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Effect of Foliar Treatments with Calcium and Nitrogen on Oregano Yield

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Abstract: Oregano, *Origanum vulgare* L., is a perennial herbaceous plant belonging to the *Lamiaceae* family. Oregano shows variations in biomass yield and essential oil (EO) content due to the influence of abiotic and biotic factors. The aim of this study was to assess the effect of different foliar applications based on calcium (Ca) and nitrogen (N) on morphological and productive parameters in oregano. Tests were carried out in Sicily (Italy) in 2020–2021. In each year, eight foliar applications were applied. Only flowers and leaves were used for the extraction of the EO. For all parameters in the study, except for plant height and inflorescence length, the highest values were found in treated plants with respect to the control. In plants treated with calcium and nitrogen, an increase of between 0.6 and 1.6 t ha^{-1} was observed for fresh yield, and an increase of between 0.5 and 0.9 t ha^{-1} was observed for dry yield. The increase in biomass yield led to an increase in EO yield of between 4 and 12 kg ha^{-1}. The results highlight that foliar treatments with Ca in combination with N enable growth in crop production in environments that show poor water availability.

Keywords: oregano; foliar application; calcium; nitrogen; yield; essential oil

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1. Introduction

Increasing interest in medicinal and aromatic plants (MAPs) has been observed worldwide [1], not only regarding their content of bioactive molecules (important resources for the pharmaceutical and food industries), but also regarding the development of sustainable and multifunctional cropping systems [2]. The Mediterranean area represents the center of diversification for many MAPs, which is a result of different climatic and soil conditions and the high adaptability of these species [3]. In this area, oregano represents one of the most important MAPs [4].

Origanum vulgare ssp. *hirtum* (sin.: *O. heracleoticum* L.) is a perennial herbaceous plant widely distributed throughout Europe and North Africa both as a spontaneous and cultivated plant [5,6]. In addition to the use of fresh and dried leaves and flowers to flavor traditional Mediterranean dishes [6], oregano provides therapeutic benefits for human health due to its antioxidant, antimicrobial and antifungal properties [7–9].

Both in cultivated and spontaneous plants, oregano shows significant variations in essential oil (EO) yield and quality due to the effects of biotic and abiotic factors [10–14]. The development and use of an appropriate cultivation technique can limit these effects, improving the production and qualitative performance [7,15,16].

In the Mediterranean region, oregano is often unirrigated since this area is characterized by prolonged periods of drought [5]. Water scarcity is one of the main factors leading to a decrease in crop production and inducing physiological and biochemical changes in plants. Stomatal closure is the first impact of water stress and causes the inhibition of carbon dioxide absorption and a decrease in photosynthesis; carbohydrate synthesis and distribution to belowground plant parts are negatively affected by water stress [17].

Oregano tolerates water stress due to morphological adaptation [18]. Similar to other MAPs, prolonged periods of water stress during the developmental stage may cause alterations in physiological and metabolic processes [19,20]. According to many authors, limited water availability can have a negative effect on photosynthetic processes and transpiration, leading to a significant fall in growth and yield parameters [5,19].

In addition, low water availability tends to reduce nutrient absorption and translocation to the buds due to the slowing down of the transpiration process; limited water flow can occur while the availability of nutrients, such as potassium (K), nitrogen (N) and calcium (Ca), around the root area is scarce [21].

Therefore, it is essential to obtain nutrient absorption and photosynthetic metabolism in order to improve crop yields.

Nitrogen is a fundamental element for plant nutrition, and it is extremely important for oregano growth [6,16,22,23]. A number of studies carried out both in pots [24] and open fields [16,25–27] showed the positive effect of nitrogen on biomass and EO yields.

As reported in the literature, the application of phosphorus and potassium can influence the production and qualitative parameters of various medicinal and aromatic plants. Potassium is involved in enzymatic activation, photosynthesis and protein synthesis [28,29]. Application of potassium increased biomass yield and the EO content in spearmint (*Mentha spicata* L.) [30] and marigold (*Calendula officinalis* L.) [31].

Phosphorus plays a crucial role in the biosynthesis of primary and secondary metabolites and essential oil synthesis of medicinal plants [32,33]. Kapoor et al. [34] in fennel (*Foeniculum vulgare* Mill.), Trivino and Johnson [35] in marjoram (*Origanum majorana* L.) and Ramezani et al. [33] in basil (*Ocimum basilicum* L.) observed an increase in EO yield with P-fertilization.

Calcium plays a vital role in plant development and growth and plays a structural role in the cell wall and membranes. It is a counter-cation for inorganic and organic anions in the vacuole and is an intracellular messenger in the cytosol [36]. Various studies indicate the importance of Ca^{2+} in the regulation of photosynthesis [37,38]. The function of proteins in the photosynthetic process and their dependence on Ca^{2+} have been studied. In particular, the role of Ca^{2+} in mitigating stress from water scarcity was investigated in mouse-ear cress (*Arabidopsis thaliana* (L.) Heynh) [39] and corn (*Zea mays* L.) [40].

Like other elements, Mg takes part in the photosynthesis process and in the synthesis of nucleic acids and adenosine triphosphate in plants [41–43]. The availability of Mg can mitigate metabolic alterations and increase yield and quality in crops [44,45].

