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Animal and plant-derived protein hydrolysates positively affect yield traits but produce contrasting response on chemicals of organic rosemary (*Salvia rosmarinus* Spenn.) grown under rainfed conditions

--Manuscript Draft--

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Abstract:	<p>Rosemary (<i>Salvia rosmarinus</i> Spenn.) is a perennial crop which is well known for its antibacterial, anti-inflammatory, and antioxidant properties. It is widely documented that the biosynthesis of bioactive compounds in this plant is strongly influenced by endogenous and exogenous factors. To enhance yield production and reduce the effects of these factors on open field crops, certain agronomic practices can be optimized through the use of innovative products. The foliar application of protein hydrolysates is recognized as a good practice to achieve this. A two-year study was carried out to investigate the impacts of two different protein hydrolysates (animal-based and plant based) on the yield traits and chemical parameters of rosemary. A randomized complete block design was used with three replicates. The results highlighted that both biostimulants produced a substantial increase in fresh biomass between 1.4 and 3.9 t ha⁻¹, and in dry biomass between 0.5 and 1.3 t ha⁻¹, in comparison with the control plants. It was found that the lowest dose of plant-based protein hydrolysates significantly increased essential oil yields, producing yields of over twenty kilograms per hectare. It is worth noting that the application of both protein hydrolysates did not modify the aromatic profile of the essential oil. In addition to this, contrasting responses were observed when considering the effect of these biostimulants on the antioxidant activity, phenolic content, and rosmarinic acid. The results of this study demonstrate that protein hydrolysates improve the yield of rosemary plants cultivated under organic agriculture conditions.</p>

Additional Information:	
Question	Response

Dear Editor,

Please find enclosed the manuscript: “Animal and plant-derived protein hydrolysates positively affect yield traits but produce contrasting response on chemicals of organic rosemary (*Salvia rosmarinus* Spenn.) grown under rainfed conditions” by, Davide Farruggia, Mario Licata, Giuseppe di Miceli, Giovanni Urso, Francesco Salamone, Johannes Novak to be submitted as a new full-length article to Special Issue in Italian Journal of Agronomy entitled “Innovations For Sustainable Crop Production In The Mediterranean Region” edited by Prof. Dr. Giorgio Testa, Dr. Susana Carvalho and Dr. Aspasia P. Efthimiadou.

All co-authors have seen, read and agree with the contents of the manuscript and there is no financial interest to report. The paper has not been published in whole or in part elsewhere. Therefore, it is not currently under consideration in another journal. I attest to the validity and legitimacy of the data and I agree to its submission to the mentioned journal.

This paper focused on investigate the influence of two foliar protein hydrolysates (one animal-derived and one plant-derived), applied with two different doses, on the morphological, productive, and chemical properties of rosemary cultivated in the Mediterranean region without irrigation. During the two years of field experiments, both protein hydrolysates significantly improve yield traits in rosemary plant, plant-derived protein hydrolysate performs well when applied at the lowest dose. Both protein hydrolysates do not modify the aromatic profile of the essential oil but determine contrasting effects on some chemical properties of rosemary. These results support the idea that the application of small doses of biostimulants obtained from plants can be considered an innovative and sustainable coltuivation practice for rosemary production.

We believe that the paper fits the aims and scope of Italian Journal of Agronomy because it highlights the importance of the application of innovative e sustainable crop management practices to increase productivity and chemical components. Rosemary is one of the most cultivated medicinal and aromatic plants over the world, often under stressful conditions. Foliar application of plant-derived protein hydrolysate can be a sustainable way to increase yields and enhance some characteristics of the secondary metabolites derived from rosemary grown in a Mediterranean climate.

Since the corresponding author is a member of the Italian Society of Agronomy, we kindly ask you to apply the discount reserved for the society's affiliates.

I confirm that the authors used the Italian Journal of Agronomy style, and the manuscript meets the journal's style requirements. I attest that the manuscript has been revised by a native English lecturer (Lucie Branwen Hornsby MCIL, CL, DipTrans, Chartered Institute of Linguists, <http://www.ciol.org.uk>, contact e-mail:

lbhornsby@gmail.com) for professional language editing and has been significantly improved in all its parts.

We hope the editorial board will agree on the interest of this study.

Sincerely yours,

Giuseppe Di Miceli on behalf of the authors

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Animal and plant-derived protein hydrolysates positively affect yield traits but produce contrasting response on chemicals of organic rosemary (*Salvia rosmarinus* Spenn.) grown under rainfed conditions

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Highlights

- Protein hydrolysates significantly improve yield traits in rosemary plant.
- Plant-derived protein hydrolysate performs well when applied at the lowest dose.
- Animal and plant-derived protein hydrolysates do not modify the aromatic profile of the essential oil.
- Protein hydrolysates determine contrasting effects on some chemical properties of rosemary.

Abstract

Rosemary (*Salvia rosmarinus* Spenn.) is a perennial crop which is well known for its antibacterial, anti-inflammatory, and antioxidant properties. It is widely documented that the biosynthesis of bioactive compounds in this plant is strongly influenced by endogenous and exogenous factors. To enhance yield production and reduce the effects of these factors on open field crops, certain agronomic practices can be optimized through the use of innovative products. The foliar application of protein hydrolysates is recognized as a good practice to achieve this. A two-year study was carried out to investigate the impacts of two different protein hydrolysates (animal-based and plant based) on the yield traits and chemical parameters of rosemary. A randomized complete block design was used with three replicates. The results highlighted that both biostimulants produced a substantial

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increase in fresh biomass between 1.4 and 3.9 t ha⁻¹, and in dry biomass between 0.5 and 1.3 t ha⁻¹, in comparison with the control plants. It was found that the lowest dose of plant-based protein hydrolysates significantly increased essential oil yields, producing yields of over twenty kilograms per hectare. It is worth noting that the application of both protein hydrolysates did not modify the aromatic profile of the essential oil. In addition to this, contrasting responses were observed when considering the effect of these biostimulants on the antioxidant activity, phenolic content, and rosmarinic acid. The results of this study demonstrate that protein hydrolysates improve the yield of rosemary plants cultivated under organic agriculture conditions.

Keywords: biostimulant, essential oil, crop yield, organic agriculture, *S. rosmarinus*.

1. Introduction

Rosemary (*Salvia rosmarinus* Spenn.) is an evergreen perennial shrub, native to the Mediterranean region (Mwithiga et al., 2022). Similar to other medicinal and aromatic plants (MAPs), this species is used in various culinary traditions all over the world due to its many health benefits and intense aroma (Aziz et al., 2022). Rosemary has numerous bioactive compounds that can enhance the digestive process, reduce inflammation and provide antioxidant benefits (Veenstra and Johnson, 2021). Furthermore, it is rich in carnosic and rosmarinic acid, both of which have strong antioxidant properties. These chemicals strengthen the health-promoting properties of the species by helping to scavenge free radicals and provide defense against oxidative stresses (Topal and Gulcin, 2022; Li Pomi et al., 2023). The essential oils (EO) are used for their pleasant aroma and therapeutic benefits, influencing mood, cognition, and overall well-being (Pirzad and Mohammadzadeh, 2018; Fierascu et al., 2021; Tuttolomondo et al., 2021). The main compounds, such as cineole and camphor, contribute to its preservative properties, making it valuable for food preservation and as a natural disinfectant (Jafari-sales and Pashazadeh, 2020; Günther et al., 2022). The biosynthesis of bioactive compounds takes place through metabolic pathways by using intermediate products of primary metabolism. These compounds accumulate in plant cells either due to biochemical inefficiencies or as part of a normal physiological process, (Julsing et al., 2006). The products of secondary metabolism are multifunctional and help restore cellular properties when specific events disrupt the balance of the plant organism (Borges et al., 2017). These substances are commonly stored in various parts of the plant, such as the flowers, leaves, roots, and seeds, and their concentration levels can vary depending on environmental conditions, genetic factors, and phenological stages (Julsing et al., 2006; Virga et al., 2020; Tuttolomondo et al., 2021; Huang et al., 2022). Moreover, yield and qualitative parameters

