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# Biostimulants Improve Plant Performance of Rosemary Growth in Agricultural Organic System

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**Abstract:** Rosemary (*Rosmarinus officinalis* L.) is an evergreen and a xerophytic shrub that is frequently employed in culinary, decorative, and industrial applications. It shows significant variations in biomass and essential oil (EO) yield due to effects of biotic and abiotic factors. Biostimulants are widely used in agriculture, and in organic agricultural systems, they may contribute significantly to the stability and/or to the increase in crop yields, ensuring respect for the environment. The aim of this study was to assess the effect of different types of biostimulants on the yield performance of rosemary. Four commercial formulations of biostimulants based on *Eklonia maxima*, *Ascophyllum nodosum*, fulvic acids, and protein hydrolysates were used for the tests. Water was used as a control. Six applications were performed. At harvest, several morphological and productive parameters were determined. All parameters were affected by biostimulant application. The highest fresh and dry yields were obtained with the application of fulvic acids (13.1 t $ha^{-1}$ of fresh biomass and 4.3 t $ha^{-1}$ of dry biomass) and protein hydrolysates (13.6 t $ha^{-1}$ of fresh biomass and 4.4 t $ha^{-1}$ of dry biomass) with values 60% higher than that of the control. Regarding the EO content in the dry biomass, the highest value (1.72% $v/w$) was found in control plants, whilst the lowest (1.14%) was observed in plants treated with fulvic acids. In the case of the EO yield, the lowest value (46.5 kg $ha^{-1}$) per unit area was found in the control. The application of *Eklonia maxima* and protein hydrolysates produced the highest EO yield values, 65 kg $ha^{-1}$ and 66.5 kg $ha^{-1}$, respectively. This study highlights the use of biostimulants to increase the yield performance of rosemary when grown in an organic agricultural system.

**Keywords:** *Rosmarinus officinalis* L.; medicinal and aromatic plants; foliar biostimulants; yield; essential oil



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## 1. Introduction

Medicinal and aromatic plants (MAPs) are usually grown in marginal lands with the use of lower energy inputs with respect to other open field crops, helping to reduce soil degradation and allowing farmers to increase their income [1–3]. These plants are largely known for the high content of bioactive metabolites, such as essential oils (EOs), phenols and flavanols. These metabolites show antibacterial, anti-inflammatory, antioxidant, anti-tumor, antidiabetic and other properties, and have been largely investigated in previous studies [4,5].

The literature reports that MAPs can show different rates of biomass production and bioactive compounds due to the effects of endogenous and exogenous factors [6–9]. For example, it is well known that fertilizers can affect the quantitative and qualitative characteristics of MAPs [10–12]. However, the increase in commercial production of MAPs due to fertilizers requires the application of an optimal dose of fertilizer [13]. Farmers must add nutrients to the soil, such as synthetic or natural fertilizers, to enrich it and thus obtain adequate yields [14,15]. The use of such fertilizers in agriculture boosts the output; however, their incorrect use has a negative influence on soil productivity and the quality of the environment [16]. This is of great importance in particular in areas where water availability is limited and the management of those water resources represents a priority [13,17,18]. A number of authors [10,19,20] report that, in the developmental stage of MAPs, prolonged water stress conditions may determine changes in physiological and metabolic processes and negatively affect the photosynthetic and transpiration processes, causing a considerable drop in growth and yield performance. The use of non-conventional and innovative cultivation practices could improve the agronomical and productive performance of crops [21–23].

The application of biostimulants in agriculture represents a sustainable and successful practice to improve nutrient use efficiency and to obtain yield stability, also under sub-optimal conditions [24,25]. As reported in European Regulation 2019/1009 "https://eur-lex.europa.eu/eli/reg/2019/1009/oj (accessed on 15 June 2023)", biostimulants are any "substance(s) and/or micro-organisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality". Plant biostimulants are widely used in various cropping systems, including organic, conventional, and integrated agricultural systems [26]. The use of microbial and non-microbial biostimulants has spread quickly as these substances stimulate vegetative growth and enhance tolerance to abiotic and biotic stresses [27]. These products are considered as safe for both humans and animals and environmentally friendly and might represent a sustainable solution to help mitigate problems in agriculture caused by climate change [28,29].