As mineral nutrition is essential for oregano growth, it is possible to increase biomass and EO yields by foliar application of calcium and nitrogen. The aim of this study was to assess the effect of foliar fertilization with different levels of calcium, alone and in combination with nitrogen, on plant height, inflorescence length, chlorophyll content, relative water content (RWC), total fresh yield, total dry yield, inflorescence dry yield, leaf dry yield, stem dry yield, EO content and EO yield of an accession of Sicilian oregano.

2. Materials and Methods

2.1. Experimental Site and Plant Material

Tests were carried out in 2020/2021 at a local farm in Alia (Sicily, Italy) (560 m a.s.l., 37°44′12.61″ N, 13°44′43.77″ E Google Earth). The soil was classified as clay soil (58% clay, 22% silt, 20% sand) with a pH of 7.20, electrical conductivity of 0.73 dS m^{-1}, organic matter content of 1.8%, C content of 10.2 g kg^{-1}, N content of 0.8 g kg^{-1}, P of 231 mg kg^{-1} and K of 488 mg kg^{-1} (USDA classification: typic chromoxerert fine).

A local ecotype, named "Villaba", previously classified as *Origanum vulgare* ssp. *hirtum* (sin.: *O. heracleoticum* L.), was used for the tests.

A low-input cultivation technique was carried out under rainfed conditions, in accordance with commonly used practices in Sicily for oregano. Agamic propagation was accomplished by dividing the bushes. The plants were set into the ground at the beginning of spring 2018 with planting distances of 2.20 × 0.50 m.

Before transplanting, organic fertilization was carried out: 2 t ha^{-1} of manure was distributed and buried at an approximate depth of 40 cm. No pesticides were used, and weed control was carried out by surface tillage at the beginning of spring and before harvesting.

2.2. Weather Data

Data on rainfall and air temperature were recorded at a weather station belonging to the Sicilian Agro-Meteorological Information Service [46]. The station is located approx. 600 m from the experimental field. The station is equipped with a datalogger and various sensors for the measurement of air temperature (TAM platinum PT100 sensor, heat resistance with anti-radiation screen) and total rainfall (PPR sensor with tilting bucket rain gauge). Data regarding average daily maximum and minimum temperatures (°C) and total decadal (10-day period) precipitation (mm) have been taken into consideration.

2.3. Foliar Treatments

During the tests, 8 foliar applications (FAs) of different levels of calcium and calcium in combination with nitrogen were made. Calcium was applied in the form of CaO (containing 48% Ca), and nitrogen was applied in the form of urea (containing 46% N). The first treatment was applied at the onset of stem elongation in both years during the first week of April; the others were carried out weekly up to 15 days before harvest. In comparison with the control treatment (FAO), which provided water only, the following treatments were tested: CaO 8 g L^{-1} (FA1); CaO 12 g L^{-1} (FA2); CaO 8 g L^{-1} + urea 16 g L^{-1} (FA3); CaO 12 g L^{-1} + urea 16 g L^{-1} (FA4). A portable sprayer with an operating pressure of 250 kPa was used, and 1200 L of water ha^{-1} was applied for each treatment.

2.4. Plant Measurement

During the test period, the main plant measurements were taken between vegetative growth and harvest. During the cycle, plant height and chlorophyll content (SPAD unit) were measured. At harvest, relative water content (RWC), inflorescence length, total fresh yield, total dry yield, inflorescence dry yield, leaf dry yield, stem dry yield, EO content and EO yield were determined.

The chlorophyll content was measured using a portable double wavelength meter (SPAD 502, chlorophyll meter, Minolta Camera Co., Ltd., Osaka, Japan). Thirty fully developed leaves were used per plot. The instrument automatically averaged these readings.

The relative water content of the leaves was estimated by taking leaf discs (5 mm diameter) from fresh leaf tissue. After the initial fresh weight (FW) was recorded, the leaf discs were floated in distilled water for 1 h to record the turgid weight (TW). Subsequently, the leaf discs were dried in an oven for 24 h and the dry weight (DW) was recorded [47]. The RWC was calculated using the following equation:

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

2.5. Collection of Plant Material

In both years, plants were harvested during blooming. Plants were cut at 5 cm above ground level and then dried in a shaded and ventilated environment for about 10 days at a temperature of 25–30 °C. The plant material was manually separated into stems, leaves and flowers. The stems were not examined in the subsequent analysis due to their low EO content. EO extractions include only the dried flowering tops (flowers and leaves) of the plants.

2.6. Essential Oil Extraction

EO content was obtained by hydrodistillation of 100 g of dried leaves and flowers. Extraction was carried out until increases in EO concentrations were no longer observed (approximately 3 h). The EO samples were stored at 4 °C.

2.7. Experimental Design

A randomized complete block design with three replicates was adopted. Each block comprised 5 plots of 3.3 m^2. Treatments (FA0-FA5) were applied to each parcel randomized in the block. The plots were well spaced in the block; plastic panels were used to delimit each plot and to avoid drift during foliar applications.

2.8. Statistical Analysis

Biometric and production data were compared using analysis of variance (ANOVA). Statistical analysis was performed using MINITAB 19 for Windows. Data on plant height and chlorophyll content were analyzed using repeated measures ANOVA. However, data relating to single dates of observations were subjected to one-way ANOVA. The difference between the means was determined using Tukey's test ($p \leq 0.05$). The percentage data for RWC were subjected to arc-sine transformation.