1 of the crop are also influenced by agronomic factors, including fertilization and irrigation practices,
2 plant density, soil management, and harvest time (Sabatino et al., 2013; Chrysargyris et al., 2022;
3 Nurzyńska-Wierdak et al., 2023; Farruggia et al., 2024a). Understanding the impact of these practices
4 on the plant could help optimize the yield traits of rosemary, promoting sustainability and maximizing
5 economic value.
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8 The availability of micro- and macro elements in the soil is widely considered as fundamental in
9 medicinal and aromatic plants (MAPs), affecting plant growth and increasing the synthesis of
10 secondary metabolites (Farruggia et al., 2023; Nurzyńska-Wierdak et al., 2023). The application of
11 biostimulants can influence both primary and secondary metabolism in plants and improve tolerance
12 to adverse climate and soil conditions, as confirmed by previous studies (Ertani et al., 2013; Lucini
13 et al., 2015; Amato et al., 2024; Farruggia et al., 2024a). Protein hydrolysates (PH) are a type of
14 biostimulant obtained from the chemical or enzymatic hydrolysis of animal or plant-based proteins
15 (Colla et al., 2015). Collagen derived from tanning-industry waste is the most frequently used source
16 of animal protein hydrolysates and legumes are currently used to generate plant protein hydrolysates,
17 as reported by various authors (Colla and Rouphael, 2015; Du Jardin, 2015). PH are a mixture of
18 polyprotein hydrolysates, oligo-protein hydrolysates and amino acids (Rouphael et al., 2017) and can
19 be applied to leaves or roots to promote plant physiological response, increase crop yields, and
20 improve plant tolerance to stress conditions (Rouphael et al., 2022; Di Miceli et al., 2023). Di Miceli
21 et al. (2023) observed increases in growth, yield and quality parameters in eggplant (*Solanum*
22 *melongena* L.) treated with plant-derived PH under open field conditions. Similar results were
23 obtained by Sabatino et al. (2021) treating lettuce (*Lactuca sativa* L.) with plant PH. These authors
24 observed significant improvements in yield and yield-related features, nutritional and functional
25 traits, as well as nitrogen indices. there seem to be controversial findings in literature regarding the
26 use of animal/plant PH, and, in some cases, negative effects have been observed (Ertani et al., 2009;
27 De Lucia and Vecchiatti, 2012; Moe, 2013; Corte et al., 2014; Colla et al., 2015; Visconti et al., 2015;
28 Wilson et al., 2015; Colla et al., 2017; Pérez-Aguilar et al., 2024).
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30 There are currently very few studies on the effects of foliar application of PH on rosemary yields and
31 its chemical composition and studies which compare the effects of different types of PH on rosemary
32 are notably lacking. With this in mind, the aims of this study were: (i) to evaluate the effect of two
33 different PHs (animal-derived and plant-derived) on morphological, and yield parameters of rosemary
34 plants grown under organic farming conditions; (ii) to investigate the impact of PH application on
35 EO characteristics; (iii) to determine how antioxidant activity, rosmarinic acid (RA), and total
36 phenolic (TP) varied depending on the PH dose.
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1 The following experimental hypothesis was taken into consideration: the foliar application of two
2 different protein hydrolysates leads to increases in biomass and EO yields, enhances the aromatic
3 profile of the EO, and produces positive effects on other chemicals in rosemary plants grown under
4 rainfed conditions.
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8 **2. Materials and methods**

9 *2.1. Test site, experimental field and cultivation practices*

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12 The trials were carried out at an organic farm (37°22'32.71"N, 13°38'33.59"E), situated in the
13 countryside of Aragona, South-Western Sicily (Italy) during the growing seasons 2021–2022 and
14 2022-2023.
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16 The soil has a sandy clay loam texture (48% sand, 26% silt, and 26% clay) and shows 1.1% organic
17 matter, 1.3% total nitrogen, 22.4 ppm assimilable phosphate, 330.0 ppm assimilable potassium, with
18 a pH of 7.2. It is classified as Regosols (typic xerorthents), in accordance with the United States
19 Department of Agriculture (USDA). The climate of the site is warm temperate with dry and hot
20 summers and mild winters, following the classification provided by Köppen–Geiger (Kottek et al.,
21 2006).
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23 The experimental field of rosemary was established in February 2019 by transplanting herbaceous
24 cuttings obtained from mother plants. The distance between rows and between plants on the same
25 row was 2.5 and 1.0 m, respectively, ensuring a density of 4000 plants ha⁻¹. Fertilization was carried
26 out by applying 2.0 t ha⁻¹ of cattle manure (0.5% of N, 0.2% of P₂O₅, 0.7% K₂O) prior to
27 transplantation. Rosemary plants were grown under rainfed conditions and weed control was
28 mechanical. In both years, harvesting occurred once per year by cutting the plant to 0.30 m above
29 ground level. Plants were manually harvested during the third 10-day period of June.
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32 *2.2. Weather data*

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34 During the two-year study, average maximum and minimum air temperatures and total rainfall were
35 monitored from a weather station, owned by the Sicilian Agro-Meteorological Information Service
36 (SIAS, 2024), and situated close to the farm. Figure 1 (A and B) shows the temperature and rainfall
37 trends (10-day periods).
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47 **Figure 1.** Temperature and rainfall trends during the first (A) and the second (B) year of study.
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A total rainfall of 611 mm (2021–2022) and 538 mm (2022–2023) was recorded during the growth period; however, the distribution of the rainfall differed considerably over the two years. During the 1st growing season, 80% of rainfall (480 mm) was mainly concentrated in October to January, whilst only 15% (92 mm) was recorded at the onset of vegetative growth through to the harvest date. During the 2nd growing season, rainfall was spread over a greater period from September to March. In spring, May was the wettest month with well-distributed rainy days. Temperature trends influenced the phenological stages of rosemary and were largely consistent with the average temperature of the study area. In both growing seasons, an increase in minimum and maximum temperatures was detected from pre-flowering stage to harvest. When comparing the two growing seasons, the highest maximum temperatures were recorded in the 2nd growing season.

2.3. Treatments

Two protein hydrolysates were used for foliar applications:

- Animal protein hydrolysate (APH), derived from hydrolyzed animal epithelium, with organic nitrogen 8.0%, organic carbon 27.0%, amino acids 50.0% and free amino acids 15%.
- Plant protein hydrolysate (PPH), obtained from *Fabaceae* with amino acids and plant peptides (31%), organic nitrogen (5%) and organic carbon (25%).

For each biostimulant, two doses were applied. Taking into consideration the nitrogen (N) content of each protein hydrolysate, each biostimulant dose was formulated to ensure all treatments provided the same total quantity of N (Table 1).

Table 1. The protein hydrolysates doses.

Biostimulant	Doses for each application [L hL ⁻¹]	Abbreviation
Animal protein hydrolysates	0.100	APH-100
	0.200	APH-200
Plant protein hydrolysates	0.150	PPH-150
	0.300	PPH-300

A total of 4 foliar applications were performed during the vegetative growth stage. Control (C) treatment plants were sprayed with only water. 400.0 L of water ha⁻¹ were used for each application and the first event was carried out in the first 10-day period of April. Foliar treatments were made using a portable hand-sprayer equipped with a flat fan nozzle and with an operating pressure of 250.0 kPa. To avoid drift and contamination of nearby plots, plastic panels were used to delineate each plot

1 during application. A single operator managed foliar sprays to ensure uniform application and
2 consistent dosage, and treatments were applied early in the morning to guarantee optimal foliar
3 absorption (Ruiz-Navarro et al., 2019). The plot size was 50 m² (2.5 m × 20.0 m) with 20 plants per
4 plot. These 20 plants were taken into consideration for the subsequent measurements. A randomized
5 complete block design with three replications was adopted as experimental design.
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10 *2.4. Morphological traits and biomass yield*

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14 When the plants were harvested, plant height, relative water content (RWC), chlorophyll content
15 (CC), and total fresh yield (FY) were recorded. Harvested plants were dried in a shaded and ventilated
16 environment for approximately 10 days at a temperature of 25–30 °C and total dry yield (DY) was
17 determined.
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21 Chlorophyll content was measured using a Dualex Scientific (Force A, Orsay, France) portable
22 chlorophyll meter, and the device was used on 40 leaves per plot.
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25 Relative waste content was evaluated by applying the method described by Farruggia et al. (2024b)
26 and the following equation by Alyemini et al. (2018):
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$$29 \quad RWC = \frac{FW - DW}{TW - DW} \times 100$$

30 where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight.
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36 *2.5. Essential oil content, composition and yield*

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39 For each plot, 500.0 g of air-dried plant material were hydro-distilled for 3 h to obtain essential oils,
40 in accordance with the Ph. Eur. 7.0, 20812 (European Pharmacopoeia, 2008). For each treatment, 3
41 EO extractions were performed. EO concentration was calculated by dividing EO volume for each
42 sample by biomass weight. EO yield was calculated by multiplying EO content by the total dry yield.
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66 *2.6. Determination of chemical parameters*

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room temperature. After being filtered, the extracts were stored at $-20\text{ }^{\circ}\text{C}$ for further examination.

