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Seaweed extract and fulvic acid application affect the biomass performance, the essential oil yield and composition of Sicilian oregano grown in an organic agricultural system

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ABSTRACT

Origanum vulgare ssp. *hirtum* is one of the taxa used as ‘oregano’ and is medicinal and aromatic plant (MAP) utilized worldwide in various sectors. Nowadays, agricultural systems are moving towards sustainable, organic, and environmentally friendly agricultural production and biostimulants have been used in recent years as a potential strategy. The objective of this research was to investigate the influence of seaweed extract based on *Aschophyllum nodosum* (AN) and fulvic acid (FA), applied with two different doses, on morphological parameters, fresh and dry yield, essential oil (EO) traits, total phenolic content (TPC), antioxidant activity (AA) and rosmarinic acid content (RAC) of organic oregano. Two-year experiment was conducted using a randomized complete block design with one main factor (foliar biostimulant) and 3 replicates. In AN- and FA-treated plants, increases in fresh biomass between 0.3 and 8.4 t ha^{-1} and in dry biomass between 0.5 and 3.0 t ha^{-1} were observed. The application of the lowest doses of fulvic acid (FA4) produced the highest EO contents (3.49 % and 3.46 %) and EO yields (73.3 and 97.4 kg ha^{-1}) in both years. The application of the lowest doses of FA produced an increase (on average 4 %) in thymol content. Biostimulant application generated contrasting response, and, in some cases, a reduction in some chemical parameters compared with control plants. The findings of this study contribute to add novelty in cultivation of oregano. The application of low doses of AN and FA allows to increase yields and improve some qualities of the secondary metabolites from organic oregano.

1. Introduction

Oregano (*Origanum vulgare* ssp. *hirtum*) is an annual/perennial shrub native to the Mediterranean region, and it is well-known for the aromatic, taste, and medicinal properties of the aerial parts and flowers (Emrahi et al., 2021; Kosakowska et al., 2019; Morshedloo et al., 2017; Sarikurkcu et al., 2015; Tuttolomondo et al., 2013). As a medicinal and aromatic plant (MAP), this crop provides a great deal of health benefits that are largely acknowledged and listed in the official Pharmacopoeia of many countries (Bora et al., 2022; 2015). The biological activity of oregano, the properties of which have been exploited in the care of various diseases, has been the subject of in-depth investigation (Emrahi et al., 2021; Lombrea et al., 2020; Fraj et al., 2019). The antibacterial, antifungal, anti-inflammatory, antimicrobial, and antioxidant activities of this species have been reported by several authors investigating the properties of oregano under the effects of abiotic and biotic factors (Abdali et al., 2023; Hancioglu et al., 2021; Ninou et al., 2021; Morshedloo et al., 2017; Lukas et al., 2015; Sarikurkcu et al., 2015) and, in many countries, thanks to these many properties, this aromatic herb is used as a remedy in traditional healing systems (Matłok et al., 2020; Lukas et al., 2015). Oregano is also grown worldwide for its essential oils (EOs) and the diversity in composition of the EOs may influence the quality, biological activity, and properties of this species (Aytaç et al., 2022; Węglarz et al., 2020). Essential oils are concentrated volatile liquids produced as secondary metabolites by different organs of the plant (Rostro-Alanis et al., 2019) and commonly stored in canals, epidermic cells, glandular trichomes, and secretory cells (Bora et al., 2022). Carvacrol and thymol are the predominant components of the

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EOs and, based on their prevalence, two principal chemotypes can be usually ascribed to this species (Aytaç et al., 2022; Kimera et al., 2021; Laothaweerungsawat et al., 2020; Napoli et al., 2020; Matłok et al., 2020; Sakkas and Papadopoulou 2017). However, as stated by (Zinno et al., 2023; Napoli et al., 2020; Lukas et al., 2015; Crocoll et al., 2010), other chemotypes have been recognised worldwide, based on the relative amounts of other compounds. Oregano is usually extremely rich in EOs and can exhibit highly variable oil concentrations due to a number of reasons, as studies confirm (Abdali et al., 2023; Farruggia et al., 2023; Aytaç et al., 2022; Ninou et al., 2021; Węglarz et al., 2020). Literature highlights that, in general, genetic factors, environmental factors, and growing practices have a significant effect on the quantity and quality of EOs and extracts of MAPs (Farruggia et al., 2024a; Tuttolomondo et al., 2021; Bistgani et al., 2019; Ninou et al., 2017; Baranauskienė et al., 2013).

As reported by several authors (Kosakowska et al., 2019; Murillo-Amador et al., 2013), oregano can be successfully cultivated in organic farms located in temperate areas in accordance with European guidelines. Some authors (Danesh-Shahraki et al., 2023; Aytaç et al., 2022; Murillo-Amador et al., 2015; Said-Al Ahl et al., 2009) sustain that the use of unconventional farming practices may greatly enhance crop production performances in terms of quality and yield. In particular, many studies have assessed how organic fertilisers can positively affect the crop yield, the EO yield, and the quality of oregano grown using organic farming methods (Kimera et al., 2021; Matłok et al., 2020; Kosakowska et al., 2019; Murillo-Amador et al., 2015, 2013).

The application of plant biostimulants, which has recently received global interest, is feasible, innovative, and compatible with organic farming practices (Sani and Yong 2021; De Pascale et al., 2017). As an alternative to common fertilisation, foliar fertilisation with the use of biostimulants can provide additional benefits, helping to minimize nutrient runoff and leaching, reduce the environmental impact, and preserve the soil health. As well reported by Szpunar-Krok (2022), and Fernández and Brown (2013), foliar fertilisation provides a more direct and effective way of supplying nutrients to plants than conventional soil-based fertilisation techniques. Biostimulant foliar application is being utilized more and more in modern agriculture to replace traditional foliar fertilisers (Rouphael and Colla, 2020; Yakhin et al., 2017;). Foliar biostimulants are utilized as an innovative and eco-friendly strategy to increase crop yields, nutrient-use efficiency, resistance to various abiotic stressors, and plant growth (Rakkammal et al., 2023; Di Miceli et al., 2023). Various biostimulants, microbial and non-microbial, can be used in organic production systems and positively affect primary and secondary metabolism (Farruggia et al., 2024a; Abdali et al., 2023; Baltazar et al., 2021). They contain physiologically active substances and allow a reduction in the amount of agricultural inputs used compared with traditional foliar fertilisers (Consentino et al., 2023; Yaseen and Takacs-Hajos, 2022; Rouphael and Colla, 2020; Tadros et al., 2019). Many studies have demonstrated the advantages of applying biostimulants to horticultural and open field crops, highlighting how they lead to an enhancement of the qualitative and yield characteristics of these crops, as well as to improved photosynthetic, and water/nutrient absorption rates (Farruggia et al., 2024b; Abdali et al., 2023; Sun et al., 2023; Rahimi et al., 2022).

Among the available biostimulants, this study has taken into consideration two specific products, like seaweed extracts (SWE) and fulvic acids (FA), based on their uncommon foliar application on MAPs. Seaweed extracts, such as those based on *Aschophyllum nodosum* (AN), have been widely used on several crops, also grown in organic systems, with excellent results (Villa e Vila et al. 2023; Rajendran et al., 2022; Arioli et al., 2015; Shukla et al., 2019). In fact, many studies highlight that the application of seaweed extracts permit to affect some productive and qualitative traits of open field and greenhouse crops in terms of nutrient uptake and water absorption, photosynthetic rate improvement, and increasing in production of hormonal substances (Ammar et al., 2022; Ali et al., 2021; Khan et al., 2009). Tursun (2022) demonstrated that foliar application of seaweed extract as an organic fertiliser positively impacted on biomass yield of coriander (*Coriandrum sativum* L.). Rasouli et al. (2022) reported that the application of seaweed extract improved the morphological, productive, and biochemical traits of lettuce (*Lactuca sativa* L.).

