a | 13.37 b | 11.71 b | 31.18 a | 3.63 a | 8.54 ab | 5.05 b | 1.11 a |
| S ₄ | 29.46 a | 48.71 a | 0.70 a | 15.29 a | 19.41 a | 26.54 c | 3.08 c | 8.02 b | 6.10 a | 0.94 a |
| Plant habitus (H) | | | | | | | | | | |
| H ₁ | 39.29 a | 30.29 c | 1.29 a | 18.69 a | 6.45 b | 27.96 b | 3.58 b | 8.90 a | 5.77 a | 1.20 a |
| H ₂ | 22.47 b | 50.73 a | 0.44 b | 10.83 c | 5.37 b | 33.12 a | 3.80 a | 8.54 a | 5.38 b | 0.79 b |
| H ₃ | 16.15 c | 36.37 b | 0.46 b | 13.62 b | 30.18 a | 25.23 c | 2.74 c | 8.39 a | 4.85 c | 0.85 b |
| Interactions (significance) | | | | | | | | | | |
| Y × I | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Y × S | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Y × H | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| I × S | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| I × H | n.s. | * | n.s. | n.s. | * | n.s. | n.s. | n.s. | n.s. | n.s. |
| S × H | n.s. | * | n.s. | n.s. | * | * | * | n.s. | n.s. | n.s. |
| Y × I × S | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Y × I × H | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Y × S × H | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| I × S × H | * | n.s. | * | n.s. | * | n.s. | n.s. | n.s. | n.s. | n.s. |
| Y × I × S × H | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test; n.s. = not significant; * = significant at $p \leq 0.05$.

Table 3. Production characteristics of rosemary plants in response to year, irrigation, peat-alternative substrate and plant habitus.

| Treatment | Fresh Weight (g plant ⁻¹) | Dry Weight (g plant ⁻¹) | EO Content (%) |
|-----------------------------|---------------------------------------|-------------------------------------|----------------|
| Year | | | |
| Y ₁ | 98.35 a | 28.25 a | 0.67 a |
| Y ₂ | 96.66 a | 27.61 a | 0.67 a |
| Irrigation | | | |
| I ₁ | 76.31 b | 21.38 b | 0.72 a |
| I ₂ | 118.76 a | 34.49 a | 0.62 b |
| Substrate | | | |
| S ₁ | 89.78 b | 26.01 b | 0.68 a |
| S ₂ | 82.83 b | 22.99 b | 0.66 b |
| S ₃ | 84.63 b | 22.89 b | 0.68 a |
| S ₄ | 133.28 a | 39.74 a | 0.66 b |
| Plant habitus | | | |
| H ₁ | 100.08 a | 35.05 a | 0.68 a |
| H ₂ | 92.43 b | 27.01 b | 0.67 ab |
| H ₃ | 94.01 b | 21.75 c | 0.65 b |
| Interactions (significance) | | | |
| Y × I | n.s. | n.s. | n.s. |
| Y × S | n.s. | n.s. | n.s. |
| Y × H | n.s. | n.s. | n.s. |
| I × S | n.s. | n.s. | n.s. |
| I × H | n.s. | n.s. | n.s. |
| S × H | * | * | * |
| Y × I × S | n.s. | n.s. | n.s. |
| Y × I × H | n.s. | n.s. | n.s. |
| Y × S × H | n.s. | n.s. | n.s. |
| I × S × H | * | n.s. | n.s. |
| Y × I × S × H | n.s. | n.s. | n.s. |

Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey's test; n.s. = not significant; * = significant at $p \leq 0.05$.

The interaction between irrigation factors, substrate and plant habitus determined significant differences for height, height-to-diameter ratio, number of secondary branches and plant fresh weight. Observing the various test treatments, the greatest values for height were obtained when irrigation level I₂ interacted with the control substrate and the biotype with erect habitus, whereas the lowest values for height were found with the interaction I₁-by-S₁-by-H₃. The greatest average values for height-to-diameter ratio were determined with the interaction I₂-by-S₄-by-H₁ and I₂-by-S₂-by-H₁. The highest number of secondary branches, however, was found in the interaction irrigation level I₂ with the control substrate and prostrate habitus biotype, whereas the lowest average values were found with the interaction I₁-by-S₄-by-H₁.

Regarding production parameters, it is worth noting that the interaction I₂-by-S₄-by-H₃ presented higher average values for fresh weight (174.72 g), whilst the interaction I₁-by-S₂-by-H₂ presented the lowest averages (64.57 g).

4. Discussion

In this study, over two years of tests, three Sicilian biotypes of *Rosmarinus officinalis* L. were evaluated growing in pots with differing habitus types and two levels of irrigation. Four substrates were compared containing different ratios of peat and compost to perlite.

All the biotypes showed good adaptation capacities to the irrigation conditions and to the different substrate types in both years, showing significant differences for all of the morphological and production characteristics in the study. The general appearance of the

plants was monitored regularly and, for both levels of irrigation over the two years, no signs of lack of water or water stress were noted. Various observations have been made in scientific literature on the effects of irrigation on the morphological and production characteristics of rosemary in open-field conditions. Some authors [59] stated that the use of surface or underground micro-drip irrigation systems does not exert a significant effect on the characteristics of rosemary plants. It was demonstrated [23] that the quality of the irrigation water did not have a significant effect on rosemary. In contrast, other authors [60] observed that irrigation significantly influenced the main characteristics of rosemary plants grown in pots. In our study, it was noted that the interval of time between irrigation events significantly influenced the characteristics of rosemary. In general, plant growth was negatively affected by water stress, probably due to a decrease in the stomatal aperture, which limits the circulation of CO₂ in the leaves and reduces photosynthetic activity, as reported in the literature [61].