The extracts were used to determine:

- antioxidant activity (AA), expressed as milligram trolox equivalents per gram of dry weight (mg t.e. g^{-1} dw), using the method described by Chizzola et al. (2008);
- rosmarinic acid (RA) content, as described Farruggia et al. (2024c);
- total phenolic (TP) content, expressed as milligram caffeic acid equivalents per gram of dry weight (mg c.a.e. g^{-1} dw), adopting the method provided by Lamien-Meda et al. (2010).

2.7. Statistical analyses

Data for each year were subjected to one-way analysis of variance (ANOVA). Differences between means were compared using Tukey's test ($p \leq 0.05$). In the linear model/ANOVA, foliar treatments were used as fixed effects. Before ANOVA, the RWC data were subjected to arcsine transformation. Homogeneity of variance and normality of all data were checked using Levene's test and the Shapiro-Wilk test. Statistical analyses were performed using software MINITAB 19 (State College, PA, USA) for Windows.

3. Results

3.1. Morphological traits and biomass yield

Data on morphological traits of rosemary plants subjected to foliar applications with animal and plant-based protein hydrolysates are shown in Table 2.

ANOVA revealed that, in both years, all morphological parameters were significantly affected ($p \leq 0.01$) by foliar treatments with both biostimulants, with the exception of RWC (Table 2).

During the 1st year, no significant differences in plant height were found between APH- and PPH-treated plants at any dose of application. However, substantial variations were observed when APH- and PPH-treated plants were compared to control plants, highlighting the effect of biostimulants on plant growth. In general, plant height ranged from 46.5 cm to 71.8 cm, depending on the use or not of biostimulant. The highest average value (71.8 cm) was recorded in the 2nd year for plants treated with PPH-150 every two weeks. In the 2nd year, it is worth noting that, for each protein hydrolysate, the best performance was achieved by applying of the lowest dose of biostimulant.

Concerning chlorophyll content, significant differences were found in both years between control plants and treated-plants, and between APH- and PPH-treated plants. In the 2nd year, regardless of the

dose, foliar application of APH and PPH produced higher chlorophyll content in rosemary plants compared to the 1st year. The highest chlorophyll content (34.6 µg cm⁻²) was obtained with the application of PPH-300 during the 2nd year, confirming the effectiveness of the biostimulants. The lowest content (28.4 µg cm⁻²) was detected on average in control plants. When comparing the two years, no significant differences were found between control plants and APH-100-treated plants, whilst differences were observed with the slightly higher application of APH-200, highlighting the importance of the dose in this type of biostimulant.

In the case of RWC, ANOVA showed a significant effect ($p \leq 0.01$) for the foliar application of APH and PPH on rosemary plants in the 1st year alone. The highest RWC values were determined in APH-200- (79.8%) and PPH-150-treated plants (79.6%). It should be noted that control plants showed higher RWC than APH-100-treated plants, highlighting the limited effect of this dose regarding APH. In the 2nd year, no increases in RWC values were observed in treated plants compared to control plants.

Table 2. Influence of foliar treatment on rosemary plant height, chlorophyll content and relative water content (RWC) during the two-years research.

Foliar treatment	Plant height [cm]		Chlorophyll content [µg cm ⁻²]		RWC [%]	
	I year	II year	I year	II year	I year	II year
C	48.3 b	46.5 d	29.6 b	28.4 c	79.3 ab	77.2 a
APH-100	60.6 a	68.2 bc	29.8 b	30.0 c	76.9 b	82.6 a
APH-200	60.2 a	66.0 c	31.9 a	33.4 ab	79.8 a	80.8 a
PPH-150	60.4 a	71.8 a	31.2 ab	32.7 b	79.6 a	79.4 a
PPH-300	60.3 a	67.0 b	30.8 ab	34.6 a	79.2 ab	74.0 a
<i>p</i> -value	0.000	0.000	0.007	0.000	0.022	0.149

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

ANOVA results indicated that foliar treatment with APH and PPH had significant effects ($p \leq 0.01$) on FY and DY in both years (Figure 2). FY results differed based on the year, type of biostimulant and application dose. In the 1st year, APH-100- (6.0 t ha⁻¹), PPH-150- (6.5 t ha⁻¹) and PPH-300-treated plants (6.0 t ha⁻¹) obtained the highest biomass yields on average. This trend changed for the 2nd year and the best biomass yield performance was observed in the PPH-150-treated plants (6.8 t ha⁻¹).

When comparing DY over the two years, applications of APH and PPH produced similar trends of biomass yield both with the lowest and highest dose. This confirms that treatments with biostimulants increased the crop yield compared to control plants. In particular, PPH-150-treated plants showed the

highest crop yields both in the 1st (2.5 t ha⁻¹) and 2nd year (2.4 t ha⁻¹). It is worth mentioning the fact that the lowest dose of both biostimulants produced the highest biomass yields in rosemary plants.

Figure 2. Influence of foliar treatment on fresh and dry yield (DY) of rosemary.

Means and standard deviations are shown. The values with different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

3.2. Essential oil content, composition and yield

Table 3 and figure 3 summarize the results of ANOVA for essential oil content and yield. The statistical test revealed that foliar treatments with APH and PPH significantly affected ($p \leq 0.01$) EO content and yield in both years. The effects of biostimulant application on EO content differed over the two years. During the 1st year, significant differences were found between control plants and treated plants. However, when comparing the various treatments, no significant variations were detected. It is worth noting that control plants produced the highest EO content (2.1%). By contrast in the 2nd year, the highest EO content (2.5%) was recorded in PPH-150-treated plants, whilst the lowest (2.2%) in control plants. Moreover, greatest EO performance was obtained with the lowest doses of APH and PPH (Table 3).

Table 3. Influence of foliar treatment on rosemary essential oil (EO) content during the two-years research.

Foliar treatment	EO content [%]	
	I year	II year
C	2.1 a	2.2 c
APH-100	1.6 b	2.4 ab
APH-200	1.6 b	2.3 b
PPH-150	1.5 b	2.5 a
PPH-300	1.5 b	2.3 bc
<i>p</i> -value	0.000	0.000

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

In the case of EO yield, similar trends were observed in both years (Figure 3). In general, control plants had similar EO yield values in both years whilst the highest performance for treated-plants were recorded on average in the 2nd year. In particular, application of the lowest doses of APH

1 generated the highest EO yields both in the 1st year (37.4 kg ha⁻¹) and 2nd year (45.9 kg ha⁻¹). On the
2 contrary, when considering foliar treatment with PPH, similar values of EO yield were obtained
3 regardless of the dose during the 1st year only. In the study period, the highest EO yield performance
4 (59.7 kg ha⁻¹) was detected in PPH-150-treated plants in the 2nd year.
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8 **Figure 3.** Influence of foliar treatment on rosemary essential oil (EO) yield during the two-years
9 research.

10 Means and standard deviations are shown. The values with different letter are significantly different for $p \leq 0.05$ according
11 to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹
12 of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant
13 protein hydrolysate.
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16 Table 4 refers to the main EO compounds. Gas chromatography-mass spectrometry detected a total
17 of 29 compounds. ANOVA showed that foliar application with APH and PPH produced significant
18 effects ($p \leq 0.01$) on few EO compounds, with variations depending on the year. For example, in the
19 1st year, borneol was the only compound whose percentage was significantly affected by protein
20 hydrolysates. The highest value (4.06%) was found in APH-200-treated plants. No significant
21 differences were found for other EO compounds when comparing control plants with treated plants.
22 During the 2nd year, the treatment factor had significant effects on percentages of tricyclene,
23 terpinolene, bornyl acetate, and α -copaene. In the case of tricyclene, the highest percentage value
24 (0.22%) was obtained in control plants. The best performance for terpinolene was obtained in control
25 plants (1.31%), APH-100- (1.31%) and PPH-300-treated plants (1.31%). Applications of both doses
26 of APH and PPH determined similar percentages for bornyl acetate, ranging from 3.56% to 3.11%.
27 Finally, the application of the highest dose of APH resulted in the highest percentage (0.54%) for α -
28 copaene, whereas no differences were found with varying doses of PPH.
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Table 4. Components of rosemary EO and p-value in response to foliar treatment during the two-years research.

