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Plant Biostimulants Increase the Agronomic Performance of Lavandin (*Lavandula x intermedia*) in Northern Apennine Range

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Abstract: Lavandin (*Lavandula x intermedia*) belongs to the *Lamiaceae* family and is a shrub cultivated in the Mediterranean region for essential oils used to produce cosmetic, aromatherapy, and pharmaceutical ingredients. Nowadays, interest in plant biostimulants is rising due to their ability to increase biomass production in a sustainable way. The scope of the present study was to assess the effects of two plant biostimulants, one based on hydrolyzed proteins (FITOSIM®) and one based on seaweed extracts (FITOSTIM ALGA®), on the cultivar Grosso for two consecutive years in three different farms, located in the Italian Tuscan-Emilian Apennine Mountains. A difference in the efficiency of treatments among farms was shown, probably due to the plant age. In 2020, FITOSTIM ALGA® increased inflorescence fresh weights (+35%), while FITOSTIM® and FITOSTIM ALGA® enhanced stem and total fresh weights (+23% and +22%, respectively) compared to the untreated control. In 2021, both treatments enhanced the fresh and dry weights of inflorescence (+47% and +38%, respectively), while FITOSTIM ALGA® also improved the total plant dry weights (+34%). The plant biostimulants did not affect the chemical composition of essential oils. Our results indicate plant biostimulants as a supplement for sustainable management practices, enhancing Lavandin's performance in mountainous agricultural areas.

Keywords: sustainability; plant biostimulant; biofertilizer; fertilization; lavandin; *Lamiaceae* family; agronomic parameters; essential oil

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1. Introduction

Aromatic plants of the genus *Lavandula* are native to the Mediterranean region and now widespread globally [1]. The genus belongs to the *Lamiaceae* family, which has 39 species and nearly 400 cultivars, including annual and perennial herbaceous or shrub crops [2,3]. The major species are lavender (*Lavandula angustifolia* Mill.), spike lavender (*Lavandula latifolia* L.), and lavandin (*Lavandula x intermedia* Emeric ex Loisel.), a sterile hybrid obtained by crossing of *L. angustifolia x L. latifolia* [4]. In Italy, the area of cultivation is widespread, from the littoral regions to the Apennines, and the optimal altitude for having a good aromatic profile is around 800 m a.s.l. [5]. Lavender and lavandin are perennial crops with a cycle that can last from 12 to 15 years. Both crops grow in slope, calcarean, well-drained, and stony soils with full sunlight. Plants of the *Lavandula* genus are among the most cultivated aromatic species in Europe [6]. Besides their use as ornamental plants, *Lavandula* herbs are cultivated for the production of essential oils (EOs) and resins, due to the richness of secondary metabolites, such as linalyl acetate, camphor, linalool, lavandulyl acetate, and lavandulol, in their flowers [7]. Resins and EOs have different uses: perfumes, cosmetics, medicines, pharmaceuticals, and insecticides [8].

Lavandin is characterized by vigorous growth, and greater rusticity and yields than lavender [5]. Among lavandin varieties, the genotype 'Grosso' is well suited for cultivation in marginal areas, such as the north-central Apennines Mountains [9]. The global production of lavandin is largely concentrated in France, where it occupies an area of 16,000 hectares, producing 1000 tons per year, which is 90% of the worldwide market [10].

Modern agriculture needs to modify the management of crops in a sustainable way, reducing environmental risks and the use of non-renewable resources [11]. In mountain environments, where landscape variation is evident along with altitude, the agroecological management of farms involves numerous challenges [12]. Additionally, mountain areas are fragile environments, which are suffering more than others the impacts of climate changes [13,14]. Despite physical and climatic difficulties, mountain agriculture may contribute to the production of quality goods and services for humans and the environment [15]. In this scenario, plant biostimulants have been proposed as sustainable management tools to overcome abiotic stresses, maintaining and/or improving the crop yield and quality [16,17].

Plant biostimulants in agriculture are defined as fertilizers able to stimulate the plant nutrition processes by enhancing nutrient use efficiency, to increase tolerance to abiotic stresses, and to improve the quality of production [18,19]. Plant biostimulants are products based on natural raw materials, such as hydrolyzed proteins and amino acids from animal and plant byproducts, microalgae and seaweed extracts, humic substances, plant extracts, and microorganisms [20].