Seaweed extracts obtained from *Eklonia maxima* and *Ascophillum nodosum* can affect the productive response of crops by improving the absorption of nutrients and water, increasing the photosynthesis rate and promoting compounds, such as auxins, cytokinins, and gibberellins [30]. A number of studies [31,32] report that the application of various seaweed extracts to MAPs and other crops can increase their resistance to various abiotic stresses, such as drought, high temperature, and a lack of nutrients in the soil.

Abiotic stress may be mitigated using fulvic acids as a biostimulant [33]. These products also have a positive impact on several molecular processes, including protein synthesis, photosynthetic activity, and enzyme activity [34,35]. Their use can also produce positive effects on plant growth and increase the content of photosynthetic pigments, carotenoids, and total phenols, as well as nitrogen, phosphorus, and potassium (NPK) concentration [33,34]. In *Origanum vulgare* subsp. *hirtum*, Aytaç et al. [35] obtained an increase in yields and essential oil content with the application of humic acid to soil.

Protein hydrolysates represent another type of biostimulant, and their application can improve primary and secondary plant metabolism [36]. They are mixtures of polyprotein hydrolysate, oligo-protein hydrolysate, and amino acids [37] and can be applied to the leaf or root. Protein hydrolysates can promote various plant physiological responses, increase crop yield and quality, and enhance the tolerance of plants to drought, high temperatures, lack of nutrients, salinity, and unfavorable soil pH [38]. Paul et al. [39] claimed that protein hydrolysate application on tomato plants can be considered as a sustainable crop enhancement technology for agricultural productivity under water-limited conditions. Some studies carried out in different countries [31,37,40–42] highlight the effectiveness of biostimulants on yield and on the qualitative characteristics of greenhouse and open-field crops; however, findings concerning MAPs and rosemary are less abundant.

*Rosmarinus officinalis* L. (*Lamiaceae*) is a plant that grows widely throughout Europe, Asia, and Africa, and the Mediterranean basin is one of its preferred growth regions [43]. In this area, rosemary is often grown in organic farming and the demand for organic herbs is increasing worldwide [44]. In recent years, this plant captures the attention of researchers, consumers, health professionals, and companies because it is known to be an important source of bioactive chemicals with potential health advantages [43]. Rosemary possesses a wide range of significant therapeutic and functional qualities, including antibacterial, antidiabetic, anti-inflammatory, anticancer, and antioxidant effects [45]. Additionally, like many other Mediterranean species, rosemary is a widely used herb by food companies, and its extracts are added to a variety of products to enhance their organoleptic characteristics and oxidative durability [5]. As a result, its usage is supported and encouraged, and also, the academic community has focused on rosemary cultivation in recent years. Regarding biostimulant application on rosemary, the few studies conducted [46–48] have evaluated the effect of a single type of foliar biostimulant, and the information is very limited. On this basis, this study's objective was to evaluate the impact of four different biostimulants on the morphological and productive parameters of *Rosmarinus officinalis* L. organically cultivated in a Mediterranean environment without irrigation.

## 2. Materials and Methods

### *2.1. Experimental Site and Cultivation Practices*

Tests were conducted on a local farm located in Aragona (Sicily, Italy) (330 m a.s.l., 37°22′32.71″ N, 13°38′33.59″ E Google Earth), during the growing seasons of 2020–2021 and 2021–2022. The soil was categorized as Regosol [United States Department of Agriculture (USDA) classification: typic xerorthents] and sandy clay loam (48% sand, 28% clay and 24% silt) with a pH of 7.4, 16 g kg$^{-1}$ organic matter, 1.22% total nitrogen, 20.4 ppm assimilable phosphate, and 364 ppm assimilable potassium. Agamic propagation was carried out by dividing the bushes. The plants were transplanted at the beginning of spring 2019. The plant density was 4000 plants ha$^{-1}$. The distance between rows and within rows was 2.50 m and 1.00 m, respectively. Rosemary plants were managed under rainfed conditions adopting a low-input growth method following the common agronomic practices of the cultivation area. Before transplanting, the experimental field received organic fertilization through the distribution of 2 t ha$^{-1}$ of manure which was buried at a depth of 0.30 m. No pesticides were applied in either year. Weeds were controlled mechanically at the beginning of spring and before harvesting. In both years, plants were harvested during the third 10-day period of June.

### *2.2. Weather Data*

Rainfall and temperature data were taken from a meteorological station that belonged to the Sicilian Agro-Meteorological Information Service [49]. The station has a datalogger and sensors for measuring air temperature (TAM platinum PT100 sensor, heat resistance with anti-radiation screen) and total rainfall (PPR sensor with tilting bucket rain gauge). Data about the average daily maximum and minimum temperatures (°C) and total decadal (10-day period) precipitation (mm) have been considered.