Compounds	RI _{calc}	RI _{lit}	I year						II year					
			Foliar treatment						Foliar treatment					
			C	APH-100	APH-200	PPH-150	PPH-300	Significance	C	APH-100	APH-200	PPH-150	PPH-300	Significance
tricyclene	919	921	0.11	0.11	0.22	0.26	0.16	n.s.	0.22 a	0.18 ab	0.15 b	0.18 ab	0.19 ab	*
a-thujene	923	924	0.18	0.20	0.18	0.20	0.22	n.s.	0.24	0.22	0.18	0.22	0.21	n.s.
a-pinene	931	932	35.52	36.75	35.44	38.59	42.93	n.s.	36.89	32.92	33.60	35.29	33.25	n.s.
camphene	943	946	3.93	4.36	3.96	4.36	4.59	n.s.	4.18	3.66	3.51	3.85	3.79	n.s.
thuja-2,4(10)-diene	949	953	1.02	1.01	0.86	1.04	1.03	n.s.	0.90	0.93	0.89	0.89	0.88	n.s.
sabinene	972	969	1.08	1.20	1.16	1.21	1.27	n.s.	2.50	2.18	2.16	2.34	2.19	n.s.
b-myrcene	990	988	1.33	1.32	1.26	1.17	1.35	n.s.	1.19	1.08	1.08	1.10	1.09	n.s.
a-phellandrene	1002	1002	0.32	0.33	0.29	0.31	0.34	n.s.	0.39	0.38	0.34	0.37	0.38	n.s.
a-terpinene	1014	1014	0.79	0.83	0.77	0.81	0.88	n.s.	1.02	1.00	0.93	1.00	1.01	n.s.
p-cymene	1022	1024	1.55	1.55	1.43	1.41	1.52	n.s.	0.59	0.53	0.51	0.49	0.56	n.s.
1.8 cineol	1029	1026	27.00	26.83	27.01	27.79	25.45	n.s.	24.43	24.20	24.92	25.08	24.44	n.s.
γ-terpinene	1055	1054	0.96	1.01	1.01	1.20	1.29	n.s.	1.73	1.75	1.68	1.72	1.80	n.s.
terpinolene	1085	1088	0.75	0.81	0.73	0.63	0.73	n.s.	1.31 a	1.31 a	1.22 b	1.27 ab	1.31 a	*
linalool	1099	1098	1.93	1.65	1.90	1.39	1.21	n.s.	1.61	1.86	1.92	1.72	1.83	n.s.
chrysanthenone	1121	1124	0.47	0.46	0.52	0.39	0.37	n.s.	0.79	0.83	0.71	0.71	0.77	n.s.
camphor	1138	1141	2.36	2.26	2.34	2.11	1.85	n.s.	1.96	2.07	2.12	1.96	2.08	n.s.
pinocarvone	1155	1160	0.48	0.39	0.48	0.36	0.27	n.s.	0.33	0.36	0.36	0.34	0.36	n.s.
borneol	1160	1165	3.83 ab	3.29 ab	4.06 a	3.05 ab	2.41 b	*	3.40	4.03	4.04	3.60	3.97	n.s.
terpinen-4-ol	1172	1174	0.63	0.50	0.65	0.39	0.28	n.s.	0.44	0.52	0.50	0.46	0.49	n.s.
α-terpineol	1186	1186	2.03	1.64	2.03	1.30	1.11	n.s.	1.21	1.49	1.50	1.31	1.45	n.s.
myrtenol	1192	1194	0.22	0.28	0.31	0.17	0.10	n.s.	0.23	0.28	0.27	0.24	0.27	n.s.
verbenone	1205	1204	6.10	5.12	5.64	4.75	3.75	n.s.	5.52	6.22	6.28	5.62	6.44	n.s.
carvacrol methylether	1243	1241	0.08	0.14	0.13	0.06	0.05	n.s.	0.29	0.37	0.27	0.29	0.34	n.s.
geranial	1262	1264	0.61	0.21	0.68	0.14	0.14	n.s.	1.38	1.91	1.92	1.57	1.75	n.s.
bornyl acetate	1287	1285	1.96	2.06	2.00	1.83	1.51	n.s.	2.54 b	3.56 a	3.48 a	3.11 a	3.40 a	*
α-copaene	1371	1374	0.19	0.11	0.35	0.19	0.37	n.s.	0.35 b	0.50 ab	0.54 a	0.39 ab	0.44 ab	*
β-caryophyllene	1419	1417	4.07	4.70	3.93	4.16	4.50	n.s.	3.65	4.48	4.29	4.08	4.36	n.s.
α-humulene	1455	1452	0.28	0.23	0.29	0.19	0.17	n.s.	0.45	0.58	0.52	0.50	0.54	n.s.
caryophyllene oxide	1589	1582	0.08	0.05	0.08	0.04	0.04	n.s.	0.08	0.13	0.09	0.08	0.09	n.s.

RI_{calc}: Retention indices relative to C9-C27 n-alkenes from a HP-5MS-column; RI_{lit} = Retention Indices based on Adams (2007). ** = significant at 0.01 probability level; * = significant at 0.05 probability level; n.s. = not significant. Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

3.3. Antioxidant activity, rosmarinic acid and total phenolic contents

Results of ANOVA revealed that foliar treatment significantly affected ($p \leq 0.05$) antioxidant activity (AA) and phenolic content (PC) of rosemary extracts in both years, as shown in Table 5. During the 1st year, the highest AA values were recorded in control plants and PPH-300-treated plants, 140.8 and 138.8 mg t.e. g⁻¹, respectively. Conversely, in the 2nd year, the application of APH-200 generated the highest AA value (144.1 mg t.e. g⁻¹). In both years, the best performances were obtained by applying the highest doses of both biostimulants. Regarding PC, in the 1st year, the highest PC (109.2 mg c.a.e. g⁻¹), on average, was recorded in control plants. It is interesting to observe that no significant differences were found between the various treatments regarding either biostimulant. On the contrary, during the 2nd year, APH-100- and PPH-150-treated plants produced the highest PC values, with 81.2 and 82.1 mg c.a.e. g⁻¹, respectively. It is also worth noting that the best performances were obtained when using both biostimulants at their lowest dose. As regards the rosmarinic acid content, ANOVA showed that the foliar treatment factor had a significant effect during the 2nd year only. The highest percentage (1.6%) was recorded in control plants and APH-200- and PPH-300-treated plants. By contrast, the highest doses of APH and PPH produced the best results for this parameter.

Table 5. Influence of foliar treatment on rosemary phenolic content (PC), antioxidant activity (AA) and rosmarinic acid (RA).

Foliar treatment	PC		AA		RA	
	[mg c.a.e. g ⁻¹]		[mg t.e. g ⁻¹]		[%]	
	I year	II year	I year	II year	I year	II year
C	109.2 a	78.5 ab	140.8 a	136.7 ab	1.6 a	1.6 a
APH-100	97.3 b	81.2 a	111.1 b	124.4 b	1.4 a	1.2 b
APH-200	97.6 b	69.8 c	121.2 b	144.1 a	1.5 a	1.6 a
PPH-150	97.3 b	82.1 a	111.1 b	124.9 b	1.5 a	1.2 b
PPH-300	95.3 b	75.3 b	138.8 a	135.6 ab	1.6 a	1.6 a
<i>p</i> -value	0.000	0.000	0.000	0.013	0.300	0.000

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

4. Discussion

4.1. Morphological and yield traits

The management of available resources and the correct choice of agronomic practices are of fundamental importance for the agricultural sector to endure high quality standards for products.