Fulvic acids permit to alleviate exogenous stresses, such as drought and thermal stress (Farruggia et al., 2024b; Aytaç et al., 2022). These substances are organic chemicals formed when dead biota decomposes in soil and contain a high number of carboxylic groups, a high number of phenolic compounds, and a low number of aromatic structures (do Rosário Rosa et al. 2021; Canellas et al., 2015). The FA application has been tested to increase flavonoids, glutathione, ascorbate by activating genes involved in their metabolism, with the aim of reducing the harmful consequences of drought stress (Fang et al., 2020; Suh et al., 2014). Several authors observed an increase in productive and quality features of rosemary (*Rosmarinus officinalis* L.) (Farruggia et al., 2024a) and oregano (*Origanum vulgare* subsp. *hirtum*) (Aytaç et al., 2022), treated with foliar or radical humic substances, but data on the impact of FA foliar application on yield and chemical traits of oregano plants cultivated at field conditions are lacking in literature.

In the Mediterranean region, oregano is often cultivated in low input or organic systems and without irrigation (Virga et al., 2020). Limited water availability is one of the major factors contributing to agricultural yield declines and to physiological and biochemical alterations in plants (Giannoulis et al., 2020; Morshedloo et al., 2017; Yadav et al., 2014). The limited water absorption of plants also causes a limited uptake of nutrients, which are already limited in low-input cultivation systems, such as organic (Farruggia et al., 2024a; Shahrajabian et al., 2021). In this scenario, foliar biostimulant application appears to be one of the novel approaches to obtain improvements in the organic cultivation of oregano also under rainfed conditions. For these reasons, the aims of this study were: (i) to investigate the effects of AN and FA at different dosages on morphological and yield traits of oregano grown under organic farming conditions, (ii) to assess the impact of AN and FA at different dosages on EO content, yield and composition, (iii) to evaluate the influence of AN and FA at different dosages on total phenolic content (TPC), antioxidant activity (AA), and rosmarinic acid content (RAC). Specifically, the next hypothesis was examined: the application of different doses of foliar biostimulants improves the agronomic characteristics of oregano grown in rainfed conditions and modulates the chemical characteristics of the extract obtained from this species.

2. Materials and methods

2.1. Test site

The study was conducted in an organic farm located in Aragona, a rural community in Western Sicily (Italy) during two growing seasons (2021–2022 and 2022–2023). This farm is specialised in the production of oregano plants.

The soil is sandy clay loam (45 % sand, 25 % silt and 30 % clay) with 1.3 % organic matter, 1.2 % total nitrogen, 19.1 ppm assimilable phosphate, 341.0 ppm assimilable potassium, pH of 7.2. The soil is classified as Regosols (typic xerorthents) in accordance with the United States Department of Agriculture (USDA). In accordance with the climate classification by Köppen–Geiger (Kottek et al., 2006), the climate of the site is warm temperate with dry and hot summers.

2.2. Weather data

During the trials, 10-day total rainfall and average maximum and minimum air temperatures were detected by a weather measurement station owned by the Sicilian Agro-Meteorological Information Service (SIAS, 2023) and located close to the farm. Temperature and rainfall trends during the two years are shown in Fig. 1 (A and B).

Total rainfall ranged from 611 mm (2021–2022) to 538 mm

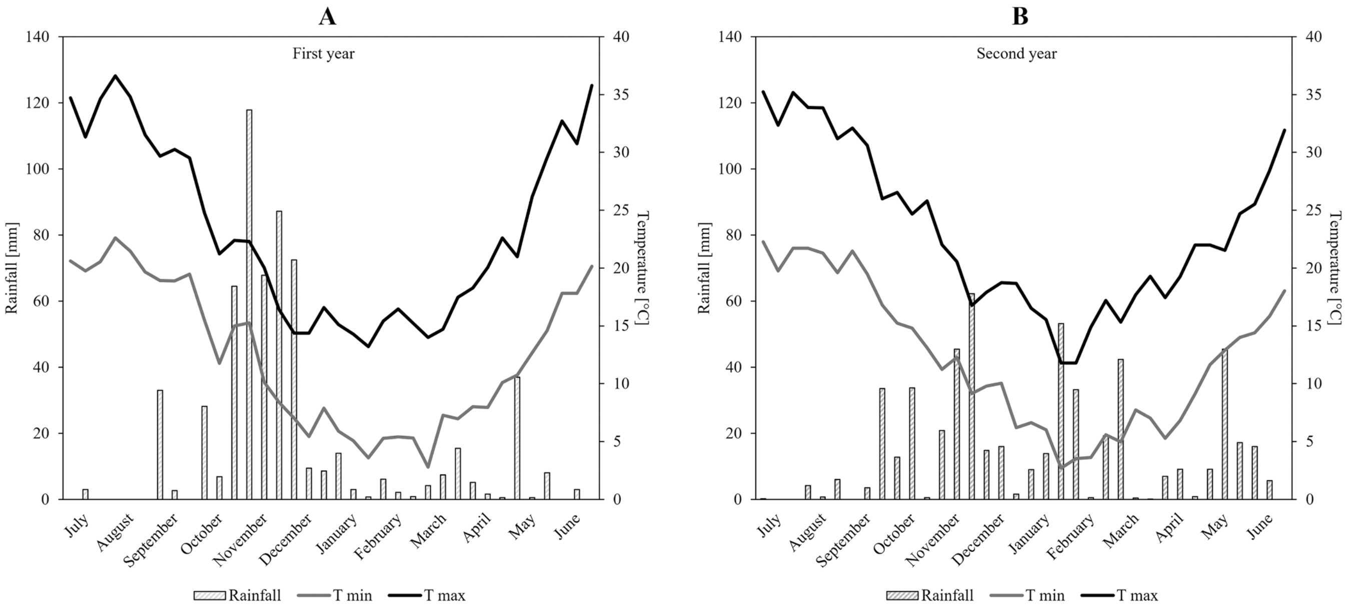

Fig. 1. Temperature and rainfall trends during the first (A) and the second (B) year.

(2022–2023). Average rainfall over the 2-year period was 574 mm. In the 1st-growing season, approximately 80 % of rainfall (480 mm) was recorded from October to January. From the beginning of the vegetative growth phase till the harvest date, 92 mm of rainfall was observed. The highest rainfall event (37 mm) happened during first 10-day period of May.

In the 2nd-growing season, rainfall levels were well spread from September to March. Throughout the biostimulant application phase and up to harvest, 110 mm of rainfall were measured. The highest rainfall event occurred in the second 10-day period of May (45 mm). The temperature trends in both years were comparable to the study area's average temperature. An increase in minimum and maximum temperatures was observed from pre-flowering stage until harvest. In general, higher maximum temperatures were observed in the second growing season, from May until harvest, compared with the first growing season.

2.3. *Experimental field and cultivation practices*

The experimental field of oregano was established in March 2019. Agamic propagation consisted in dividing the bushes. A plant density of 10000 plants ha^{-1} was obtained, adopting 2.0 m between rows and 0.5 m within rows. The field was fertilised using 2.0 t ha^{-1} of cattle manure (0.5 % of N, 0.2 % of P_2O_5, 0.7 % K_2O, approximately) prior to transplantation. Oregano plants were organically cultivated under rainfed conditions. Every year, weeds were mechanically controlled at the start of spring and before harvest.