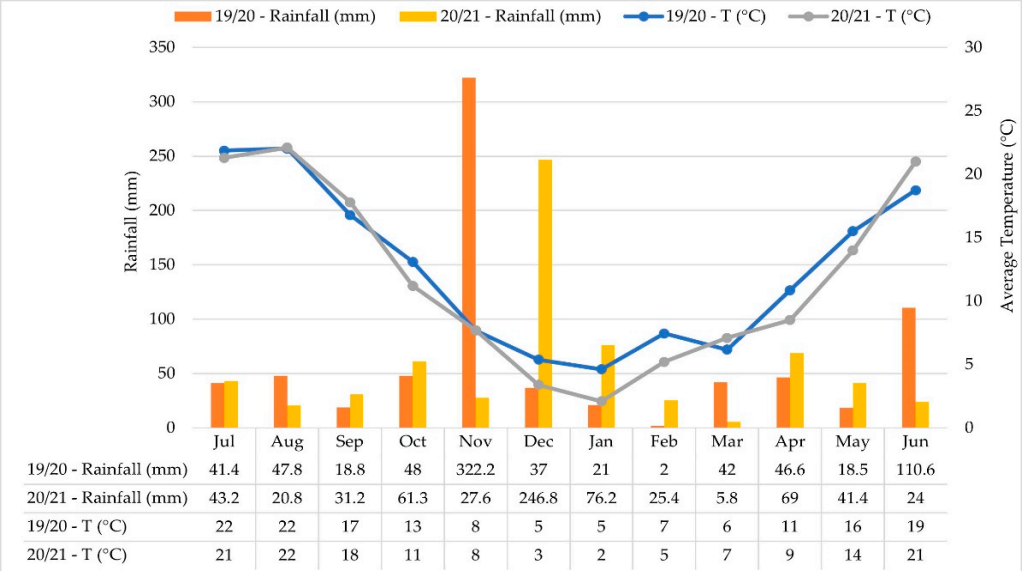
In the literature, there are many examples of the positive effects of plant biostimulants on diverse crops, and in a previous study, our group demonstrated the effects of plant biostimulants on the quality and chemical composition of EOs extracted from lavandin cultivated in the Tuscan-Emilian Apennines [21]. Foliar applications of amino acids and seaweed-based extracts did not induce significant changes in the relative abundance of the main mono- and sesquiterpenes of the EOs. Conversely, the yield of EOs per plant was increased after the application of seaweed extracts [21]. Plant biostimulants based on seaweed and plant extracts were tested on cuttings of lavender, showing beneficial effects on root formation and branching [22]. The positive effects were ascribed to the contents of amino acids, salicylic acid, sterols, and gibberellins inside the plant biostimulant products [23,24]. Tanase et al. [25], testing spruce and beech bark extracts on lavender from the sowing to the flowering stages, noticed that the extracts had phytohormone-like activity effects comparable to those of auxins and cytokinins. Foliar applications of microalgae extracts on lettuce showed an improvement of the photosynthetic activity by stimulating the biosynthesis of chlorophyll and carotenoid pigments [26,27]. On the other hand, the use of plant hydrolyzed proteins affected the elongation of corn coleoptile, due to the presence of tryptophan (a metabolic precursor of indoleacetic acid pathways), and the shoot length of dwarf pea due to gibberellin-like activity [28]. Several authors have also reported the effects of hydrolyzed proteins, seaweed, and microalgae extracts on plants under stress conditions [19,29]. In particular, hydrolyzed proteins can increase the plant's tolerance to drought and temperature stresses thanks to the presence of amino acids, such as proline, glutamate, betaine, and peptides [28,30,31]. On the other hand, betaines and cytokinins are considered the molecules related to the antistress effect of microalgae and seaweeds extracts [32], even though the mechanism is still not fully understood [24].

Although extensive literature exists on the topic of plants biostimulants, to the best of our knowledge, there have been few works on their use on lavandin cultivation in mountainous areas. In the present work, the effects of two plant biostimulants, one based on hydrolyzed proteins and one based on seaweed extracts, were investigated in terms of morphological and agronomical data during two different years (2020 and 2021). Furthermore, the quality and the chemical composition of the extracted EOs in summer 2021 were analyzed and compared to those of our previous report [21].