1 Nutrition thus plays a key role in the growth and development of crops, and the application of low
2 doses of PHs can increase the nutritional availability of substrates, nutrient absorption and improve
3 nutrient usage efficiency also in MAPs (Nardi et al., 2016; Paul et al., 2019).
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5 During both years, the application of the two PHs led to increases in plant height of between 12 and
6 25 cm compared to untreated plants. These values were consistent with those reported by Singh and
7 Wasnik (2013) and Mostafa (2019) who assessed the effect of organic and inorganic fertilizers on
8 growth, and chemical composition of rosemary plants. Some authors observed similar plant heights
9 and chlorophyll content in rosemary plants when using different biostimulants (Waly et al., 2019; Al-
10 Fraihat et al., 2023; Farruggia et al., 2024b). Carillo et al. (2022) report that root devolvement can be
11 improved by the application of bioactive peptides contained in plant-derived protein hydrolysates,
12 which can elicit auxin and/or gibberellin-like activities. This, in turn, indirectly enhances the
13 efficiency of nutrient uptake and exploitation, plant growth, and yield, as sustained by Colla et al.
14 (2017). In additional, PHs have been demonstrated to positively affect chloroplast size and
15 abundance within cells, which in turn encourages chlorophyll production, as well documented by
16 Abdali et al. (2023). Several studies have reported that chlorophyll levels increased following the
17 application of foliar PHs (Omer et al., 2013; Sabatino et al., 2021; Fraihat et al., 2023). Bandurska
18 (2022) reports that photosynthetic activity and stomatal conductance in plant tissues are strongly
19 correlated to leaf relative water content (RWC). RWC values found in this study were consistent with
20 those provided by other studies carried out on rosemary plants when grown under different agronomic
21 conditions (Munné-Bosch et al., 1999; Farruggia et al., 2024b). However, contrary to the findings of
22 Elansary et al. (2019) and Rahimi et al. (2022), the present study did not observe any increase in
23 RWC in biostimulated plants compared to control plants.
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25 Paul et al. (2019) highlight that low doses of protein hydrosylates can be considered as a sustainable
26 practice to increase agricultural production under water stress conditions. In our study, foliar
27 application of both protein hydrolysates obtained significant increases in biomass per unit area. A
28 number of direct and indirect physiological processes, such as promoting enzymatic action,
29 prompting hormone-like activity, and modulating the plant root system, improve a plant's uptake
30 capacity of water and nutrients. These processes may be linked to increases in crop yields promoted
31 by protein hydrolysates under favorable or unfavorable growth conditions (Vultaggio et al., 2024).
32 Some authors (Colla et al., 2017; Jiménez-Arias et al. 2022; Iacuzzi et al., 2024) sustain that, even
33 under conditions of limited water availability, peptide and amino acid content is essential for protein
34 biosynthesis, energy production, and the generation of molecules with high biological activity.
35 However, in this study, crop yields were lower than those reported by other authors who investigated
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1 the effects of different types of fertilizers (Singh and Wasnik, 2013) and foliar biostimulants
2 (Farruggia et al. 2024b) on rosemary.

3 It is reasonable to suggest that the significant increase in biomass yields, mainly observed in plants
4 treated with the lowest dose of PPH, are related to the tryptophan content. Tryptophan serves as a
5 precursor of indole-3-acetic acid, a regulator of shoot and root expansion in plants, as documented
6 by Sabatino et al. (2021) in a study carried out on lettuce. In addition, plant-based PHs contain
7 peptides and amino acids, such as glutamate and aspartate, which can act as signaling molecules for
8 rhizosphere microbiota or function as iron chelators, facilitating iron uptake in plants and enhancing
9 their Fe-nutritional status, as reported by Carillo et al. (2022).
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17 *4.2. Essential oil characteristics*

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21 The differing trend in EO yield over the two years could be related to factors influencing the synthesis
22 of secondary metabolites (Tawfeeq et al., 2016; Tarasevičienė et al., 2021; Chrysargyris et al., 2022;
23 Medeiros et al., 2024) and to biostimulants (Nardi et al., 2016; Colla et al., 2017; Paul et al., 2019).
24 Significant increases in biomass production observed in PPH-treated plants resulted in the best EO
25 yield in both years. This result is particularly attractive for achieving greater yields of bioactive
26 compounds (Napoli et al., 2020; Tarasevičienė et al., 2021; Angane et al., 2022). As several studies
27 confirm (Nardi et al., 2016; Colla et al., 2017; Colla and Rouphael, 2019; Consentino et al., 2020; Di
28 Miceli et al., 2023), the high peptide or amino acid content of protein hydrolysates influences various
29 metabolic processes, including the synthesis of secondary metabolites and overall plant performance.
30 Considering EO composition and the number of EO compounds, several biotic and abiotic factors
31 should be taken into account as they significantly influence these characteristics, as previously
32 reported by many authors (Elansary et al., 2019; Jafari Khorsand et al., 2022; Formica et al., 2024).
33 In this study, consistent with previous studies (Jiang et al., 2011; Micić et al., 2021; Formica et al.,
34 2024), the predominant compounds in rosemary EO were α -Pinene and 1,8-Cineole. However, their
35 percentage content was not influenced by APH and PPH treatments. Vosoughi et al. (2018) sustain
36 that foliar application of biostimulants can affect gene regulation and enzyme activity in the
37 secondary metabolic pathway. It is worth noting that, in this study, biostimulants only affected the
38 percentage content of some minor compounds (e.g. borneol, tricyclene, terpinolene, bornyl acetate,
39 and α -copaene). In contrast, the same authors observed a change in the EO profile of MAPs when
40 created with foliar biostimulants (Truzzi et al., 2021; Amato et al., 2024; El-Hefny and Hussien,
41 2025).
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4.3. Chemical parameters

Literature reports that chemical parameters such as antioxidant activity, rosmarinic acid content, and total phenolic content are affected by a number of different variables (Tawfeeq et al., 2016; Rahmani et al., 2019). It has been demonstrated that protein hydrolysates contain a combination of amino acids useful for plant growth and to enhance qualitative characteristics, especially under unfavourable environmental conditions (Al-Fraihat et al., 2023). Wang et al. (2022) stated that amino acids are recognized as biostimulant molecules that significantly reduce damage caused by abiotic stress and support plant growth. The ability of MAPs to produce secondary metabolites is well known to be influenced by stressful conditions (Kulak et al., 2020; Farruggia et al., 2023). In this study, contrasting responses for chemical parameters were detected, dependent on the type of biostimulant and application dose. It is not easy to explain these phenomena in relation to each chemical parameter analyzed in the study. Consequently, our findings suggest that the impact of biostimulants on secondary metabolites should not be generalized. It is also important to emphasize that stress and/or stress-relief conditions should be thoroughly considered. These statements are further supported by the results of previous studies carried out on various MAPs. Rahimi et al. (2022) and Saia et al. (2021) reported that the application of biostimulants could improve the quantity of secondary metabolites in thyme (*Thymus vulgaris* L.) and basil (*Ocimum basilicum* L.). On the contrary, Cardone et al. (2025) found contrasting responses in thyme plants treated with various foliar biostimulants. The authors attributed these results to the possibility that, in the case of thyme, the tested products may not have the same ability to affect antioxidant capacity and total phenolic content.