Oregano plants were manually harvested once a year, making a cut at 5.0 cm above ground level. The harvest occurred at full blooming phase, during the second 10-day period of June every year.

2.4. *Treatments*

Two biostimulant formulations were foliar applied:

- *Ascophyllum nodosum* (AN), having organic carbon (10.0 %), organic nitrogen (1.0 %), phytohormones (30 %), and organic substances naturally contained in seaweed extracts.
- Fulvic Acid (FA), that was extracted from leonardite and having organic carbon (30 %) and organic nitrogen (0.5 %).

For each biostimulant, two doses were taken into consideration in order to supply the same total amount of nitrogen avoiding any differences in nitrogen content (Table 1). Control (C) treatment was only water. Four applications, using 400.0 L of water ha^{-1} for each event, were performed from vegetative growth stage until pre-flowering stage. Yearly, the first application was carried out during the first week of April. The other foliar treatments were performed every 10 days. Foliar applications were made through a portable hand-sprayer with an operating pressure of 250.0 kPa and equipped with a flat fan nozzle. Each plot was delimited during application with a plastic panel to prevent drift and contamination of adjacent plots. To ensure uniformity in the amount of biostimulant applied to the oregano, foliar treatments were carried out by a single operator.

Foliar applications were performed early in the morning, when temperature, relative humidity and stomatal aperture were ideal for foliar absorption (Ruiz-Navarro et al., 2019). The plot size was 30 m^2 (2.0 m × 15.0 m) with 15 plants per plot, that were taken into consideration for the subsequent measurements. For preventing drift during foliar sprays, plastic panels were utilized to separate each plot. The experimental scheme was a randomized complete block design using three replications.

2.5. *Morphological and yield traits*

At harvest, some traits such as plant height, relative water content (RWC), chlorophyll content, total fresh yield, were determined. After harvest, plants were dried in a shaded and ventilated environment for approx. 10 days at a temperature of 25–30 °C and total dry yield were determined. Stems, leaves, and flowers were manually separated, and

Table 1
Biostimulants doses.

Foliar biostimulant	Abbreviation	Doses for each application [L hL^{-1}]	Total amount[a] [L ha^{-1}]
Ascophyllum nodosum	AN4	0.250	4
Ascophyllum nodosum	AN2	0.125	2
Fulvic acids	FA8	0.500	8
Fulvic acids	FA4	0.250	4

[a] = total quantity of biostimulant applied.

each fraction has been weighed. The stems were not used for EO extraction because of the marginal amounts of EO.

Chlorophyll content was measured using a Dualex Scientific (Force A, Orsay, France) portable Chlorophyll meter. For each plot, 30 leaves have been considered. The device calculated the mean of the values.

The relative water content of leaves was assessed by taking 30 fresh leaves from each plot and the fresh weight (FW) was measured. For 24 hours, the leaves floated in distilled water within a falcon tube. After that, leaves were removed from the water and put on absorbent paper to eliminate the excess water, and the turgid weight (TW) was recorded. The leaves were then dried in an oven for 24 hours, and the dry weight (DW) was noted (Alyemeni et al., 2018). The following equation was used to determine the RWC:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

2.6. EO extraction and analysis

Essential oils were extracted by hydro-distillation of air-dried plant material (500.0 g) per each plot for 3 h, according to Ph. Eur. 7.0, 20812 (European Pharmacopoeia, 2008). In total 3 essential oil extractions were carried out per each treatment. The EO content was calculated dividing the volume of EO obtained by the weight of the biomass samples. The EO yield was estimated multiplying the EO content by the total dry yield. The EO samples were stored at −18°C. Prior to gas chromatography–mass spectrometry (GC/MS), the EOs were diluted 1:100 with hexane and moved to GC vials. The EO components were assessed with a HP 6890 gas chromatograph connected with the quadrupole mass spectrometer HP5972 MSD (Hewlett-Packard, Palo Alto, CA, USA). The operating parameters reported by Farruggia et al. (2024b) were utilized. The EO compounds retention indices (RI) were calculated and compared with those of n-alkane hydrocarbons (RI standard for GC, Sigma-Aldrich, Vienna, Austria). Mass spectra and retention indices were compared with data reported in the literature in order to identify each compound. Peak-area normalization was used to obtain the composition, and the response factor of each component was taken to be equal to 1.

2.7. Determination of some chemical parameters

Using 25.0 mL of 70.0 % aqueous methanol, 0.15 g of the finely ground dry biomass were extracted in an ultrasonic bath DU-32 (Argo Lab, Carpi, Italy, operating at 40 kHz at 120 W) for 30 minutes at room temperature. After being filtered, the extracts were stored at −20°C for further examination.

The extracts were used to determine:

- Total phenolic content, expressed as milligram caffeic acid equivalents per gram dry weight (mg c.a.e. g^{-1} dw), following the method described by Lamien-Meda et al. (2010).
- Antioxidant activity, expressed as milligram trolox equivalents per gram dry weight (mg t.e. g^{-1} dw), following the method described by Chizzola et al. (2008).
- Rosmarinic acid content, as reported Farruggia et al. (2024b).

2.8. Statistical analyses

One-way analysis of variance (ANOVA) was conducted to compare the data per year. The difference between means was carried out using Fisher's LSD test ($p \leq 0.05$). Foliar biostimulants were used as fixed effects in the linear model/ANOVA. Before ANOVA, the RWC data were subjected to arcsine transformation. Prior to statistical analysis, Levene's test and the Shapiro-Wilk test were used, respectively, to check the homogeneity of variance and normality of all the data. Principal components analysis (PCA) was also carried out on the productive and chemicals parameters and on the most represented EO compounds (over 2.0 % on average). In order to determine the ideal amount of principal components (PC), factors having eigenvalues over 1.0 were taken into account. The software MINITAB 19 (State College, PA, USA) for Windows was used for statistical analyses.

3. Results

3.1. Morphological and yield traits

ANOVA showed that foliar biostimulant significantly influenced ($p \leq 0.01$) plant height, chlorophyll content, and RWC during both years (Table 2).

The highest plant height values (51.0 and 65.2 cm) were observed in AN4-treated plants in both years. During the second year, the application of FA4 produced the highest plant height (64.0 cm).

Regarding chlorophyll content, the highest value (31.3 μg cm^{-2}) was observed in AN4-treated plants in the first year and in AN2-treated plants (34.8 μg cm^{-2}) in the second year. The lowest plant height and chlorophyll content values were recorded in control plants (Table 2).

Concerning the RWC, in the first year the highest value (68.6 %) was observed in AN2-treated plants while the lowest in control plant and AN4-treated plants (Table 2). In the second year, comparable RWC values have been recorded in control plants, AN4-, FA8-, and FA4-treated plants with values ranging from 87.4 % to 89.0 %.

In both years, statistical analysis of inflorescence and leaf percentages, and stem percentages revealed a significant influence ($p \leq 0.01$) of the foliar biostimulant factor (Fig. 2). The application of two doses of *A. nodosum* produced the highest percentage value for inflorescences (74.7 %) and leaves (74.1 %) and, consequently, the lowest percentage value for stems during the first year (Fig. 2. A). However, control plants generated the highest percentage values for inflorescences and leaves (66.8 %) during the second year (Fig. 2. B).