3. Results

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

Total annual rainfall was 424 mm in 2020 and 642 mm in 2021. During the period February–May 2020, rainfall was 135 mm, of which 98 mm was distributed in the second 10 days of March (Figure 1).

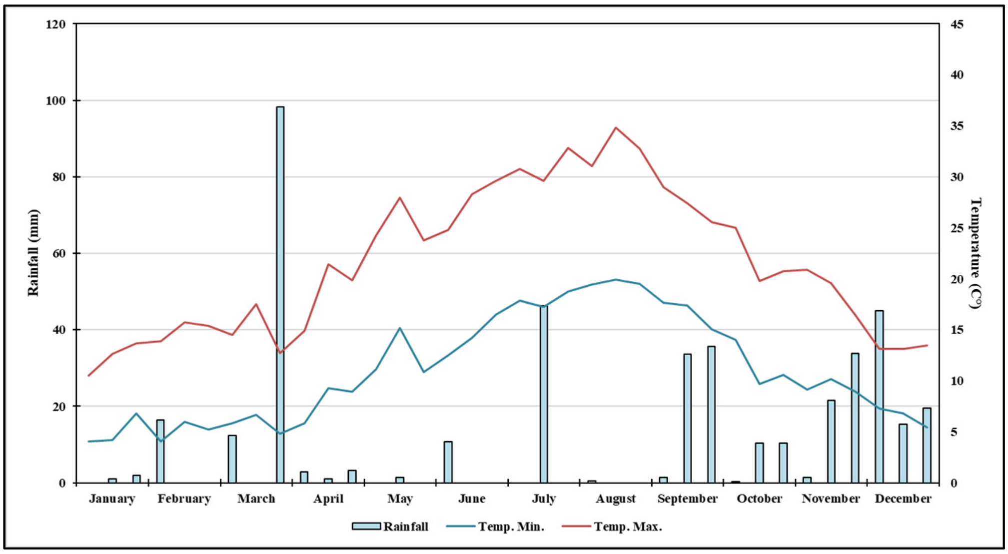


Figure 1. Rainfall and temperature trends in the year 2020.

A rainfall of 153 mm, with a more uniform distribution, was observed during the same period in 2021 (Figure 2).

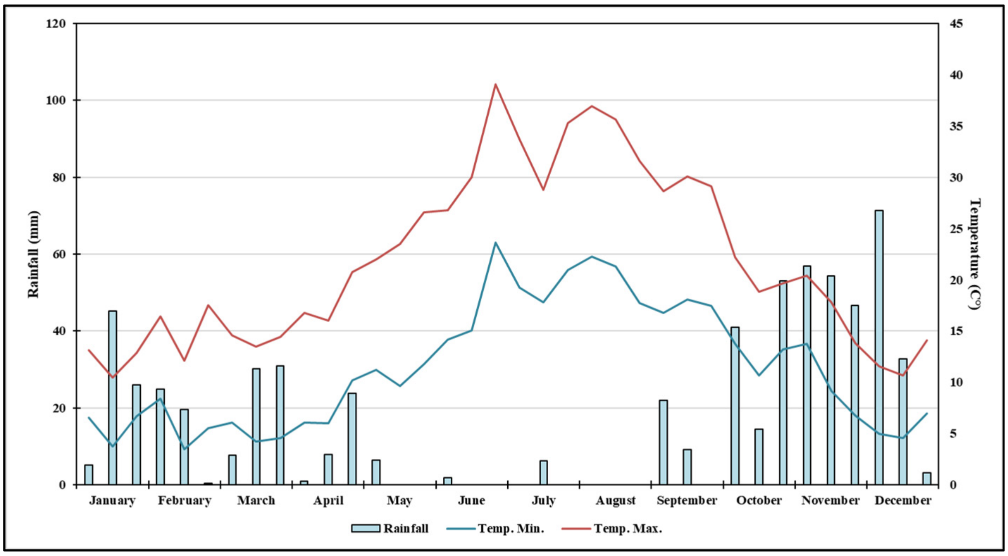


Figure 2. Rainfall and temperature trends in the year 2021.

In both years, average minimum temperatures were below 10 °C until the end of April, increasing thereafter. Furthermore, over the two years, average maximum temperatures showed a similar trend up to the first 10 days of May. A drop in the average maximum temperature was then observed during the second and third 10 days in May 2020, from 28 to 24 °C, rising to approx. 30 °C immediately thereafter. However, in the same period of 2021, average maximum temperatures increased steadily, reaching approx. 30 °C at harvest time.

3.2. *Effects of Year and Foliar Treatment on Morphological and Yield Parameters of Oregano*

The results of ANOVA showed that the year did not determine significant effects on plant height, inflorescence length, chlorophyll content and RWC. The year factor showed significant differences ($p \leq 0.01$) regarding the following parameters: total fresh yield, total dry yield, inflorescence dry yield, leaf dry yield, stem dry yield, EO content and EO yield (Table 1).

Table 1. Effects of year (Y), foliar application (FA) and their interaction (Y × FA) on morphological and yield parameters.