Although little is known about the effects of cultivation techniques on potted rosemary, it should be emphasized that almost all the studies focus on investigating the influence of substrate on plant growth and production. The composition of the substrate and, in particular, a decrease in the peat content in the mix, had significant effects on the growth and on the production parameters of the plants. Peat represents the most frequently used growing substrate due to its excellent chemical and physical properties for plants grown in pots and its stability over time. Gruda [62] affirms that peat is the standard constituent of substrates used in the production of ornamental plants in pots and that other constituents may vary by 20% to 50%. However, several authors note that high-quality peat is expensive and can cause environmental problems due to the depletion of unrenowned resources [38,55]. Compost represents an interesting substrate alternative to peat and can improve the physical, chemical and biological properties of the substrate [56,63]. Raviv [64] highlights that compost is a bioresource and can be a valid alternative to peat, despite the fact it is potentially a waste material. However, the qualitative properties of compost are strongly linked to its maturity and stability. Rinaldi et al. [38] tested a number of substrates by mixing increasingly greater amounts of eight different composts in place of peat, with a fixed inert material. The authors observed that the most suitable substrates for rosemary growth contained compost at a rate of up to 70%. De Lucia et al. [36] studied four composts, obtained from agro-industrial, urban and green wastes, as growing media components in *Rosmarinus officinalis* L. and obtained improved quality rosemary plants by substituting peat with 30% compost. Our study also assessed the chemical–physical properties of the substrates as these characteristics tend to change as the compost content increases and the peat content decreases. In fact, the particle size of the growing substrate and the geometry of the pot need to be carefully considered in order to balance water availability and root aeration [65]. As reported in some studies [24,66], the decrease in plant height in rosemary grown in pots, using substrates with decreasing quantities of peat, is of interest in order to increase the ornamental value of the rosemary as a reduction in height is a desirable ornamental characteristic. In this study, the greater length and width of the main branches was obtained using a substrate with the greatest compost content. According to Rinaldi et al. [38], compost from pruning residues or mixes with agro-industrial or urban waste are of most interest in rosemary cultivation.

In our study, the factor year did not have significant effects on the rosemary characteristics. This result was also confirmed by some authors [67], who state that the composition and variability of rosemary mainly depend on the genetic background and origin rather than on the environmental conditions and geographic location.

The factor irrigation, however, did have a significant effect on the percent content of the EO. As highlighted in the literature, irrigation influences the morphological and physiological characteristics determining the yield of the plants and also has a bearing on the quantity of some of the principal components of the essential oils (EOs) [22,68–71]. In particular, the percentage of EO increased following limited water availability, in agreement with the results of Pirzad and Mohammadzadeh [27].

The factor substrate affected the percent content of EO. This was confirmed by a number of studies that demonstrated that the quantitative characteristics of EOs of various species in the *Lamiaceae* family, such as *Thymus caespitosus* Brot. [72], *Ocimum basilicum* L. [73] and *Lavandula angustifolia* Mill. [15], are greatly affected by the composition of the substrate. It is important to note that the production of EO depends on genetic characteristics, as revealed by this study and in agreement with some authors [67,74–79] who stated that the genetic pool contributes to a greater degree than other factors to determine both the quality and the quantity of EOs.

Rosemary presents a variety of different habitus types, morphological traits, flower colours and aromatic properties [80]. Our study confirms results reported by Flamini et al. [81], who, in a recent study on the evaluation of the agronomic and production characteristics of two rosemary biotypes, state that the best performances are produced by biotypes with an erect habitus. Plant height, in particular, is a morphological characteristic under genetic control; however, its manifestation could depend on environmental factors, such as altitude, air temperature and solar radiation, but also growth techniques, such as irrigation, fertilization and, in the case of pot plants, also the substrate used. The high significance of the interactions substrate-biotype and irrigation-substrate-biotype on the agronomic and production parameters could play an important role in the production of plants with high ornamental value but also in the production of EOs, confirming results of other authors [15,70,81–83].

5. Conclusions

The results of this study show that the use of peat-alternative substrates can represent a valid opportunity for the cultivation of rosemary in pots, and, in general, for the cultivation of numerous medicinal and aromatic plants. The use of compost could allow a partial or total replacement of peat, leading to environmental benefits (the harvesting of peat as a substrate has a strong environmental impact) energy and economic benefits (peat is an expensive and functional material). It could also foster the use of biomass from agro-industrial activities and recycled municipal organic waste. In this study, although the best agronomic results were obtained using the substrate with greater peat content, it is worth highlighting that the substrates with 20% and 30% compost also gave excellent performance results, confirming the idea of a partial replacement of peat. The erect habitus biotype showed the best adaptation capacity to the various treatments. Considering the various interactions between the main factors, the substrate-by-irrigation-by-biotype interaction had significant effects on the morphological and production characteristics of the rosemary plants.

Further research is required, however, to assess both the performance of the various components of the substrate and, more specifically, exactly when the various agronomic factors interact with the substrate. A longer period is also needed to evaluate the morphological and production characteristics. Finally, the various biometric and production characteristics of the genotypes, together with the substrate types, could be of use when selecting rosemary biotypes with good adaptability to cultivation in pots for ornamental purposes, thus favouring the expansion of this species.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2077-0472/11/1/13/s1>, Table S1: Interactions between the various treatments of the main factors in the study.

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