5. Conclusions

For medicinal and aromatic plants, exploring innovative cultivation practices is crucial when attempting to optimise crop yields and quality standards whilst still adhering to sustainability criteria. This study assessed the impact of two different protein hydrolysates (one animal-based and one plant-based) on yield traits and chemical parameters in rosemary. The main findings highlight that foliar applications using the lowest dose of plant protein hydrolysate produce the highest productivity, both in terms of biomass and essential oil yields. Furthermore, the use of animal or plant protein hydrolysates does not determine any significant changes in the aromatic profile of the essential oil. This means that both can be used whilst maintaining the chemical profile of rosemary stable over time. By exploiting the benefits of biostimulants, essential oil production can meet both consumer needs and those of producers. However, the application of animal and plant protein hydrolysates at

1 different doses differentially affects antioxidant capacity, rosmarinic acid content, and total phenolic
2 content in rosemary. It is worth noting, therefore, that stress events should be evaluated carefully,
3 avoiding any generalization regarding their effects on secondary metabolites. Although further
4 studies are needed to better investigate the effects of variations in dose of each protein hydrolysate
5 on physiological processes, it can be concluded that they contribute to making rosemary cultivation
6 less conventional, thus reducing chemical fertilizer needs and producing significant environmental
7 benefits.
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38 All authors listed have significantly contributed to the development and the writing of this paper.
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42 **Data availability statement**

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45 Data will be made available on request.
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49 **Additional information**

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52 No additional information is available for this paper.
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56 **Competing interest statement**

1
2 The authors declare that they have no known competing financial interests or personal relationships
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4

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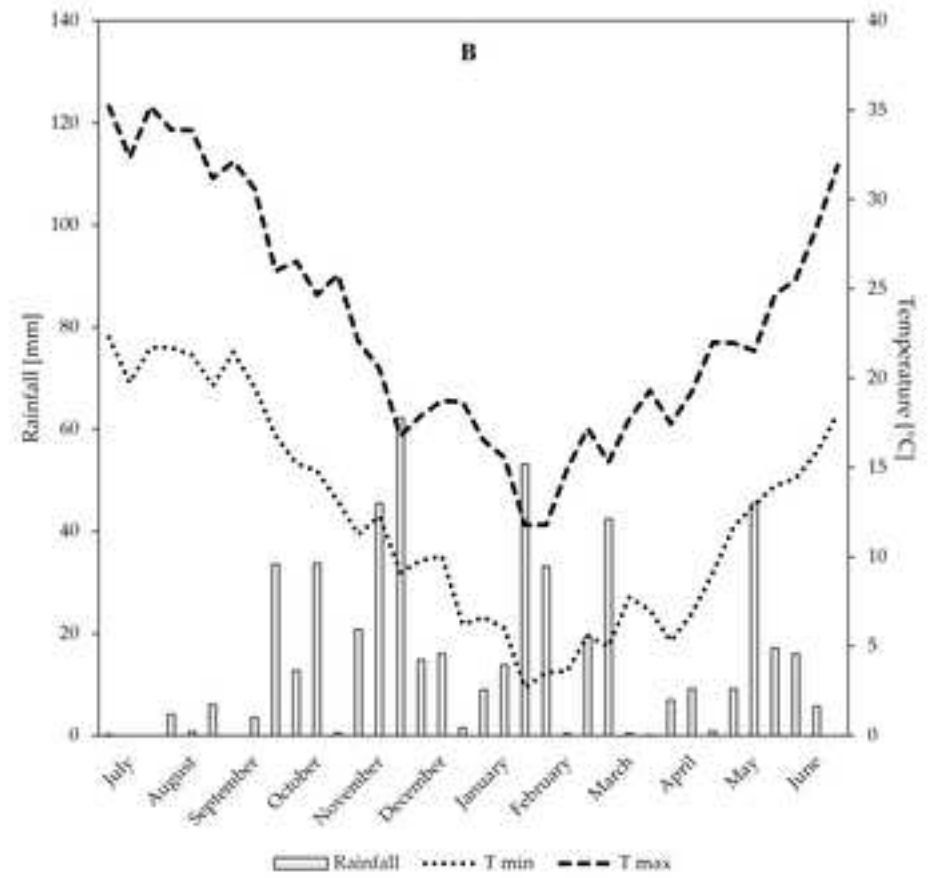
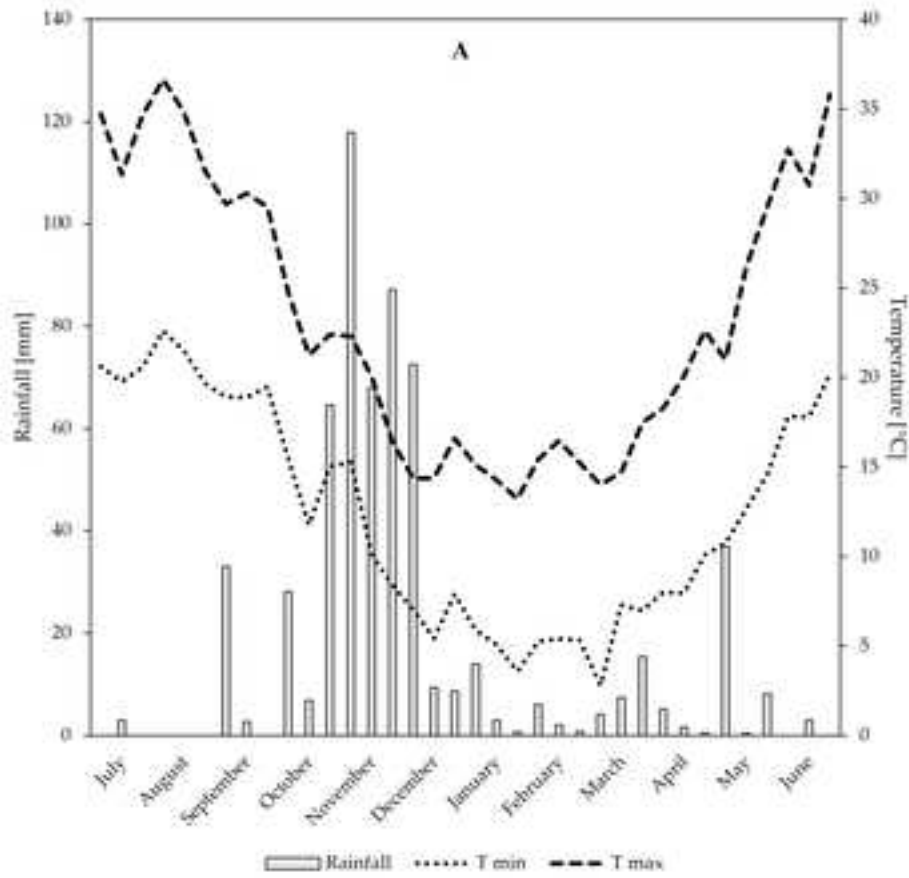
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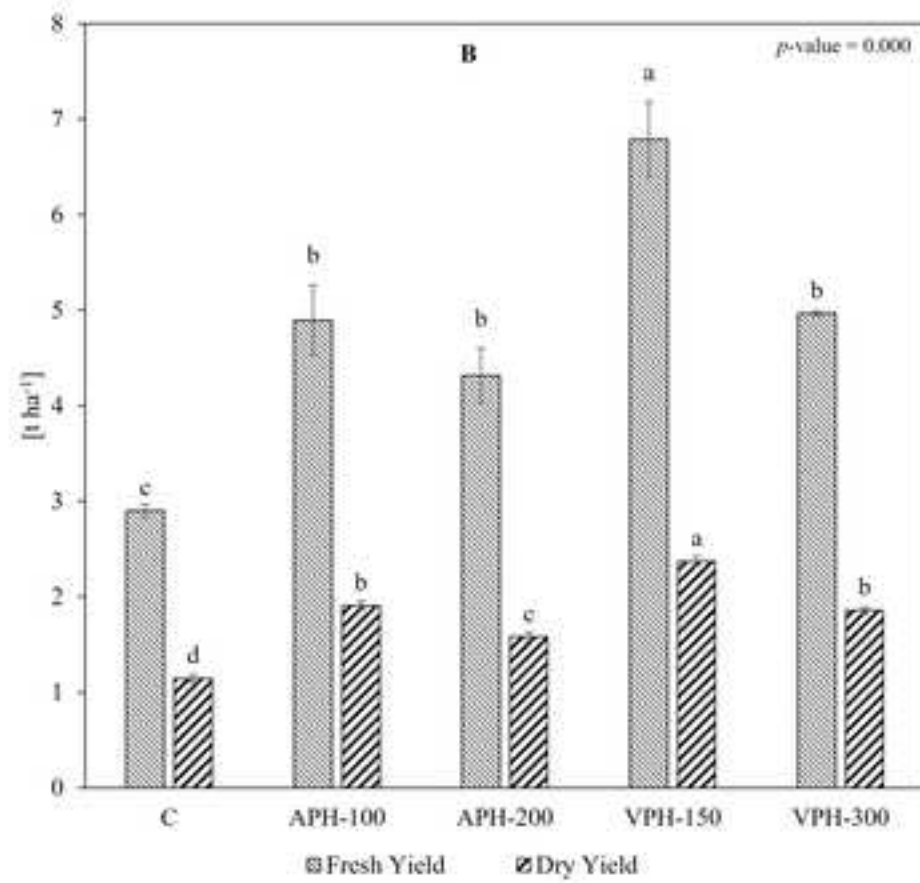
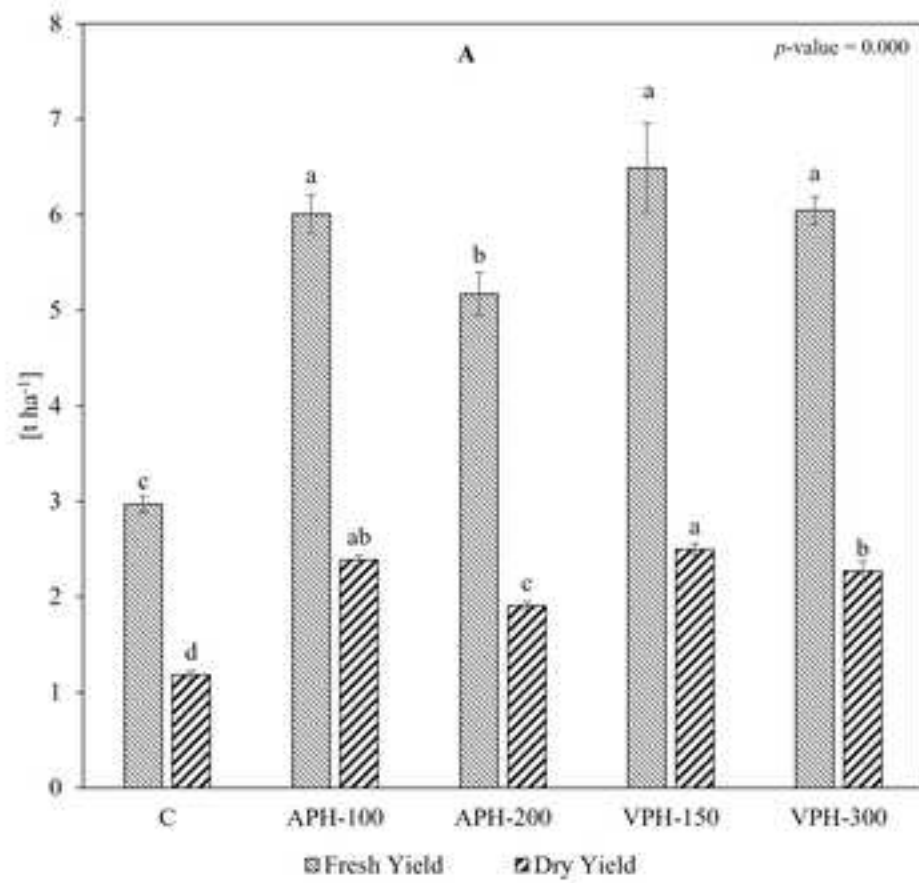
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Figure 1