	Plant Height (cm)	Inflorescence Length (cm)	Chlorophyll Content (SPAD)	RWC (%)	Total Fresh Yield ($t\ ha^{-1}$)	Total Dry Yield ($t\ ha^{-1}$)	Inflorescence Dry Yield ($t\ ha^{-1}$)	Leaf Dry Yield ($t\ ha^{-1}$)	Stem Dry Yield ($t\ ha^{-1}$)	EO Content ($\%_{w/w}$)	EO Yield ($kg\ ha^{-1}$)
Year (Y)											
2020	n.s.	n.s.	n.s.	n.s.	4.13 B	2.19 B	0.58 B	0.58 B	1.03 B	1.60 B	18.63 B
2021	n.s.	n.s.	n.s.	n.s.	4.87 A	2.68 A	0.79 A	0.73 A	1.17 A	1.78 A	27.38 A
F	0.41	0.05	1.74	0.81	17.75	51.09	41.90	49.88	17.34	20.70	27.65
p-value	0.53	0.82	0.20	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Foliar Application (FA)											
FA0	n.s.	n.s.	42.6 B	80.0 C	3.48 C	1.82 C	0.49 B	0.53 B	0.79 C	1.60 B	16.48 B
FA1	n.s.	n.s.	48.8 A	88.8 A	4.14 BC	2.31 B	0.58 B	0.68 A	1.05 B	1.55 B	20.17 B
FA2	n.s.	n.s.	48.8 A	85.9 B	5.11 A	2.70 A	0.76 A	0.73 A	1.21 A	1.60 B	24.55 A
FA3	n.s.	n.s.	50.2 A	86.6 AB	5.02 A	2.73 A	0.82 A	0.68 A	1.25 A	1.89 A	28.42 A
FA4	n.s.	n.s.	51.0 A	88.1 AB	4.73 AB	2.60 AB	0.75 A	0.64 A	1.21 A	1.82 A	25.40 A
F	0.25	1.91	16.54	31.31	11.94	24.51	14.46	10.35	25.07	12.14	9.31
p-value	0.91	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Y × FA											
F	0.49	1.37	1.19	23.42	1.09	2.74	0.50	4.16	3.35	18.62	44.23
p-value	0.74	0.28	0.34	0.00	0.39	0.36	0.74	0.013	0.03	0.00	0.00

FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with different letters are significantly different at $p \leq 0.05$. n.s. = not significant.

Foliar application (FA) had significant effects ($p \leq 0.01$) on most of the parameters in the study except for plant height and inflorescence length (Table 1).

Treated plants (FA1–FA4) showed a significant increase in chlorophyll content compared to the control test (FA0). The FA4 treatment, although not different from FA1, FA2 and FA3, recorded the highest value with 51.0 SPAD units (Table 1).

At harvest, the highest RWC value was observed in FA1 (88.8%), followed by FA4 (88.1%) and FA3 (86.6%), and the lowest in FA0 (80.0%) (Table 1).

All foliar application levels (FA1–FA4) resulted in increased total fresh and dry yields compared to the control (Table 1). The best yield performances were recorded for FA2, FA3 and FA4 with average values of approximately 5 t ha^{-1} of total fresh biomass and 3 t ha^{-1} of total dry biomass. These results were then followed by FA1; however, it displayed a value of 4.14 t ha^{-1} for biomass fresh yield, which was statistically similar to the control (3.48 t ha^{-1}). Inflorescence dry yield and stem dry yield followed the same trend (Table 1). Regarding leaf dry yield, all treatments (with values between 0.64 and 0.73 t ha^{-1}) were significantly different from the control (0.53 t ha^{-1}) (Table 1).

The highest EO content was found in plants treated with FA3 (1.89%) and FA4 (1.82%), statistically different from FA1 (1.55%), FA2 (1.60%) and the control (1.60%) (Table 1).

The highest EO yields were obtained from plants treated with FA2, FA3 and FA4, with values between 24.5 and 28.4 kg ha^{-1}. Lower EO yields (values below 20 kg ha^{-1}) were observed in plants treated with FA1 and FA0 (Table 1).

The interaction between year (Y) and foliar application (FA) significantly affected ($p \leq 0.01$) RWC, EO content and the EO yield. Other significant differences ($p \leq 0.05$) were also found for leaf dry yield and stem dry yield (Table 1).

The highest RWC was observed in plants treated with FA1 during 2020, statistically not different from FA2 and FA4 during the same year, and FA3 during 2021. The lowest RWC value was found in FA0 during the first year (Figure 3). During the second year, values ranging from 85% (FA0–FA2) to 87.5% (FA3) were found (Figure 3).

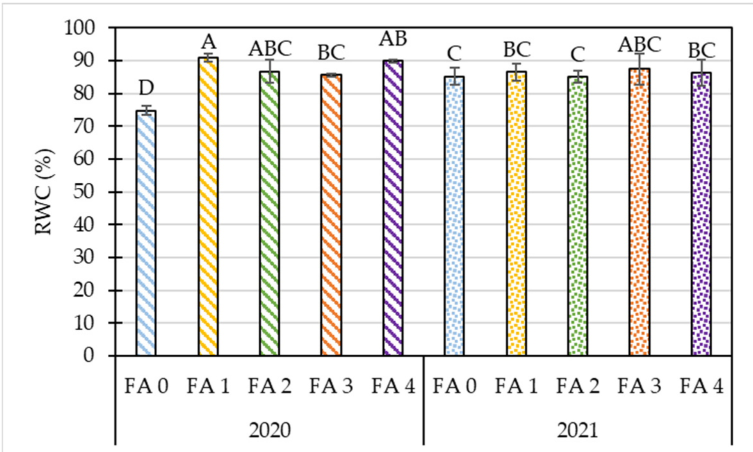


Figure 3. Influence of interaction Y × FA on relative water content (RWC). FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with different letters are significantly different at $p \leq 0.05$ according to Tukey's test. Bars represent the standard deviation.