### *2.3. Treatments*

Four commercial biostimulant formulations were used for the tests, based on *Eklonia maxima* (Kelpstar®, Mugavero fertilizers, Termini Imerese, Italy), *Ascophyllum nodosum* (Algastar®, Mugavero fertilizers, Termini Imerese, Italy), fulvic acids (Niger L®, Mugavero fertilizers, Termini Imerese, Italy), and protein hydrolysate (Tyson®, Mugavero fertilizers, Termini Imerese, Italy). Water (C) was used as the control. A total number of 6 foliar applications of biostimulants were made. The first application was performed during the first week of April in each year; the others were then carried out every two weeks. The biostimulant dosage was planned in order to provide the same total amount of N, considering the N content of each type of product. The doses are listed in Table 1.

For each foliar application, 400 L of water ha$^{-1}$ was used. A portable sprayer with an operating pressure of 250 kPa was adopted. Foliar treatments were applied at dawn, when temperature (10–12 °C), relative humidity (91–66%), and stomatal aperture were optimal for foliar absorption [50]. A randomized complete block design with three replicates was used for the tests. Year (Y) and biostimulants (B) were used as fixed effects in the linear model/ANOVA. Each block comprised 5 plots of 25 m$^2$. Foliar treatments were applied for each randomized plot in the block. The plots were well spaced in the block. During foliar applications, each plot was defined by plastic panels, which also prevented drift.

**Table 1.** Doses of foliar biostimulants.

| Biostimulant | Dose [1] [L hL$^{-1}$] | Total Amount [2] [L ha$^{-1}$] |
|---|---|---|
| EM = *Eklonia maxima* | 0.25 | 6 |
| AN = *Ascophyllum nodosum* | 0.25 | 6 |
| FA = Fulvic acids | 0.05 | 12 |
| PH = Protein hydrolysate | 0.50 | 1.2 |

[1] For each application; [2] for the 6 total applications in 2400 L of water.

### 2.4. Plant Measurement

At harvest, plant height, number and diameter of primary stems, relative water content (RWC), chlorophyll content, total fresh yield, total dry yield, EO content, and EO yield were determined. In both years, plants were harvested during the third week of June. Young shoots formed in the year of harvest were collected to determine yield parameters. The plant material was dried in a shaded and ventilated environment for approximately 10 days at a temperature of 25–30 °C. Plant dry-matter weight was then calculated.

The chlorophyll content was measured using Dualex 4 Scientific (Force A, Orsay, France) portable Chlorophyll meter. For each plot, thirty fully grown leaves were used. These readings were automatically averaged by the device.

The RWC of leaves was determined as follows. Thirty fresh leaves per plot have been taken, and the fresh weight (FW) was recorded. The leaves were floated in a falcon tube with distilled water for 24 h. The leaves were then removed from the water and placed on absorbent paper to remove the excess water, and the turgid weight (TW) was recorded. The leaves were then dried in an oven for twenty-four hours, and the dry weight was noted [51]. The following formula was used to determine the RWC:

$$RWC = (FW - DW/TW - DW) \times 100 \quad (1)$$

### 2.5. Essential Oil Extraction

EO was obtained by hydro distillation in a Clavenger-type apparatus (Albrigi Luigi, Verona, Italy) of air-dried plant material (500 g) and 6 L of water. The extraction was carried out for 3 h in accordance with international guidelines [52]. After, the EO samples were stored at 4 °C.

### 2.6. Statistical Analysis

Statistical analyses were performed using the package MINITAB 19 (State College, PA, USA) for Windows. Data were compared using analysis of variance (ANOVA). The difference between means was analyzed using Fisher's LSD test ($p \leq 0.05$). The percentage data for RWC were subjected to arcsine transformation. Pearson's correlation analysis was carried out to evaluate the relationships between the morphological and productive parameters of rosemary plants.

## 3. Results

### 3.1. Analysis of Rainfall and Air Temperature Trends at the Experimental Site

Air temperature and rainfall trends are shown in Figure S1. Over the two years, annual rainfall levels were 493 mm (2020–2021) and 611 mm (2021–2022). In the first growing season, rainfall was mainly distributed from December to March, reaching a peak during the first 10-day period of December (96 mm). In the second growing season, rainfall was mainly distributed from October to December. Significant rainfall occurred in the first 10-day period of May (37 mm). In both years, temperatures trends were similar and consistent with the average temperature of the experimental area (Figure S1).