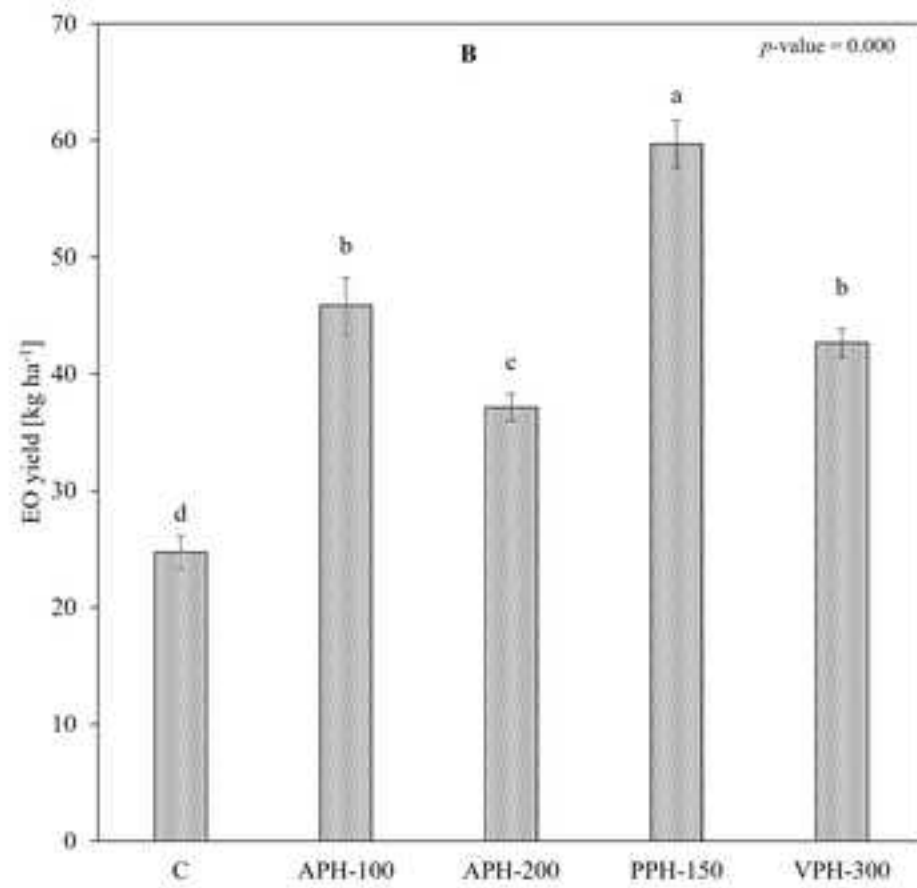
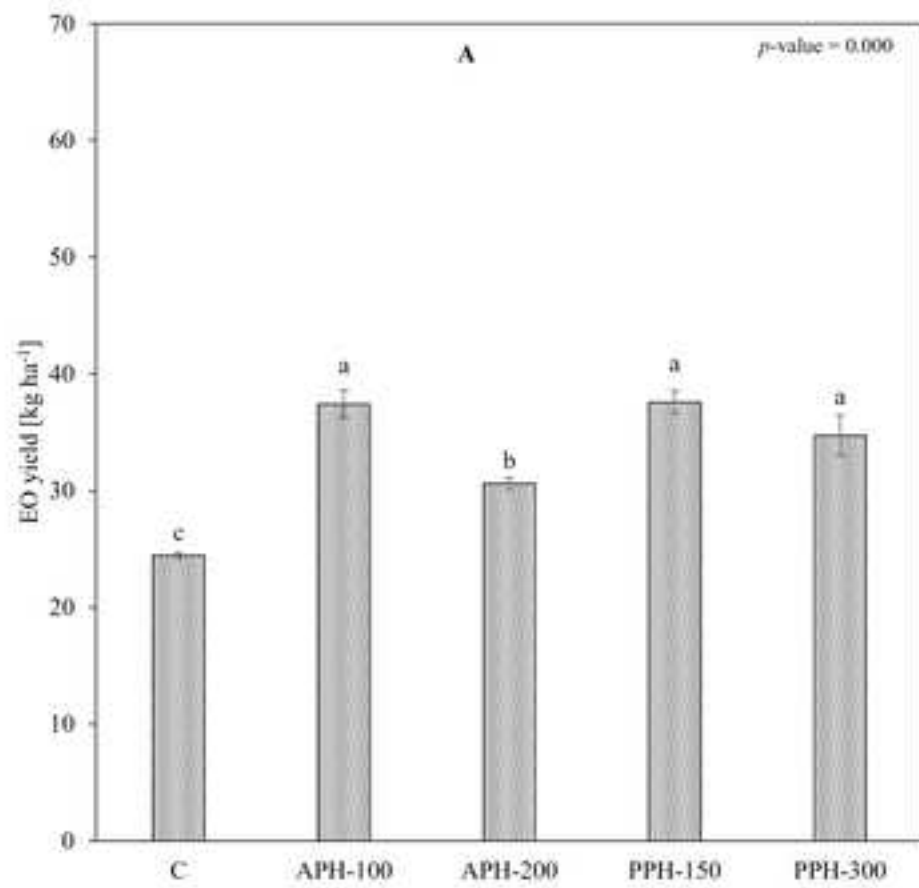


Table 1. Biostimulants doses.

Biostimulant	Doses for each application [L hL⁻¹]	Abbreviation
Animal protein hydrolysates	0.100	APH-100
	0.200	APH-200
Plant protein hydrolysates	0.150	PPH-150
	0.300	PPH-300

Table 2. Influence of foliar treatment on rosemary plant height, chlorophyll content and relative water content (RWC) during the two-years research.