In 2021, leaf dry yield was higher in all treatments except for the control, which did not show variation over the two years. In 2020, in addition to FA4, FA0 recorded the lowest leaf dry yield. This is then followed, in ascending order, by FA1, FA3 and FA2, although these treatments were statistically equal (Figure 4). During the two years, plants treated with FA2 maintained the same apical position.

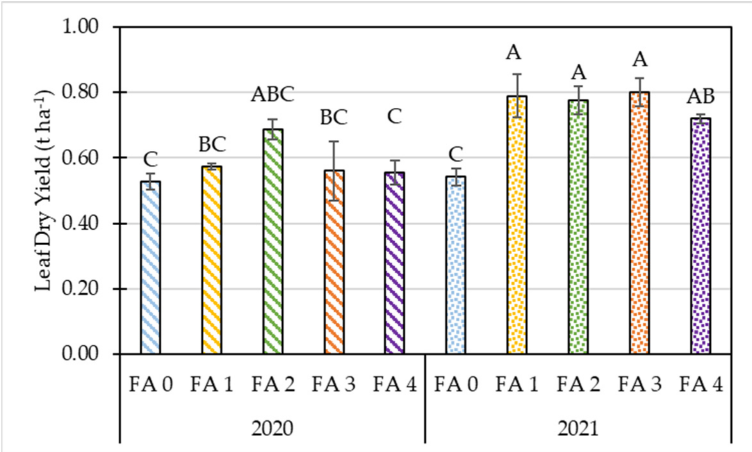


Figure 4. Influence of interaction Y × FA on leaf dry yield. FA 0: only water; FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with different letters are significantly different at $p \leq 0.05$ according to Tukey's test. Bars represent the standard deviation.

Stem dry yield had a similar trend to leaf dry yield. FA2, FA3 and FA4, although with lower values recorded in 2020, showed no significant differences over the two years (Figure 5).

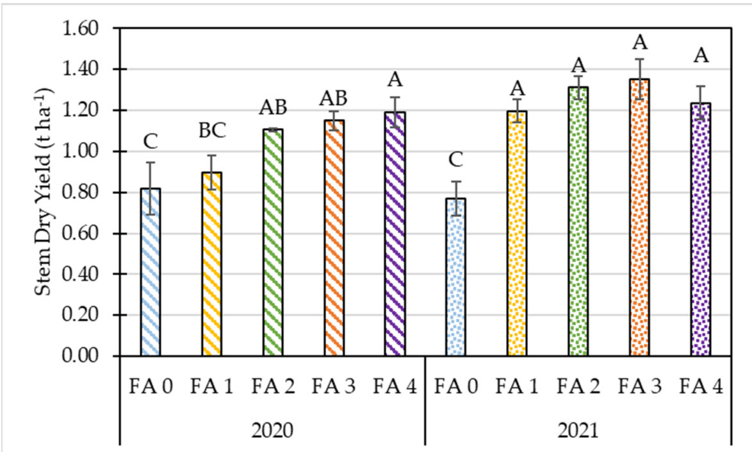


Figure 5. Influence of interaction Y × FA on stem dry yield. FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with different letters are significantly different at $p \leq 0.05$ according to Tukey's test. Bars represent the standard deviation.

Regarding the EO content, the highest values were observed in 2020 in plants treated with FA0, FA3 and FA4 and in 2021 in plants treated with FA1, FA2, FA3 and FA4, with peaks of 1.95%. The lowest values (between 1.33% and 1.43%) were obtained in FA1 and FA2 in 2020 and FA0 in 2021 (Figure 6).

The highest EO yields were obtained in 2021, with values of between 26.6 and 33.5 kg ha^{-1}, excluding the control; the lowest values were observed in FA0, FA and, FA2 during the first year, not statistically different from FA0 during the second year (Figure 7).

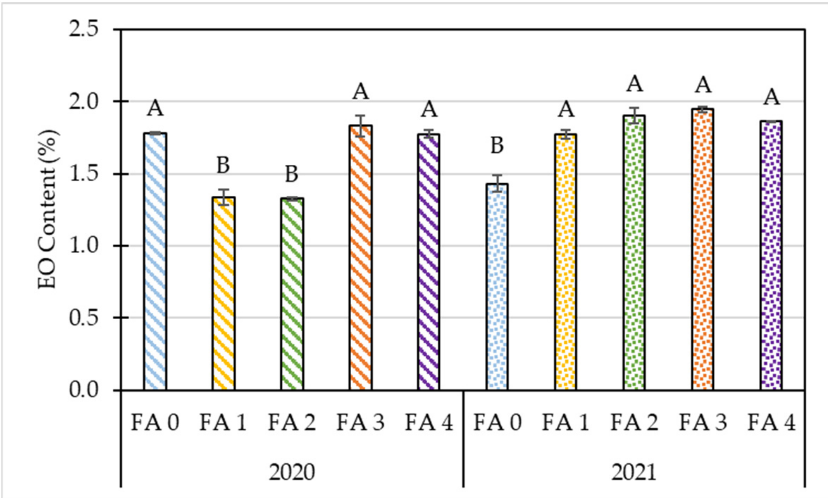


Figure 6. Influence of interaction Y × FA on essential oil (EO) content. FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with different letters are significantly different at $p \leq 0.05$ according to Tukey's test. Bars represent the standard deviation.