### 3.2. Effects of Year and Biostimulants on Morphological and Yield Parameters of Rosemary

The year (Y) factor significantly influenced plant height, stem diameter, chlorophyll content, and RWC (Table 2). The highest values of stem diameter and chlorophyll content were observed in the first year, while the highest values of plant height and RWC were in the second year (Table 2).

**Table 2.** Morphological and yield parameters in response to year (Y), biostimulants (B), and their interaction Y × B.

| | Plant Height | Primary Stem | Stem Diameter | Chlorophyll Content | RWC | Total Fresh Yield | Total Dry Yield | Essential Oil Content | Essential Oil Yield |
|---|---|---|---|---|---|---|---|---|---|
| | [cm] | [n.] | [mm] | [μg cm$^{-2}$] | [%] | [t ha$^{-1}$] | [t ha$^{-1}$] | [% *v*/*w*] | [kg ha$^{-1}$] |
| Year (Y) | | | | | | | | | |
| 2020–2021 | 52.0 ± 1.3 b | n.s. | 3.1 ± 0.3 a | 29.1 ± 0.6 a | 67.0 ± 0.9 b | n.s. | n.s. | n.s. | n.s. |
| 2021–2022 | 54.7 ± 1.5 a | n.s. | 8.8 ± 0.2 b | 28.3 ± 0.6 b | 70.1 ± 1.8 a | n.s. | n.s. | n.s. | n.s. |
| Biostimulant (B) | | | | | | | | | |
| C | 48.8 ± 0.6 d | 8 ± 0.2 d | 7.4 ± 0.2 d | 25.7 ± 0.4 c | 60.3 ± 0.7 d | 8.3 ± 0.2 c | 2.7 ± 0.1 d | 1.72 ± 0.03 a | 46.5 ± 1.96 d |
| EM | 52.8 ± 2.1 c | 19 ± 1.2 a | 9.6 ± 0.2 ab | 28.2 ± 0.3 b | 68.6 ± 1.4 c | 12.3 ± 0.8 b | 4.0 ± 0.2 b | 1.61 ± 0.05 b | 65.0 ± 3.85 a |
| AN | 53.7 ± 0.5 bc | 12 ± 0.5 c | 9.1 ± 0.3 c | 29.2 ± 0.8 b | 71.1 ± 1.1 b | 11.8 ± 0.3 b | 3.9 ± 0.2 c | 1.44 ± 0.03 d | 55.9 ± 1.93 b |
| FA | 54.0 ± 2.0 b | 17 ± 0.5 b | 9.7 ± 0.3 a | 29.4 ± 1.0 b | 67.8 ± 0.8 c | 13.1 ± 0.6 a | 4.3 ± 0.2 a | 1.14 ± 0.04 e | 48.5 ± 3.66 c |
| PH | 60.5 ± 0.3 a | 13 ± 0.4 c | 9.2 ± 0.2 bc | 31.0 ± 0.5 a | 74.2 ± 2.0 a | 13.6 ± 0.3 a | 4.4 ± 0.1 a | 1.52 ± 0.13 c | 66.5 ± 6.99 a |
| *p*-value | | | | | | | | | |
| Y | 0.000 | 0.493 | 0.028 | 0.022 | 0.000 | 0.730 | 0.751 | 0.378 | 0.131 |
| B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Y × B | 0.000 | 0.198 | 0.116 | 0.002 | 0.000 | 0.001 | 0.000 | 0.010 | 0.010 |

Means and standard errors are reported. Values with different letters are significantly different at $p \leq 0.05$. n.s. = not significant. C = control; EM = *Eklonia maxima*; AN = *Ascophyllum nodosum*; FA = fulvic acids; PH = protein hydrolysate.

Analysis of variance revealed that factor (B) had a highly significant effect ($p \leq 0.01$) on all parameters (Table 2). The highest value of plant height (60.5 cm) was recorded in PH-treated plants, while the highest number of primary stems (19) was in EM-treated plants. The stem diameter was on average higher in FA-treated plants, followed in descending order by EM, PH, and AN (Table 2). PH-treated plants showed the highest chlorophyll content (31.0 μg cm$^{-2}$) and RWC (74.2%), on average. The lowest chlorophyll content (25.7 μg cm$^{-2}$) and the lowest RWC (60.3%) were observed in C-plants (Table 2).