Based on ANOVA outcomes, the foliar biostimulant had a significant effect ($p \leq 0.01$) on total yields, fresh and dry, during both years (Fig. 3). The foliar application of AN2 and two doses of fulvic acid created the highest total fresh yield (with values ranged from 4.6 to 4.8 t ha^{-1}) and total dry yield (with values ranged from 2.6 to 2.7 t ha^{-1}) in the first year (Fig. 3. A). During the second year, the highest total dry yield was found in oregano plants treated with both FA doses (Fig. 3. B). The application of all biostimulants led to obtain similar total dry yields during the second year (values ranged from 4.6 to 4.9 t ha^{-1}). The lowest yields were observed in control plants in both years (Fig. 3).

Regarding EO content and EO yield, ANOVA revealed that foliar biostimulant produced significant differences ($p \leq 0.01$) in both years (Table 3). The highest EO content (3.93 % and 3.46 %) and EO yields (73.3 and 97.4 kg ha^{-1}) were obtained in FA4-treated plants (Table 3). The lowest EO content was observed in control plants and in AN2-

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

Foliar application	Plant height		Chlorophyll content		RWC	
	[cm]		[μg cm^{-2}]		[%]	
	I year	II year	I year	II year	I year	II year
C	37.0c	44.5c	25.4 e	29.5 d	58.5c	88.1 a
AN4	51.0 a	65.2 a	31.3 a	33.3c	59.6c	89.0 a
AN2	42.5 b	61.2 b	30.7 b	34.8 a	68.6 a	84.0 b
FA8	41.7 b	60.0 b	27.1c	32.9c	55.3 d	87.5 a
FA4	42.5 b	64.0 a	26.4 d	34.1 b	62.3 b	87.4 a
p-value	0.000 **	0.000 **	0.000 **	0.000 **	0.000 **	0.004 **

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to LSD test. ** = significant at 0.01 probability level. C = control (only water); AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acids; FA4 = 4 L ha^{-1} of fulvic acids.

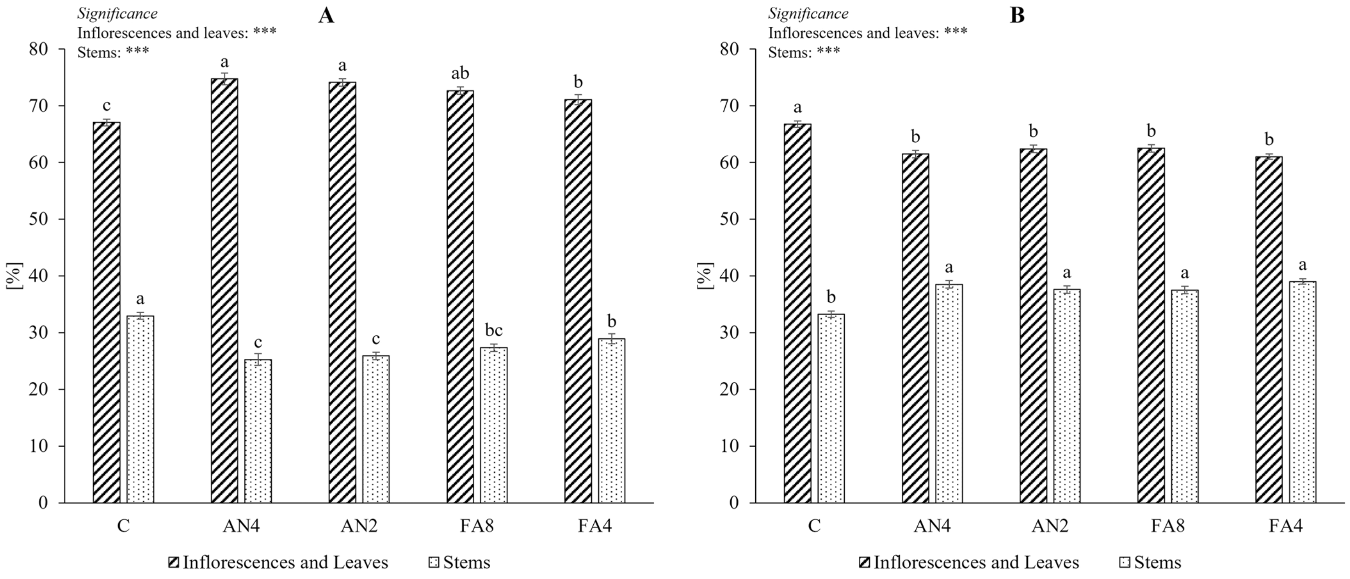

Fig. 2. Influence of foliar application on oregano inflorescence and leaf percentages and stem percentages during first (A) and the second (B) year. Means and standard errors are shown. The values followed by the equal letter are not significantly different for $p \leq 0.05$ according to LSD test. *** = $p \leq 0.001$. C: control (only water); AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acid; FA4 = 4 L ha^{-1} of fulvic acid.

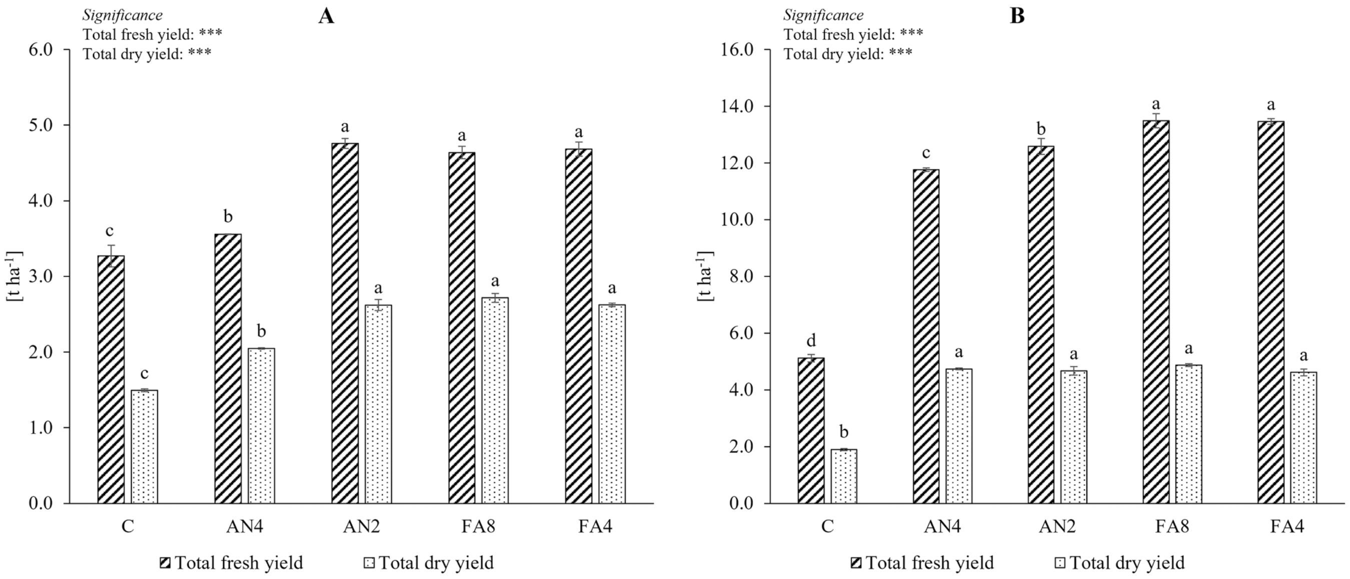

Fig. 3. Influence of foliar application on oregano total fresh yield and total dry yield during first (A) and the second (B) year. Means and standard errors are shown. The values followed by the equal letter are not significantly different for $p \leq 0.05$ according to LSD test. *** = $p \leq 0.001$. C: control (only water); AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acid; FA4 = 4 L ha^{-1} of fulvic acid.

treated plants during the first year (Table 3). The application of AN2 produced the lowest EO content during the second year (Table 3). Regarding EO yields, control plants had the lowest values (Table 3).