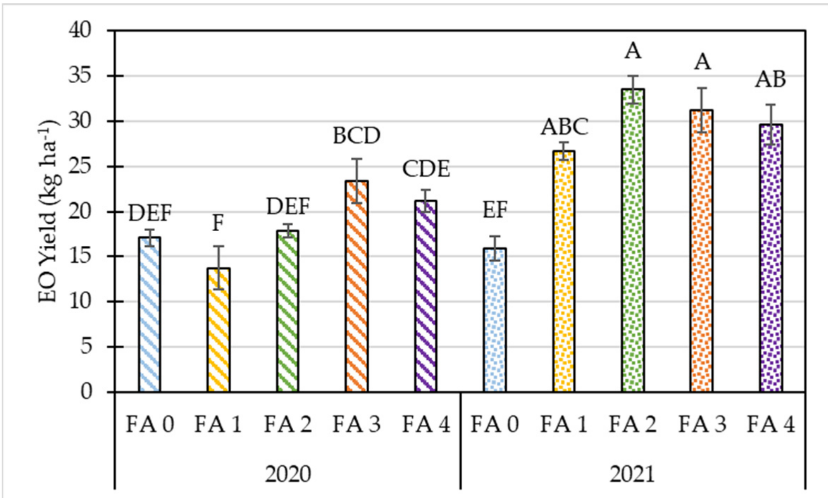
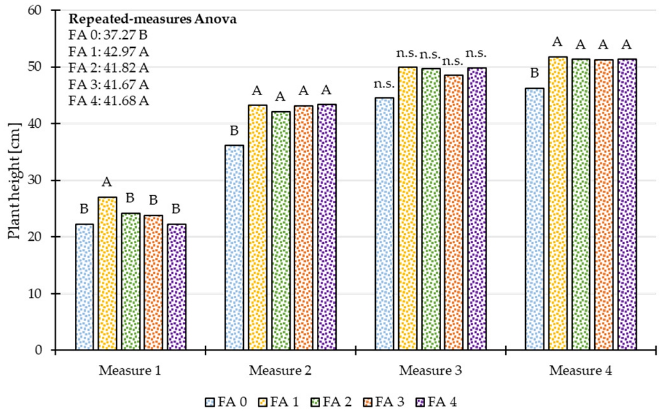


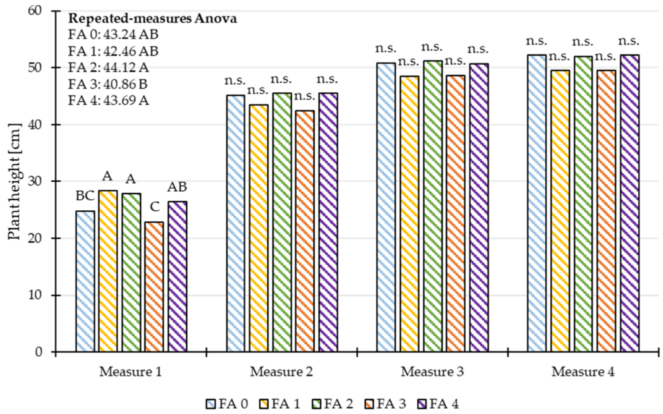
Figure 7. Influence of interaction Y × FA on Essential Oil (EO) Yield. FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with diverse letters are significantly different at $p \leq 0.05$ according to Tukey's test. Bars represent the standard deviation.

3.3. Repeated Measures ANOVA of Plant Height and Chlorophyll Content

Repeated measures ANOVA for plant height and chlorophyll content was performed to analyze the effect of foliar applications and sampling time. Regarding plant height, significant differences were found already by the first observation date; however, they were found in different ways over the two years (Figure 8).



a



b

Figure 8. Plant heights in 2020 (**a**) and 2021 (**b**). FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. n.s. = not significant. Values with different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

At each observation date, in 2020, plant height showed significant differences based on foliar applications (Figure 8a). Regarding the first date, the plants in test FA1 reached the greatest height. At subsequent observation dates, significant differences between the various foliar applications (FA1, FA2, FA3, FA4) and the control (FA0) were observed (Figure 8a). When considering all time periods, repeated measures ANOVA also showed a significant gap between the control (FA0 = 37, 27 cm) and the other foliar applications, ranging between 41 and 43 cm (Figure 8a).

In 2021, the highest plant height was found in FA1, FA2 and FA4, while the lowest was observed in FA3 and FA0. Regarding other observation dates, no significant differences were found (Figure 8b). Considering all time periods (repeated measures ANOVA), the greatest plant heights were observed in FA2 (42.46 cm) and FA4 (43.69 cm), though they were not statistically different from FA0 (43.24 cm) and FA1 (42.46 cm); the lowest was observed for FA3 (40.86 cm) (Figure 8b).

Regarding chlorophyll content, from the second observation until the final one in 2020, higher content was recorded for the different foliar applications (FA1, FA2, FA3, FA4) compared to the control (FA0) (Figure 9a). From the first observation, plants treated with FA3 were different from those treated with FA1, FA2 and FA0; treatment FA4 showed an intermediate value (Figure 9a).