The highest values of yield parameters were obtained with the application of treatments FA and PH (Table 2). Particularly, FA-treated plants obtained average values of fresh biomass and dry biomass of 13.1 and 4.3 t ha$^{-1}$, respectively. Similar average values of fresh biomass (13.6 t ha$^{-1}$) and dry biomass (4.4 t ha$^{-1}$) were found in PH-treated plants. C showed the lowest average values at 8.3 t ha$^{-1}$ for fresh biomass and 2.7 t ha$^{-1}$ for dry biomass. The lowest values for all parameters in the study, except for the EO content, were consistently observed in the control plants. Regarding the EO content, the highest average value (1.72%) was found in C, followed by those obtained with EM (1.61%), while the lowest average value (1.14%) was recorded in the FA-treated plants. However, the lowest EO yield value per unit area was observed in C. Treatments EM (65.0 kg ha$^{-1}$) and PH (66.5 kg ha$^{-1}$) showed the highest EO yield values.

The year-by-biostimulant interaction significantly affected ($p \leq 0.01$) the plant height, chlorophyll content, RWC, total fresh yield, total dry yield, EO content, and EO yield (Table 3).

In both years, the highest average values of plant height were observed in PH-treated plants. For treatments EM and FA, the tallest plants were obtained in the second year, while for AN, they were obtained during the first year. In both years, C-plants showed the lowest plant heights (Table 3).

Regarding chlorophyll content, the highest values (ranged from 30.5 to 31.5 μg cm$^{-2}$) were obtained by the interactions Y1 × AN, Y1 × PH, Y2 × FA, and Y2 × PH (Table 3). The lowest average value of the chlorophyll content (24.9 μg cm$^{-2}$) was observed in C during both growing seasons (Table 3).

**Table 3.** Morphological and yield parameters in response to interaction year (Y) × biostimulants (B).

| | Plant Height | Chlorophyll Content | RWC | Total Fresh Yield | Total Dry Yield | Essential Oil Content | Essential Oil Yield |
|---|---|---|---|---|---|---|---|
| | [cm] | [μg cm$^{-2}$] | [%] | [t ha$^{-1}$] | [t ha$^{-1}$] | [% *v*/*w*] | [kg ha$^{-1}$] |
| Y × B | | | | | | | |
| Y1 × C | 47.0 ± 0.3 f | 26.5 ± 0.3 cd | 61.6 ± 0.5 g | 8.2 ± 0.2 e | 2.7 ± 0.1 d | 1.73 ± 0.03 a | 46.1 ± 0.42 f |
| Y1 × EM | 48.2 ± 0.6 ef | 28.8 ± 0.4 b | 65.6 ± 0.7 f | 12.0 ± 0.2 cd | 3.9 ± 0.1 c | 1.64 ± 0.02 b | 63.2 ± 0.57 c |
| Y1 × AN | 54.6 ± 0.2 c | 30.6 ± 0.6 a | 68.8 ± 0.8 de | 12.0 ± 0.3 cd | 4.1 ± 0.0 b | 1.39 ± 0.03 e | 56.6 ± 0.07 d |
| Y1 × FA | 49.7 ± 0.8 e | 28.2 ± 0.4 b | 68.8 ± 0.8 de | 13.6 ± 0.3 a | 4.5 ± 0.1 a | 1.11 ± 0.02 f | 49.4 ± 0.46 e |
| Y1 × PH | 60.3 ± 0.2 a | 31.5 ± 0.6 a | 69.8 ± 0.4 cd | 13.4 ± 0.3 ab | 4.3 ± 0.1 b | 1.53 ± 0.01 cd | 65.1 ± 0.86 bc |
| Y2 × C | 44.6 ± 0.3 g | 24.9 ± 0.3 d | 59.1 ± 0.6 g | 8.5 ± 0.1 e | 2.7 ± 0.0 d | 1.71 ± 0.02 a | 46.9 ± 1.08 f |
| Y2 × EM | 57.3 ± 0.2 b | 27.7 ± 0.1 bc | 71.6 ± 0.6 bc | 12.6 ± 0.2 c | 4.2 ± 0.1 b | 1.58 ± 0.01 bc | 66.8 ± 1.34 ab |
| Y2 × AN | 52.7 ± 0.2 d | 27.7 ± 0.4 bc | 73.4 ± 0.3 b | 11.6 ± 0.1 d | 3.7 ± 0.0 c | 1.50 ± 0.01 d | 55.2 ± 0.99 d |
| Y2 × FA | 58.3 ± 0.2 b | 30.5 ± 0.5 a | 66.9 ± 0.9 ef | 12.7 ± 0.2 bc | 4.1 ± 0.1 b | 1.17 ± 0.01 f | 47.6 ± 0.84 ef |
| Y2 × PH | 60.6 ± 0.5 a | 30.5 ± 0.5 a | 78.3 ± 1.1 a | 13.9 ± 0.1 a | 4.5 ± 0.0 a | 1.51 ± 0.03 d | 68.0 ± 0.49 a |
| *p*-value | | | | | | | |
| Y × B | 0.000 | 0.002 | 0.000 | 0.001 | 0.000 | 0.010 | 0.010 |