Foliar treatment	Plant height [cm]		Chlorophyll content [$\mu\text{g cm}^{-2}$]		RWC [%]	
	I year	II year	I year	II year	I year	II year
C	48.3 b	46.5 d	29.6 b	28.4 c	79.3 ab	77.2 a
APH-100	60.6 a	68.2 bc	29.8 b	30.0 c	76.9 b	82.6 a
APH-200	60.2 a	66.0 c	31.9 a	33.4 ab	79.8 a	80.8 a
PPH-150	60.4 a	71.8 a	31.2 ab	32.7 b	79.6 a	79.4 a
PPH-300	60.3 a	67.0 b	30.8 ab	34.6 a	79.2 ab	74.0 a
<i>p</i> -value	0.000	0.000	0.007	0.000	0.022	0.149

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

Table 3. Influence of foliar treatment on rosemary essential oil (EO) content during the two-years research.

Foliar treatment	EO content [%]	
	I year	II year
C	2.1 a	2.2 c
APH-100	1.6 b	2.4 ab
APH-200	1.6 b	2.3 b
PPH-150	1.5 b	2.5 a
PPH-300	1.5 b	2.3 bc
<i>p</i> -value	0.000	0.000

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

Table 4. Components of rosemary EO and *p*-value in response to foliar treatment during the two-years research.

Peak	Compounds	RI _{calc}	RI _{lit}	I year						II year					
				Foliar treatment					Significance	Foliar treatment					Significance
				C	APH-100	APH-200	PPH-150	PPH-300		C	APH-100	APH-200	PPH-150	PPH-300	
1	tricyclene	919	921	0.11	0.11	0.22	0.26	0.16	n.s.	0.22 a	0.18 ab	0.15 b	0.18 ab	0.19 ab	*
2	a-thujene	923	924	0.18	0.20	0.18	0.20	0.22	n.s.	0.24	0.22	0.18	0.22	0.21	n.s.
3	a-pinene	931	932	35.52	36.75	35.44	38.59	42.93	n.s.	36.89	32.92	33.60	35.29	33.25	n.s.
4	camphene	943	946	3.93	4.36	3.96	4.36	4.59	n.s.	4.18	3.66	3.51	3.85	3.79	n.s.
5	thuja-2,4(10)-diene	949	953	1.02	1.01	0.86	1.04	1.03	n.s.	0.90	0.93	0.89	0.89	0.88	n.s.
6	sabinene	972	969	1.08	1.20	1.16	1.21	1.27	n.s.	2.50	2.18	2.16	2.34	2.19	n.s.
7	b-myrcene	990	988	1.33	1.32	1.26	1.17	1.35	n.s.	1.19	1.08	1.08	1.10	1.09	n.s.
8	a-phellandrene	1002	1002	0.32	0.33	0.29	0.31	0.34	n.s.	0.39	0.38	0.34	0.37	0.38	n.s.
9	a-terpinene	1014	1014	0.79	0.83	0.77	0.81	0.88	n.s.	1.02	1.00	0.93	1.00	1.01	n.s.
10	p-cymene	1022	1024	1.55	1.55	1.43	1.41	1.52	n.s.	0.59	0.53	0.51	0.49	0.56	n.s.
11	1.8 cineol	1029	1026	27.00	26.83	27.01	27.79	25.45	n.s.	24.43	24.20	24.92	25.08	24.44	n.s.
12	γ-terpinene	1055	1054	0.96	1.01	1.01	1.20	1.29	n.s.	1.73	1.75	1.68	1.72	1.80	n.s.
13	terpinolene	1085	1088	0.75	0.81	0.73	0.63	0.73	n.s.	1.31 a	1.31 a	1.22 b	1.27 ab	1.31 a	*
14	linalool	1099	1098	1.93	1.65	1.90	1.39	1.21	n.s.	1.61	1.86	1.92	1.72	1.83	n.s.
15	chrysanthenone	1121	1124	0.47	0.46	0.52	0.39	0.37	n.s.	0.79	0.83	0.71	0.71	0.77	n.s.
16	camphor	1138	1141	2.36	2.26	2.34	2.11	1.85	n.s.	1.96	2.07	2.12	1.96	2.08	n.s.
17	pinocarvone	1155	1160	0.48	0.39	0.48	0.36	0.27	n.s.	0.33	0.36	0.36	0.34	0.36	n.s.
18	borneol	1160	1165	3.83 ab	3.29 ab	4.06 a	3.05 ab	2.41 b	*	3.40	4.03	4.04	3.60	3.97	n.s.
19	terpinen-4-ol	1172	1174	0.63	0.50	0.65	0.39	0.28	n.s.	0.44	0.52	0.50	0.46	0.49	n.s.
20	α-terpineol	1186	1186	2.03	1.64	2.03	1.30	1.11	n.s.	1.21	1.49	1.50	1.31	1.45	n.s.
21	myrtenol	1192	1194	0.22	0.28	0.31	0.17	0.10	n.s.	0.23	0.28	0.27	0.24	0.27	n.s.
22	verbenone	1205	1204	6.10	5.12	5.64	4.75	3.75	n.s.	5.52	6.22	6.28	5.62	6.44	n.s.
23	carvacrol methylether	1243	1241	0.08	0.14	0.13	0.06	0.05	n.s.	0.29	0.37	0.27	0.29	0.34	n.s.
24	geranial	1262	1264	0.61	0.21	0.68	0.14	0.14	n.s.	1.38	1.91	1.92	1.57	1.75	n.s.
25	bornyl acetate	1287	1285	1.96	2.06	2.00	1.83	1.51	n.s.	2.54 b	3.56 a	3.48 a	3.11 a	3.40 a	*
26	α-copaene	1371	1374	0.19	0.11	0.35	0.19	0.37	n.s.	0.35 b	0.50 ab	0.54 a	0.39 ab	0.44 ab	*
27	β-caryophyllene	1419	1417	4.07	4.70	3.93	4.16	4.50	n.s.	3.65	4.48	4.29	4.08	4.36	n.s.
28	α-humulene	1455	1452	0.28	0.23	0.29	0.19	0.17	n.s.	0.45	0.58	0.52	0.50	0.54	n.s.
29	caryophyllene oxide	1589	1582	0.08	0.05	0.08	0.04	0.04	n.s.	0.08	0.13	0.09	0.08	0.09	n.s.

RI_{calc}: Retention indices relative to C9-C27 n-alkenes from a HP-5MS-column; RI_{lit} = Retention Indices based on Adams (2007). ** = significant at 0.01 probability level; * = significant at 0.05 probability level; n.s. = not significant. Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

Table 5. Influence of foliar treatment on rosemary phenolic content (PC), antioxidant activity (AA) and rosmarinic acid (RA).

Foliar treatment	PC [mg c.a.e. g ⁻¹]		AA [mg t.e. g ⁻¹]		RA [%]	
	I year	II year	I year	II year	I year	II year
C	109.2 a	78.5 ab	140.8 a	136.7 ab	1.6 a	1.6 a
APH-100	97.3 b	81.2 a	111.1 b	124.4 b	1.4 a	1.2 b
APH-200	97.6 b	69.8 c	121.2 b	144.1 a	1.5 a	1.6 a
PPH-150	97.3 b	82.1 a	111.1 b	124.9 b	1.5 a	1.2 b
PPH-300	95.3 b	75.3 b	138.8 a	135.6 ab	1.6 a	1.6 a
<i>p</i> -value	0.000	0.000	0.000	0.013	0.300	0.000

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to Tukey's test. C = control (only water); APH-100 = 100 mL hL⁻¹ of animal protein hydrolysate; APH-200 = 200 mL hL⁻¹ of animal protein hydrolysate; PPH-150 = 150 mL hL⁻¹ of plant protein hydrolysate; PPH-300 = 300 mL hL⁻¹ of plant protein hydrolysate.

Highlights

- Protein hydrolysates significantly improve yield traits in rosemary plant.
- Plant-derived protein hydrolysate performs well when applied at the lowest dose.
- Animal and plant-derived protein hydrolysates do not modify the aromatic profile of the essential oil.
- Protein hydrolysates determine contrasting effects on some chemical properties of rosemary.