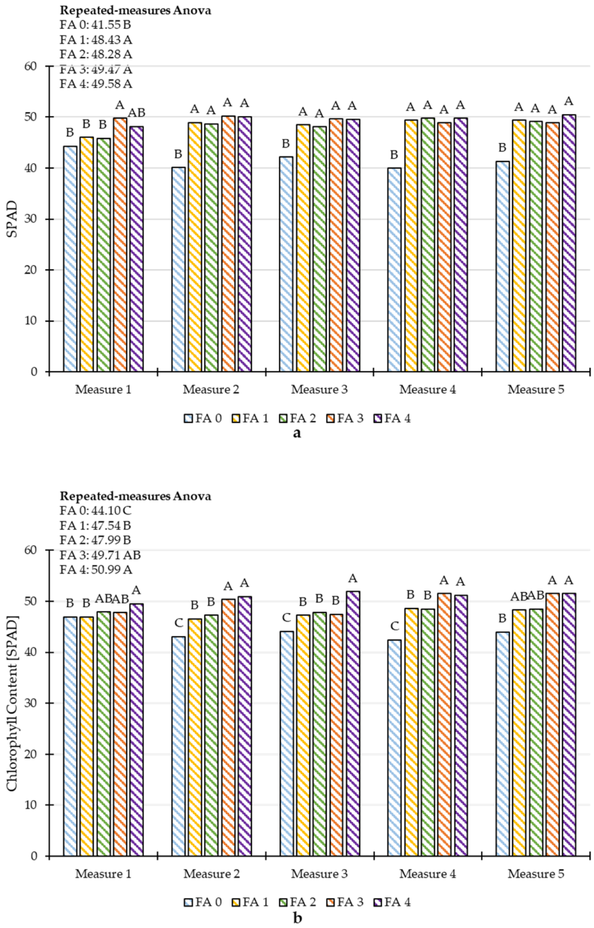


Figure 9. Chlorophyll content (SPAD) in 2020 (**a**) and 2021 (**b**). FA0: only water; FA1: 8 g L^{-1} of CaO; FA2: 12 g L^{-1} of CaO; FA3: 8 g L^{-1} of CaO + 16 g L^{-1} of urea; FA4: 12 g L^{-1} of CaO + 16 g L^{-1} of urea. Values with different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

When considering all time periods in 2020 (repeated measures ANOVA), the lowest chlorophyll content was recorded for FA0 (41.55 SPAD units) and differed from other foliar applications (range: 48.28–49.58 SPAD units) (Figure 9a).

From the first measurement in 2021, lower chlorophyll content was recorded for FA0 and FA1, and the highest chlorophyll content was recorded for FA4 (47.75 SPAD units) (Figure 9b). The same trend was found in repeated measures ANOVA; the lowest chlorophyll content was found in FA0 (44.10 SPAD units), while FA3 (49.71 SPAD units) and FA4 (50.99 SPAD units) produced the highest values (Figure 9b).

4. Discussion

In this study, the effects of calcium and nitrogen foliar applications on the agronomic and productive response of a local Sicilian population of *O. vulgare* under rainfed conditions were assessed.

The year factor had significant effects on all yield parameters, excluding plant height, inflorescence length and chlorophyll content. As highlighted by other authors [5,22,36], lower production values (obtained during the first year of cultivation) could be linked to differing rainfall distribution and lower water availability during the crop's greatest water requirement period. However, weather conditions in 2021 appeared more favorable for the cultivation of oregano due to improved rainfall amounts and distribution. In the Mediterranean area, MAPs are often grown without irrigation due to limited water resources [5].

The use of foliar fertilization can regulate and improve the physiological and metabolic processes of crops in order to improve nutrient absorption and use efficiency, stress tolerance and qualitative aspects of production [48–50]. Nitrogen fertilization has a direct effect on the yield and quality of many crops and must be modulated to ensure proper development of the plant and the profitability of agricultural activity, thereby avoiding waste [25,51,52]. In this regard, foliar application represents a useful method of nitrogen fertilization, avoiding nutrient loss and leaching in the soil and the consequent pollution of water bodies and soils [26,53]. The application of macroelements plays an important role in the management of crop systems; however, microelements, such as calcium, also play vital roles in promoting plant growth and development [54]. Calcium can improve tolerance to plant water scarcity and affect metabolism regulation [55].

In our study, all factors showed no significant effects on plant height and inflorescence length. However, these parameters are influenced by environmental conditions, cultivation methods [56–60] and irrigation [61–63]. In all experimental treatments, plant height was approximately 50 cm while inflorescence length was recorded at between 11 and 14 cm. In a similar environment, Virga et al. [5] obtained oregano plants with greater plant heights (from 51 to 68 cm) with the use of unconventional water. In different environments, Dordas [64] recorded oregano plants with greater height (57 cm and 70 cm) when applying calcium and magnesium through foliar treatments. Other authors [65] observed lower plant heights with soil organic fertilizers. Krol et al. [23] recorded plant heights of between 24 and 35 cm when applying increasing doses of N to the soil; higher values were observed in plants that were fertilized with increasing nitrogen doses. Sotiropoulou et al. [25] recorded plant heights of between 40 and 50 cm when applying different levels of nitrogen fertilization.