3.2. Essential oil profile

The EO compounds, and the ANOVA results are reported in Table 4. Thirty-nine different compounds were found. Statistical analysis revealed that foliar biostimulant significantly influenced ($p \leq 0.05$) the percentage of γ-terpinene, *trans*-sabinene hydrate, carvacrol methyl ether, carvacrol, aromadendrene, α-humulene, alloaromadendrene in the first year and the percentage of carvacrol in the second year (Table 4).

The highest γ-terpinene values were noted in control plants (16.10 %) and in AN4-treated plants (16.09 %), while the lowest value in FA4-treated plants (13.95 %) (Table 4). The highest *trans*-sabinene hydrate contents were recorded in AN4- and FA8-treated plants (0.21 %) while the lowest in control plants (0.20 %). Control plants, AN4-, and AN2-treated plants generated the highest carvacrol methyl ether (values ranged from 5.31 % to 5.47 %), and FA4-treated plants the lowest (5.08 %). The highest carvacrol content was found in control plants (0.64 %) and FA4-treated plants (0.57 %) in the first year (Table 4). The same performance was obtained by control plants and by AN2-treated plants (0.59 %) in the second year (Table 4). Considering the aromadendrene content, the highest value (0.14 %) was recorded in AN2-treated plants while the lowest (0.11 %) in control plants. The foliar application of all biostimulants produced higher values of α-humulene (ranged from 0.24 % to 0.26 %) and alloaromadendrene

Table 3
Influence of foliar application on oregano essential oil (EO) content and yield during the two-year research.

Foliar application	EO content		EO yield	
	[$\%_{v/w}$]		[kg ha^{-1}]	
	I year	II year	I year	II year
C	2.70c	2.85c	27.1 e	36.1 d
AN4	3.02 b	3.08 b	46.1 d	89.6 b
AN2	2.83c	2.16 d	55.0c	63.0c
FA8	3.14 b	2.96 bc	61.9 b	90.2 b
FA4	3.93 a	3.46 a	73.3 a	97.4 a
p-value	0.000 **	0.000 **	0.000 **	0.000 **

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to LSD test. ** = significant at 0.01 probability level. C = control (only water); AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acids; FA4 = 4 L ha^{-1} of fulvic acids.

(ranging from 0.12 % to 0.14 %) compared with those observed in control plants (0.21 % α-humulene and 0.11 % alloaromadendrene (Table 4). The highest viridiflorol contents were recorded in FA8- (0.16 %) and FA4-treated plants (0.17 %) while the lowest in control and AN4-treated plants (0.14 %).

3.3. *Chemical parameters*

ANOVA showed that foliar biostimulant significantly affected TPC and AA in both years (Table 5). The RAC was significantly influenced only in the second year (Table 5). In both years, the highest TPC was observed in control plants (128.3 mg c.a.e. g^{-1} and 112.7 mg c.a.e. g^{-1}). As reported in Table 5, the lowest total phenolic was obtained in FA4-treated plants in the first year (109.9 mg c.a.e. g^{-1}) and in AN4-treated plants in the second year (91.3 mg c.a.e. g^{-1}).

During the first year, the greatest antioxidant activity was recorded in the control, AN4- and FA8-treated plants (Table 5). In the second year, the control plant produced the highest AA (133.5 mg t.e. g^{-1}) and the highest RAC (2.4 %) (Table 5).

3.4. *Principal components analysis (PCA)*

The PCA for productive and chemical parameters and for the most represented EO compounds (over 2.0 % on average) showed two principal components (PC) with eigenvalues higher than 1. The two PC described 93.9 % of the total variance (Table 6).

The first principal component (PC1) provided 53.1 % of the total variation and it mostly showed a favourable correlation with TPC, AA, RAC, α-terpinene, γ-terpinene, neral, carvacrol methyl ether. The second principal component (PC2) gave 40.9 % of the total variation and was positively correlated with total fresh yield, total dry yield, EO yield, α-terpinene, *p*-cymene, γ-terpinene, neral, carvacrol methyl ether (Table 6).

Foliar biostimulant application generated a distinct separation with the control in the bottom right quadrant of the plot and the biostimulant in the bottom left and in the two upper quadrants of the plot. In particular, the control treatment was placed in the bottom right quadrant because of the variables TPC, AA, and RAC acid. The lowest dose of fulvic acids was in the bottom left quadrant with the variables EO content and thymol contributing, while FA8 was in the upper left quadrant with the variables EO yield, total fresh yield, total dry yield, and *p*-cymene contributing. Both AN treatments were positioned in the upper right quadrant of the plot based on other EO compounds, such as α-terpinene, *p*-cymene, γ-terpinene neral, carvacrol methyl ether (Fig. 4).

4. Discussion

4.1. *General aspects*

Oregano is currently one of the most interesting and exploited MAPs throughout the world (Lukas et al., 2015). It is a valuable source of bioactive compounds characterized by a range of pharmacologic properties, including antibacterial, antioxidant, anti-cholinesterase, and cardioprotective activities (Morshedloo et al., 2018).

The increase in yields and optimization of energy inputs for production are the main objectives of modern agriculture (Al-Karaki and Othman, 2023; Caruso et al., 2019). However, maintaining quality standards and production levels whilst reducing chemical fertiliser use are critical aspects for MAP farmers (Ninou et al., 2021; Kimera et al., 2021). To enhance nutrient absorption and utilisation effectiveness, stress tolerance, and quality features, foliar biostimulant application promotes the regulation and improvement of some physiological responses in plants (Rouphael and Colla, 2018). Using naturally produced plant biostimulants is a viable and sustainable method in both conventional and organic farming systems (De Pascale et al., 2017)

This study highlights how the agronomic performance of organically grown oregano can be improved by the foliar spray of biostimulants. Foliar biostimulants have influenced the morphological, yield and qualitative characteristics of this species which was managed without the use of irrigation water. Future studies should consider the effects of biostimulants on oregano cultivated under different climatic conditions to validate their applicability in various global agricultural environments.

4.2. *Morphological and yield traits*

In this study, similar trends in morphological and yield characteristics were observed; however, the highest values were recorded during the second year. This can be explained by the greatest vegetative growth of plants, in accordance with previous studies (Ninou et al., 2021; Goliaris, 1997).

In general, the application of biostimulants allowed the plant height to increase with respect to control plants. The highest doses of *A. nodosum* produced the greatest boost in plant height compared with control. As reported by Shukla et al. (2019), *A. nodosum* improves plant performance through the control of plant hormone signaling and boost of flavonoid, carotene, betaine, or carbohydrate biosynthesis. A wide range of complex bio-stimulatory substances, such as vitamins, minerals, and complex chemical compounds, are responsible for these effects, as described by Santaniello et al. (2017). In Greece, in oregano plants, Dordas (2009) obtained values of plant height which varied between 57.0 cm and 70.0 cm through the foliar treatments with calcium and magnesium. When adding various nitrogen rates to the soil, Król et al. (2020) reported values of plant height ranging from 24.0 cm to 35.0 cm and found the highest performance in plants which were fertilised using the highest nitrogen doses. Plant heights of 40–50 cm were recorded by Sotiropoulou and Karamanos (2010) by varying nitrogen fertilisation treatments. In a similar environment, some authors (Farruggia et al., 2023; Virga et al., 2020) observed similar plant heights to this study.