Means and standard error are reported. Values with different letters are significantly different at $p \leq 0.05$. Y1 = 2021–2021; Y2 = 2021–2022; C = control; EM = *Eklonia maxima*; AN = *Ascophyllum nodosum*; FA = fulvic acids; PH = protein hydrolysate.

In the second growing season, PH-treated plants showed the highest average value of RWC (78.3%), followed by AN-treated plants (73.4%). The lowest average values (ranged from 61.6% to 59.1%) were obtained by C in both years (Table 3).

The highest values of fresh yield were obtained with the interactions Y1 × FA, Y1 × PH, and Y2 × PH (Table 3). The highest values ranged from 13.4 to 13.9 t ha$^{-1}$. In both years, the lowest values of fresh yield (8.2 and 8.5 t ha$^{-1}$) and the lowest values of dry yield (2.7 t ha$^{-1}$) were observed in C (Table 3). FA-treated plants (4.5 t ha$^{-1}$), in 2020–2021, and PH-treated plants (4.5 t ha$^{-1}$), in 2021–2022, revealed the highest average values of dry yield (Table 3).

In both years, similar EO content values were observed. The highest values (1.73% and 1.71%) were recorded in C, while the lowest (1.11% and 1.17%) values were recorded in FA-treated plants (Table 3).

Plants treated with PH and EM showed comparatively higher EO yields over both years, however with overall higher values in 2022. In 2021–2022, PH-treated plants showed the highest value of EO yield (68.0 kg ha$^{-1}$) followed by EM-treated plants (66.8 kg ha$^{-1}$) (Table 3). The lowest average values (46.1 and 46.9 kg ha$^{-1}$) were obtained in the control in both years and in FA-treated plants (Table 3).

Concerning the first growing season, a correlation analysis (Table 4) showed that plant height was significantly correlated with chlorophyll content (r = 0.844) and RWC (r = 0.728); an increase in the total fresh yield was related to morphological parameters, such as the number of primary stems (r = 0.668), diameter of primary stems (r = 0.837), and RWC (r = 0.826). The total dry yield was positively correlated with RWC (r = 0.879) and the total fresh yield (r = 0.969). Negatively significant relationships were found between the EO content and RWC (r = −0.641), total fresh yield (r = −0.681), and total dry yield (r = −0.761). The EO yield was positively correlated with the chlorophyll content (r = 0.733). In the second growing season, a correlation analysis revealed positive relationships between morphological and yield parameters. The EO content was negatively correlated with the chlorophyll content (r = −0.757). The increase in EO yield was due to an increase in RWC (r = 0.802), total fresh yield (r = 0.655), and total dry yield (r = −0.730). In both years, no significant correlation was found between the EO content and EO yield (Table 4).