Chlorophyll content was positively influenced by different levels of foliar applications; calcium treatments and those in combination with nitrogen differed from the control, which resulted in the lowest values. The results found in this study are in accordance with those obtained by Dordas [64], who observed an increase in chlorophyll content compared to untreated plants when applying different doses of calcium through foliar treatments. In general, this author [64] obtained values that were 10% lower than those obtained in our study, both regarding the control and treated plants. The highest chlorophyll content (found in FA3 and FA4) may be due to nitrogen, which improves chlorophyll content, enzyme content and enzyme activity [66]. Furthermore, the application of Ca allowed us to obtain higher chlorophyll values than those found for untreated plants. Calcium has limited mobility inside the plant as it is blocked in the vacuoles of radical cells [67]. Calcium is moved only by xylematic means, and its availability is closely linked to the availability of water [68]. Foliar spraying makes calcium immediately available to the plant as the element penetrates through the stomata [69] and positively affects photosynthetic activity [70].

Emrahi et al. [71] found significant decreases in SPAD readings in plants subjected to severe water stress (32–35 SPAD units) compared to irrigated plants (45–48 units SPAD); this chlorophyll content of irrigated plants is similar to that obtained in this study in treated plants. Murillo-Amador et al. [56] obtained lower SPAD values (between 34 and 37 SPAD units) in Mexico; the authors cultivated *O. vulgare* in an open field and in shady conditions and administered organic fertilizers. Murillo-Amador et al. [65] also obtained similar SPAD readings in *O. vulgare* and thyme (*Thymus vulgaris* L.).

Water deficiency is the most important of the abiotic stress factors and negatively affects the development of crops [72]. Water stress causes significant changes in the morphological, physiological and biochemical properties of plants. As reported in the literature, drought stress causes the closure of stomata, a decrease in leaf water potential and turgor pressure, and a reduction in cell division, photosynthetic activity and plant biomass production [73,74].

An adequate RWC of plant tissues is a fundamental aspect in the modulation of stomatic conductance and photosynthetic activity [71]. Alterations in the water balance cause molecular changes, growth retardation and, in some cases, death of plant tissues [75,76]. The year factor did not significantly affect RWC, while foliar application of the different levels of Ca, and these in combination with nitrogen, causes an increase in RWC compared to untreated plants. According to our findings, several authors [77–79] reported that RWC decreases with poor water availability. In this study, the lowest RWC was found in both years in the control plants and in the less rainy year. In two subsp. of oregano grown in Iran, Emrhai et al. [71] found RWC values of between 51 and 59% when applying severe and moderate water stress. Morshedloo et al. [80] found RWC values similar to those obtained in this study in two different populations of oregano subjected to moderate water stress. The application of the lowest dose of Ca (FA1), and the same in combination with nitrogen (FA3), produced the highest values of RWC in the years 2020 and 2021, respectively. Improvements in plants' water conditions through the administration of Ca were observed in other species, and several authors [40,81] confirm the metabolic advantages provided by calcium applications.

In MAPs, yield and EO content are influenced by endogenous and exogenous factors [14,82–84]. The agronomic technique is one of the aspects which can be managed to improve quantitative and qualitative essence parameters [15,85,86]. As evidenced by many authors [5,16,22,23,25], the application of N in oregano may have positive effects on biomass production, EO content and EO yield.

In this study, production parameters were influenced by year and foliar treatments. The second year (2021) was more productive due to greater rainfall and good rain distribution; optimal water availability improves the production of biomass and its components in MAPs [5]. Calcium-based foliar applications, and the combination of these with nitrogen, increased dry and fresh biomass yield, flower yield, leaf yield and stem yield compared to unfertilized plants. The best productive performances were obtained by using calcium doses in combination with nitrogen, in particular, FA3. The application of calcium and nitrogen affects meristems and the synthesis of plant constituents and carbohydrates [87,88].

The results of this study agree with those of other authors [16,22,27,64,89] who found increases in production parameters upon application of various types of fertilizers, both foliar and radical. In Greece and under similar growing conditions, Dordas [64] and Giannoulis et al. [22] obtained slightly higher dry biomass yields. This could be linked to lower rainfall at the experimental site during the growing season.

The highest percentage of EO was found in 2021 with Ca applications in combination with nitrogen (FA3 and FA4). Virga et al. [5], under irrigated conditions, obtained EO contents of more than 2% and EO yields that were far higher than those found in this study due to increased water availability and consequently higher biomass production. Dordas [64] also obtained higher EO contents than those obtained in this study. Krol et al. [23], however, obtained lower EO content by applying different doses of nitrogen. In this study, EO yield increased with foliar applications of calcium in combination with nitrogen in relation to a greater biomass yield; as highlighted in the literature [64,90,91], the increase in biomass production is linked to the effect of the two elements.

5. Conclusions

The results of this study highlight that foliar application of calcium in combination with nitrogen represents a promising practice for growing oregano in areas with poor water availability. Foliar application represents a useful means of fertilization and enables improvements in the production and qualitative parameters of oregano. In particular, the application of the highest dose of calcium and the applications of the two doses of calcium in combination with nitrogen allowed obtaining an increase in biomass yield and EO yield. Further research is required to assess the effects of foliar application of nutrients on the morphological and productive characteristics of oregano and other medicinal and aromatic plants.

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