In the first year, chlorophyll content and relative water content were found to be lower than those of the second year. This fact was related to greater rainfall in the second year and, consequently, higher soil moisture than the first year; increases in water availability promoted photosynthetic activity in oregano plants in accordance with Hancioglu et al. (2021). Biostimulant application led to higher chlorophyll content. Several authors (Farruggia et al., 2024a; Ciriello et al., 2022; Abdel-Rahman and Abdel-Kader 2020) observed improvements in chlorophyll content in some species treated with foliar or radical biostimulants. Seaweed extracts contain cytokinins or cytokinin-like compounds, which may provide benefits on chlorophyll content and

Table 4

Components of oregano EO and *p*-value in response to foliar application during the two-years research.

Peak	**Compounds**	**RI$_{calc}$**	**RI$_{lit}$**	**I year**						**II year**					
				Foliar application						**Foliar application**					
				C	**AN4**	**AN2**	**FA8**	**FA4**	***Significance***	**C**	**AN4**	**AN2**	**FA8**	**FA4**	***Significance***
1	α-thujene	923	923	1.96	2.00	1.85	1.81	1.80	n.s.	0.89	1.10	1.28	1.15	1.13	n.s.
2	α-pinene	929	933	0.77	0.82	0.74	0.71	0.71	n.s.	0.54	0.49	0.58	0.54	0.49	n.s.
3	camphene	943	952	0.09	0.09	0.09	0.08	0.08	n.s.	0.12	0.07	0.08	0.08	0.04	n.s.
4	sabinene	971	973	0.17	0.17	0.15	0.15	0.14	n.s.	0.13	0.10	0.12	0.12	0.08	n.s.
5	myrcene	989	991	2.16	2.12	1.99	2.00	1.90	n.s.	1.12	1.13	1.28	1.19	1.14	n.s.
6	α-phellandrene	1002	1005	0.41	0.41	0.38	0.38	0.37	n.s.	0.24	0.25	0.28	0.26	0.25	n.s.
7	α-terpinene	1013	1018	3.82	3.80	3.59	3.58	3.38	n.s.	2.77	2.84	3.22	2.95	2.96	n.s.
8	*p*-cymene	1021	1026	6.44	6.49	6.52	6.53	6.02	n.s.	4.96	6.33	6.61	6.03	6.20	n.s.
9	limonene	1025	1031	0.53	0.52	0.49	0.49	0.46	n.s.	0.33	0.33	0.37	0.35	0.33	n.s.
10	*cis*-beta-ocimene	1036	1040	1.98	1.85	1.79	1.81	1.74	n.s.	1.76	1.47	1.62	1.56	1.31	n.s.
11	*trans*-beta-ocimene	1046	1050	0.31	0.27	0.26	0.27	0.25	n.s.	0.16	0.15	0.17	0.15	0.14	n.s.
12	γ-terpinene	1056	1059	16.10 a	16.09 a	15.21 ab	14.92 ab	13.95 b	*	13.17	13.34	15.02	14.01	14.00	n.s.
13	*cis*-sabinene hydrate	1062	1069	0.83	0.90	0.85	0.87	0.86	n.s.	0.65	0.63	0.67	0.62	0.60	n.s.
14	α-terpinolene	1084	1084	0.12	0.11	0.10	0.10	0.09	n.s.	0.11	0.11	0.12	0.11	0.10	n.s.
15	*trans*-sabinene hydrate	1094	1089	0.20c	0.21 a	0.20 bc	0.21 a	0.21 ab	**	0.22	0.24	0.26	0.22	0.19	n.s.
16	linalool	1098	1098	0.31	0.30	0.30	0.27	0.26	n.s.	0.42	0.09	0.21	0.53	0.00	n.s.
17	borneol	1160	1165	0.15	0.14	0.14	0.14	0.14	n.s.	0.56	0.11	0.24	0.56	0.00	n.s.
18	terpinen−4-ol	1172	1177	0.34	0.31	0.32	0.30	0.30	n.s.	0.14	0.09	0.10	0.12	0.08	n.s.
19	α-terpineol	1186	1185	0.13	0.13	0.12	0.12	0.12	n.s.	0.21	0.22	0.24	0.22	0.18	n.s.
20	neral	1239	1235	2.48	2.66	2.65	2.63	2.60	n.s.	3.15	3.67	4.13	3.38	3.32	n.s.
21	carvacrol methyl ether	1249	1244	5.33 a	5.47 a	5.31 a	5.30 ab	5.08 b	*	5.04	5.27	5.52	5.27	5.02	n.s.
22	thymol	1298	1290	46.29	45.63	46.98	47.32	49.96	n.s.	52.79	51.44	47.64	50.25	53.39	n.s.
23	carvacrol	1302	1298	0.64 a	0.47c	0.48 bc	0.55 ab	0.57 a	**	0.59 a	0.54 ab	0.59 a	0.43c	0.47 bc	**
24	α-copaene	1375	1376	0.12	0.12	0.12	0.08	0.11	n.s.	0.11	0.12	0.13	0.13	0.10	n.s.
25	β-bourbonene	1384	1380	0.11	0.09	0.10	0.09	0.08	n.s.	0.09	0.08	0.08	0.09	0.02	n.s.
26	β-caryophyllene	1419	1428	1.89	1.97	2.02	1.96	1.85	n.s.	2.05	1.99	2.00	2.06	1.73	n.s.
27	aromadendrene	1439	1439	0.11c	0.13 ab	0.14 a	0.13 ab	0.12 b	**	0.14	0.14	0.14	0.14	0.11	n.s.
28	α-humulene	1455	1452	0.21 b	0.24 a	0.26 a	0.25 a	0.25 a	*	0.39	0.26	0.29	0.48	0.20	n.s.
29	alloaromadendrene	1462	1461	0.11 b	0.12 a	0.14 a	0.13 a	0.13 a	**	0.19	0.20	0.20	0.19	0.16	n.s.
30	γ-muurolene	1478	1477	0.40	0.43	0.45	0.44	0.43	n.s.	0.44	0.49	0.48	0.49	0.43	n.s.
31	germacrene D	1483	1480	0.65	0.65	0.71	0.63	0.57	n.s.	1.03	1.28	1.10	1.11	1.15	n.s.
32	bicyclogermacrene	1496	1494	0.31	0.31	0.34	0.34	0.33	n.s.	0.46	0.42	0.41	0.40	0.36	n.s.
33	α-muurolene	1501	1499	0.15	0.16	0.17	0.16	0.16	n.s.	0.17	0.19	0.18	0.18	0.17	n.s.
34	β-bisabolene	1510	1509	2.02	2.07	2.21	2.09	2.02	n.s.	1.59	1.81	1.74	1.71	1.71	n.s.
35	γ-cadinene	1516	1512	0.49	0.52	0.54	0.55	0.56	n.s.	0.56	0.64	0.59	0.59	0.56	n.s.
36	δ-cadinene	1526	1524	0.84	0.87	0.92	0.93	0.92	n.s.	1.07	1.21	1.14	1.15	1.08	n.s.
37	(*E*)-α-bisabolene	1535	1549	0.09	0.10	0.10	0.10	0.11	n.s.	0.09	0.11	0.10	0.09	0.09	n.s.
38	caryophyllene oxide	1580	1581	0.19	0.26	0.26	0.28	0.26	n.s.	0.39	0.36	0.34	0.30	0.30	n.s.
39	viridiflorol	1589	1590	0.14 b	0.14 b	0.15 ab	0.16 a	0.17 a	*	0.11	0.13	0.12	0.10	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 literature.** = 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 LSD test. C = control (only water); AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acids; FA4 = 4 L ha^{-1} of fulvic acids.