**Table 4.** Pearson's correlation coefficients for morphological and productive parameters of rosemary.

| | | 2021–2022 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Plant Height [cm]** | **Primary Stems [n.]** | **Primary Stems Diameter [mm]** | **Chlorophyll Content [µg L$^{-1}$]** | **RWC [%]** | **Total Fresh Yield [t ha$^{-1}$]** | **Total Dry Yield [t ha$^{-1}$]** | **EO Content [%]** | **EO Yield [kg ha$^{-1}$]** |
| **2020–2021** | **Plant height [cm]** | | 0.797 ** | 0.931 ** | 0.865 ** | 0.762 ** | 0.975 ** | 0.974 ** | −0.586 | 0.630 |
| | **Primary Stems [n.]** | −0.115 | | 0.819 ** | 0.604 | 0.392 | 0.754 ** | 0.788 ** | −0.627 | 0.400 |
| | **Primary Stems diameter [mm]** | 0.077 | 0.779 ** | | 0.791 ** | 0.709 ** | 0.931 ** | 0.909 ** | −0.624 | 0.549 |
| | **Chlorophyll content [µg L$^{-1}$]** | 0.844 ** | 0.127 | 0.385 | | 0.617 | 0.839 ** | 0.814 ** | −0.757 ** | 0.309 |
| | **RWC [%]** | 0.728 ** | 0.382 | 0.544 | 0.680 ** | | 0.846 ** | 0.814 ** | −0.146 | 0.802 ** |
| | **Total Fresh Yield [t ha$^{-1}$]** | 0.472 | 0.668 ** | 0.837 ** | 0.574 | 0.826 ** | | 0.976 ** | −0.562 | 0.655 ** |
| | **Total Dry Yield [t ha$^{-1}$]** | 0.525 | 0.584 | 0.797 | 0.620 | 0.879 ** | 0.969 ** | | −0.489 | 0.730 ** |
| | **EO Content [%]** | −0.134 | −0.299 | −0.595 | −0.161 | −0.641 ** | −0.681 ** | −0.761 ** | | 0.233 |
| | **EO Yield [kg ha$^{-1}$]** | 0.621 | 0.469 | 0.402 | 0.733** | 0.479 | 0.541 | 0.488 | 0.191 | |

** Significant at 0.01 probability level.

## 4. Discussion

The cultivation of MAPs has become increasingly widespread throughout the world, mainly due to the very varied uses of the products [53]. The increase in crop yields by reducing the use of common fertilizers represents one of the goals of modern agriculture [54].

The results of the present study highlight the positive effects on yields and qualitative parameters of the foliar application of biostimulants on rosemary plants under rainfed conditions. In both growing seasons, the highest plant height values were found in plants treated with protein hydrolysate. The main reason for protein hydrolysate biostimulatory actions is their peptide and amino acid content [36,54]. Plants use amino acids for a variety of processes, including the synthesis of high-biological-activity compounds, energy production, and protein biosynthesis [38]. Peptides also have a significant impact on plant responses to stress, information transfer between cells, and growth and development regulation [37]. Waly et al. [47] investigated the effects of seaweed extract foliar application on rosemary using doses varying between 200 and 600 mL hL$^{-1}$ and obtained plant height values which were similar to those found in the present study. Al-Fraihat et al. [48] obtained rosemary plant heights of between 35 and 45 cm when applying different foliar amino acids. The foliar application of biostimulants allowed us to obtain a significant increase in the number and diameter of primary stems. Biostimulants can operate on the primary metabolism by raising photosynthetic activity and derived chemicals, thereby increasing plants growth [27,53]. According to other authors, applying biostimulants generates gibberellin- and auxin-like activities, which improve crop performances [38,54].

The rosemary plants treated with biostimulants obtained an increase in yield per unit area with respect to the control plants. In particular, the foliar application of fulvic acids and protein hydrolysates led to increases in fresh yield of approximately 5 t ha$^{-1}$ higher than that of untreated plants and of 1.6–1.7 t ha$^{-1}$ in terms of dried yield. This was due to the effects on the regulation of those enzymes involved in nitrogen metabolism, which improve the absorption and assimilation of this element [36]. Indeed, several physiological and biochemical processes, including the activation of enzymes involved in carbon and nitrogen metabolic pathways, the Krebs cycle and glycolysis, the elicitation of phytohormones, and increases in mineral absorption/accumulation in biostimulated plants through the modification of root morphology, contribute to improvements in crop production [29,55]. This is the case, in particular, when induced by biostimulant application under unfavorable growing conditions, such as drought stress [54,56]. The treated roots of rosemary plants can improve water absorption and the uptake of various nutrients and promote their distribution in plant tissues [53,54]. Our yields are similar to those found by Singh and Wasnik [57], who obtained yields ranging from 10 to 21 t ha$^{-1}$ when applying organic and inorganic fertilizers and amounts of nitrogen varying between 50 and 300 kg N ha$^{-1}$. In contrast, Tawfeeq et al. [46] observed a notable decrease in biomass production in rosemary plants which were treated with algae extracts.