Table 5
Influence of foliar application on oregano total phenolic content (TPC), antioxidant activity (AA), and rosmarinic acid content (RAC) during the two-years research.

Foliar application	TPC [mg c.a.e. g^{-1}]		AA [mg t.e. g^{-1}]		RAC [%]	
	I year	II year	I year	II year	I year	II year
C	128.3 a	112.7 a	157.2 a	133.5 a	3.0 a	2.4 a
AN4	119.2c	91.3 d	154.6 a	128.2 b	3.0 a	1.6 b
AN2	124.7 b	100.5 b	150.9 b	117.6c	2.8 a	1.9 ab
FA8	127.6 ab	96.6c	154.5 a	120.9c	2.9 a	1.6 b
FA4	109.9 d	95.6c	146.9 c	106.0 d	2.7 a	1.3 b
p-value	0.000 **	0.000 **	0.000 **	0.000 **	0.103 n.s.	0.034 *

Means are shown. The values followed by different letter are significantly different for $p \leq 0.05$ according to LSD test. ** = significant at 0.01 probability level; * = significant at 0.05 probability level; n.s. = not significant. c.a.e. = caffeic acid equivalent; t.e.= trolox equivalent.C = control (only water); AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acids; FA4 = 4 L ha^{-1} of fulvic acids.

Table 6
Eigenvalues, total variance explained (TVE) and cumulative total variance explained (cumulative TVE) of the two principal components (PCs) for production and chemical variables and the most relevant EO compounds.

Variables	PC1	PC2
Total fresh yield	−0.26	0.302
Total dry yield	−0.231	0.334
EO content	−0.35	−0.147
EO yield	−0.34	0.177
Total phenolic	0.315	−0.155
Antioxidant activity	0.315	−0.117
Rosmarinic acid	0.35	−0.169
α-terpinene	0.305	0.253
p-cymene	−0.047	0.429
γ-terpinene	0.314	0.24
neral	0.052	0.414
carvacrol methyl ether	0.222	0.337
thymol	−0.272	−0.296
Total eigenvalue	6.90	5.31
TVE [%]	53.10	40.90
Cumulative TVE [%]	53.10	93.90

photosynthetic capacity of plants (Rouphael et al., 2017). The chlorophyll content in the present study was similar to that reported in studies on oregano carried out with different aims (Farruggia et al., 2023; Emrahi et al., 2021; Murillo-Amador et al., 2013).

Regarding RWC, foliar application of different biostimulants allowed to achieve an increase in leaf water content during the first year. This result could be linked to the positive effect of biostimulant used on the root growth and nutrients and water uptake (Shafie et al., 2021; Khorasaninejad et al., 2018). Biostimulants improve the absorption, digestion, and translocation of micro- and macronutrients, which is why their use has been linked to improvements the yield and quality of crops (Farruggia et al., 2024b; Aytaç et al., 2022).

Abdali et al. (2023) evaluated the influence of several foliar biostimulants on biomass characteristics traits and the adaptive physiological reactions of oregano under water stress conditions. The authors found that RWC values varied between 46 % and 79 %. In a study carried out in Iran evaluating different varieties of oregano irrigated with varying water levels, Emrahi et al. (2021) obtained RWC values similar to those recorded during the first year of this study. Murillo-Amador et al. (2015) measured percentage values of RWC which were similar to those observed during the second year of the present study. These authors tested oregano plants in different environmental conditions and with different levels of organic fertilisers, obtaining RWC values ranging from 81 % to 86 %.

During the first year, an increase of 4–7 % in leaf and flower percentages was observed in biostimulated plant compared with control plants. During the second year, increased vegetative development caused a change in the percentage distribution of the different portions of the plant in favor of stems. Oregano is a perennial plant that over the years develops its root system and, consequently, increases the vegetative development of the plant (Goliaris, 1997). Greater growth with age of vegetation and the woody portion of the stems determined a total biomass yield of more than double compared with the first year (Ninou et al., 2021). The application of AN and FA produced positive impact on biomass production in both years. During the second year, the yield of plants treated with both products more than doubled compared with control plants. The second year was more effective thanks to the higher age of the plants and to increased rainfall during the biostimulant application period; adequate water accessibility enhances the biomass production and its constituent parts in MAPs (Virga et al., 2020).

During the second year, the application of FA generated the highest fresh and dry biomass yields. It is worth noting that the two doses of fulvic acid led to similar results regarding yields in fresh and dry biomass. Plant growth can be stimulated by several substances contained in these products that acts on metabolism processes by enhancing photosynthetic activity and related compounds (Bulgari et al., 2015). Several authors (Malécange et al., 2023; Bonini et al., 2020) state that biostimulants application has hormone-like effects, such those of auxin and gibberellin, that have a positive effect on biomass yield. Biostimulants contain plant growth regulators that encourage the formation of new structural biomass, and they consent to optimize the uptake, transfer, and absorption of available nutrients from the soil (Gupta et al. 2024; Basile et al., 2021).

Yildiztekin et al. (2018) and Abdali et al. (2023) affirm that the evaporation/transpiration ratio, the production of growth-promoting agents, leaf water potential, root growth, and the oregano plant's ability to absorb water are all impacted by foliar treatment. It has also been shown that the use of biostimulants increases the permeability of cell membranes, allowing potassium to enter and promoting intracellular pressure, cell division, and production (Abdali et al., 2023; Yang et al., 2022).

Nıkou et al. (2019), in a two-year study, found the highest values of fresh biomass yield in the second year. These authors obtained values of biomass yield varying from 7.1 t ha^{-1} to 17.2 t ha^{-1} using different types of organic and mineral fertilisers. In Greece, Dordas (2009) reported values of dry biomass yield which ranged between 2.9 t ha^{-1} to 4.1 t ha^{-1} by applying foliar calcium and magnesium on two oregano varieties.

In Greece, Giannoulis et al. (2020) found flower and leaf dry yields of 1.8 t ha^{-1} and 4.2 t ha^{-1} in oregano plants grown in rainfed conditions and fertilised with different nitrogen levels. Abdali et al. (2023) recorded average dry yields of approximately 1.1 t ha^{-1} in plants treated with amino acids and foliar seaweeds.

The EO content of MAPs is commonly altered by environmental and genetic factors and agronomic practices need to be modified to reduce fluctuations in secondary metabolite production (Mot et al., 2022; Bistgani et al., 2019; Ninou et al., 2017; La Bella et al., 2015). This aspect is important for companies operating on the herbal extract market which need to provide exact quantities. As reported by Amer et al. (2021), biostimulants can affect metabolic pathways and biochemical activity in plants. Some compounds contained in biostimulants modify the route of secondary metabolites, affect plastids and chlorophyll, influence ability to tolerate stress, and change the quantity and the composition of EOs (Alkharpotly et al., 2024).

Considering the results obtained over the two-year tests, the lowest doses of fulvic acid produced the highest EO content and EO yields. Dordas (2009) obtained an EO content of more than 4 % in oregano treated with foliar calcium and magnesium. Similar EO contents were

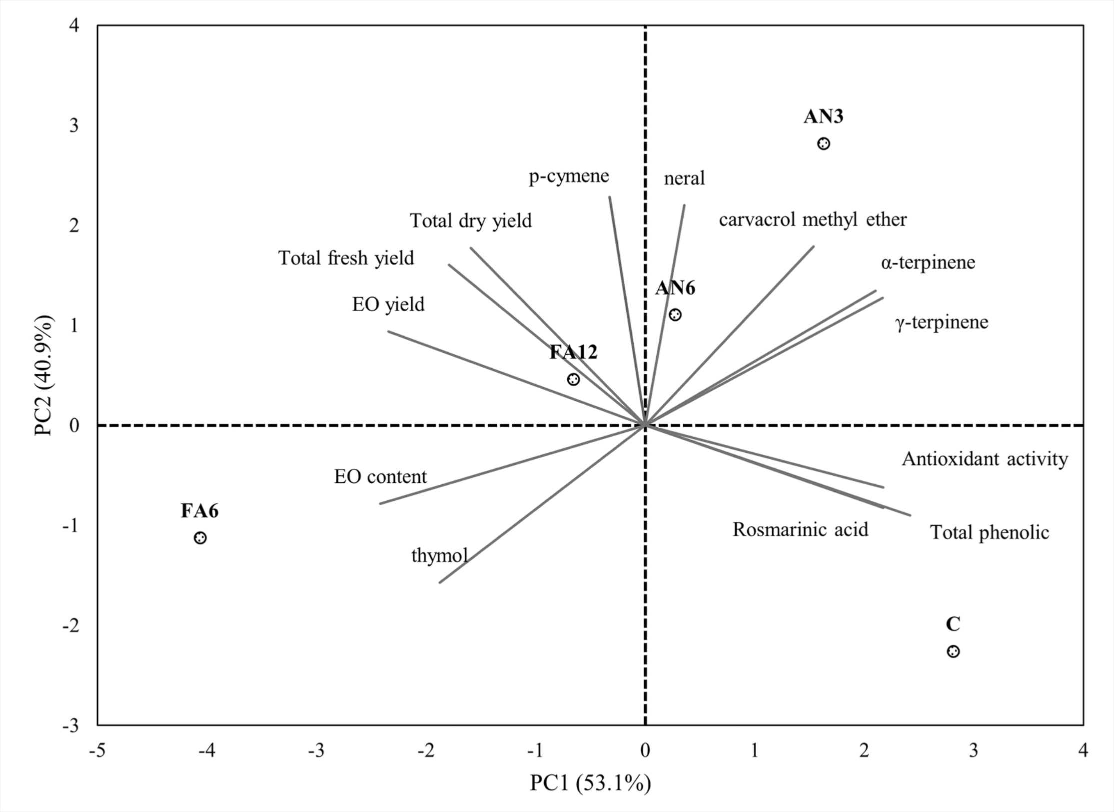

Fig. 4. Graph of principal component analysis (PCA) for productive and chemical parameters and the most represented EO compounds of oregano affected by foliar biostimulant application. AN4 = 4 L ha^{-1} of *A. nodosum*; AN2 = 2 L ha^{-1} of *A. nodosum*; FA8 = 8 L ha^{-1} of fulvic acid; FA4 = 4 L ha^{-1} of fulvic acid.

observed by Król et al. (2020) in oregano fertilised with different nitrogen doses. In addition, these findings are related to those observed by Aytaç et al. (2022) who increased oregano yields by applying different doses of humic substances to the soil. Humic substances improve conditions in the rhizosphere and can promote plant development by improving the content of natural plant growth-promoting hormones, which are related to the EO synthesis (Rahimi et al., 2022). The results are consistent with those observed by Elansary et al. (2019) and Rahimi et al. (2022) which in wild mint (*Mentha longifolia* L.) and in thyme (*Thymus vulgaris* L.), respectively, found an increase in EO content in plants treated with foliar biostimulants compared with control plants. Tawfeew et al. (2016) also observed the highest values of EO content and yield in seaweed extract-treated rosemary plants.

4.3. *Essential oil profile*

In the EO samples analysed in this study, 39compounds were discovered, of which 23 were monoterpenes and 16 sesquiterpenes. The most abundant compounds (over 2.0 % on average) were α-terpinene, *p*-cymene, γ-terpinene, neral, carvacrol methyl ether and thymol. Considering the average values of the two years, biostimulant application did not influence the ratio between monoterpenes and sesquiterpenes compared with control plants.

Many studies have been conducted on the chemical composition and content of oregano EOs, as well as their biological qualities and effects when added to food matrices (Tsitlakidou et al., 2022; Shafiee-Hajiabad et al., 2014). The EO composition is responsable for oregano aroma when used in food preparation (Asensio et al., 2015). In the present study no relevant changes were detected in the composition of EOs obtained from oregano plants treated with biostimulants and, consequently, the aromatic profile of the end-product is not altered.

According to Shahrajabian and Sun (2022), biostimulants enable plants to improve their nutrient uptake, promoting the development and proliferation of glandular trichomes and EO synthesis. Furthermore, these compounds can enhance the photosynthetic activity of EO enzymes and precursors, as reported by Rehman et al. (2016).

4.4. *Chemical parameters*

In general, TPC, AA, and RAC were found to be higher during the first year of study, and the highest values were often recorded in the control; when the highest doses of fulvic acid (FA8) were applied, a total phenolic content similar to that of control plants was generated. The application of the highest dose of both biostimulants produced similar results for antioxidant activity during the first year. Several authors (Rahimi et al., 2022; Saia et al., 2021; Bonini et al., 2020) have shown that the quantity of secondary metabolites rises in many species with exposure to microbial and non-microbial biostimulants. A number of factors produce effects on the secondary metabolite synthesis in MAPs and in oregano plants as well (Wenneck et al., 2023; Farruggia et al., 2023; Tawfeeq et al., 2016; Sharafzadeh, 2012; Figueiredo et al., 2008). Biostimulant application may have an impact on gene regulation and enzyme activity in secondary metabolic pathways (Vosoughi et al., 2018).

4.5. *Principal components analysis (PCA)*

Principal components analysis revealed a complete framework for assessing the impacts of foliar biostimulant treatment on oregano yield and quality properties. AN and FA treatments were clearly separated from control. Foliar biostimulant application negatively affected chemical parameters (total phenols, antioxidant activity, and rosmarinic acid). However, the application of foliar biostimulant produced positive effects in yields and the most represented EO compounds.

5. Conclusions

The creation and application of environmentally friendly practices that may enhance the quality and quantitative elements of crops are essential in the modern day. This study is the first to demonstrate that the application of two different biostimulants, such as seaweed extract and fulvic acid, under non-irrigated conditions can improve the production and chemical properties of Sicilian oregano, offering new solutions for organic farming in arid regions. Morphological traits and yield performance were positively influenced by foliar biostimulant applications. The highest plant growth and biomass yields were measured in plants treated with both doses of fulvic acid and with the lowest dose of *A. nodosum*. During both years, the lowest dose of fulvic acid produced the highest EO contents and, consequently, the highest EO yields. On the other hand, biostimulant treatment resulted in a drop in TPC, AA, and RAC. These results have implications for the agricultural, pharmaceutical, and food sectors. These results suggest that biostimulants should be used as a good practice to maximize crop output in unfavorable growing environments. Therefore, further research and field trials are necessary to investigate deeper how the biostimulants act on MAP primary and secondary metabolism in order to increase yields and quality but limit agronomic inputs.

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CRediT authorship contribution statement

Mario Licata: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Conceptualization. **Giuseppe Di Miceli:** Validation, Supervision, Resources. **Davide Farruggia:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giovanni Urso:** Software, Investigation, Data curation. **Johannes Novak:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Conceptualization. **Claudio Leto:** Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

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.

Data Availability

Data will be made available on request.

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