Regarding the chlorophyll content and RWC, our findings highlight that the application of biostimulants obtained higher values with respect to untreated plants. Al-Fraihat et al. [48]

found similar results in rosemary plants managed with different types of amino acids through foliar treatments, obtaining lower contents in untreated plants. Many authors [58,59] reported increases in leaf chlorophyll content in plants treated with foliar biostimulants. A crucial aspect of controlling stomatal conductance and photosynthetic activity in plant tissues is related to an appropriate RWC [60]. Modifications in the water balance can cause molecular change, growth retardation, and occasionally, plant tissue death [61,62]. The values of RWC obtained in our work were similar to those found in previous studies [60,63]. Rahimi et al. [64] and Elansary et al. [31] observed an increase in RWC by applying different types of foliar biostimulants to *Thymus vulgaris* L. and *Mentha longifolia* L., respectively. Due to the fact that biostimulants promote root development and improve the ability of plants to absorb water and nutrients, they are predicted to have a positive effect on the relative water content [65,66]. As reported by various authors [35,67], the application of fulvic acids led to increases in the yield and quality of many crops since these substances promote the absorption, assimilation, and translocation of micro- and macro-nutrients.

In our study, the highest EO content was recorded by untreated plants. On the contrary, higher biomass and EO yields were found in plants treated with protein hydrolysate, in particular. As is well known, the production of secondary metabolites and EO is affected by stressful conditions for the plants [9,46]. In the present study, the lack of irrigation and water availability in the soil increased the EO content in the control plants with respect to the plants treated with biostimulants. Elansary et al. [31] and Rahimi et al. [64] investigated the effects of the application of biostimulants on mint and thyme, respectively; however, they found increases in the EO content of plants treated with biostimulants. It is important to emphasize that the EO content in the present study in all treatments was, on average, higher than that found in previous tests on rosemary [4,11,46,68]. As reported in several studies, secondary metabolite synthesis is linked to abiotic and biotic factors, such as climate, soil, agronomic practices, post-harvest management, genetic makeup, plant age, development stage, and plant material [4,7–9,17,25,69,70]. It is also worth noting that greater increases in the biomass of those plants treated with foliar application biostimulants could lead to increases in extract yields per unit of surface area. Giannoulis et al. [23] and Truzzi et al. [71] reported similar results in lavender (*Lavandula angustifolia* Mill.) and Lavandin (*Lavandula x intermedia* Emeric ex Loisel). The results obtained in this study, in particular those obtained in plants treated with protein hydrolysates, could be valuable for growing organic plants with a greater biomass and EO yield to be used in food, pharmaceutical, and chemical industries. In organic farming systems, plants are frequently at risk of nutrient deficiencies [72]. The effectiveness of small amounts of protein hydrolysate is due to the direct and indirect effects on the plant's primary and secondary metabolism, also under stress conditions, such as limited water availability [37,38,40].

## 5. Conclusions

The results obtained in this research confirm that biostimulants represent a valid tool when seeking to increase yields in the organic farming of rosemary. In particular, the application of fulvic acids and protein hydrolysates leads to increases in plant growth and biomass yields. The highest EO yield values were found in plants treated with *E. maxima* and protein hydrolysates. It is necessary to enhance sustainable agronomic methods in order to reduce the use of chemical inputs, improve environmental quality, and boost agricultural productivity. The use of biostimulants and other bio-based products are technological advancements also for the MAP sector. These results are of extreme interest to organic companies concerned with the cultivation of rosemary for essential oil and fresh and dry biomass production. Additional investigation is necessary to evaluate the impact of foliar biostimulant application on the morphological, productive, and chemical characteristics of other MAPs.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14010158/s1, Figure S1. Temperature and rainfalls trends at the experimental site: (a) growing season 2020–2021; (b) growing season 2021–2022.

**Author Contributions:** Conceptualization, D.F. and T.T.; methodology, N.I.; software, D.F., N.T. and F.A.; validation, D.F., C.L. and T.T.; formal analysis, D.F., N.I. and T.T.; investigation, D.F, N.T. and F.A.; resources, C.L. and T.T.; data curation, D.F., N.T. and F.A.; writing—original draft preparation, D.F. and N.I.; writing—review and editing, D.F., N.I. and T.T.; visualization, N.I. and T.T.; supervision, C.L. and T.T.; project administration, C.L. and T.T.; funding acquisition, C.L. and T.T. All authors have read and agreed to the published version of the manuscript.

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