2. Materials and Methods

2.1. Field Conditions

The lavandin plants cv. 'Grosso' (*Lavandula x intermedia*) were grown in the Apennine Mountain area of Emilia-Romagna Region, Northern Italy. The trial was conducted for two consecutive years (2020 and 2021) in three different farms: "Campazzo" (CA)—Zocca, Modena, Italy (44°22′54.8″ N 11°00′06.3″ E), "Pedroni Paola" (PE)—Zocca, Modena, Italy (44°21′01.0″ N 10°59′40.9″ E), and "Preci Carlo" (PR)—Castel d'Aiano, Bologna, Italy (44°17′01.4″ N 11°00′03.1″ E). All field sites were located in the north of the Apennine ridge, where the climate conditions are typical of the temperate subcontinental climate, characterized by hot and humid summers followed by cold and harsh winters. The weather conditions during the two growing seasons are reported in Figure 1.



	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
19/20 - Rainfall (mm)	41.4	47.8	18.8	48	322.2	37	21	2	42	46.6	18.5	110.6
20/21 - Rainfall (mm)	43.2	20.8	31.2	61.3	27.6	246.8	76.2	25.4	5.8	69	41.4	24
19/20 - T (°C)	22	22	17	13	8	5	5	7	6	11	16	19
20/21 - T (°C)	21	22	18	11	8	3	2	5	7	9	14	21

Figure 1. Meteorological data for the cropping seasons 2019/2020 and 2020/2021 derived from Zocca-Monteombraro, Modena, Italy weather station (44°37′63.23″ N, 11°00′87.52″ E, 700 m a.s.l.).

Based on USDA soil texture classification, the CA site (exposure N-W) had a silty clay soil, a pH of 7.90 (in water), and 2.39% organic matter content. The PE site (exposure E) had silty claim soil, pH 7.70 (in water), and 3.21% organic matter content. The PR site (exposure S) had silt loam soil, pH 7.90 (in water), and 1.45% organic matter content (Table S1).

2.2. Growth Conditions

In the CA farm, plants were transplanted in 2013, with a spacing of 1.70 m between rows and 0.50 m between plants (11,506 plants ha^{-1}). In the PE farm, plants were transplanted in 2009, and the spacing was the same as CA (11,765 plants ha^{-1}). In the PR farm, lavandin was transplanted in 2016, with a spacing of 1.40 m between rows and 0.80 m between plants (8734 plants ha^{-1}).

The experimental layout was a randomized block design with three replications; each plot was 1 m long, and all plots were separated from each other by at least one meter. Neither fertilization nor phytosanitary treatments were carried out in any of the farms. According to local practices, lavandin was cultivated under rainfed conditions, and weeds were manually controlled. In the PR site, organic row-mulching with straw was applied.

2.3. Biostimulant Treatments

In the trial, three types of foliar treatments were assessed: two different commercial plant biostimulants, FITOSTIM® and FITOSTIM ALGA® (supplied by S.C.A.M. company,

Modena, Italy), and tap water applied as control. FITOSTIM® is a product derived from fluid hydrolyzed animal epithelium composed by amino acids, peptides, and peptones. FITOSTIM ALGA® is obtained from brown marine algae, and it is rich in vitamins, polysaccharides, betaines, amino acids, peptides, and peptones. For both products, 150 g hL^{-1} was applied in two consecutive treatments, at the beginning of blooming and during full blooming. The scope of the treatment during the blooming phase was to evaluate the effect on flowers and oil production. Freshly prepared plant biostimulant solutions were distributed by nebulization using a hand pressure sprayer at ca. 10 a.m.

2.4. Agronomic and Morphological Data Recorded

On 11 July 2020 and 09 July 2021 (that is, 15 days after the second treatment), the aerial organs of the plants were manually harvested, and different morphological parameters were recorded: number of spikelets $plant^{-1}$, fresh and dry weight of flowers, fresh and dry weight of stems, and fresh and dry weight of plants. For dry weight determination, the fresh biomass was dried at 65 °C for 4 days. Two different agronomical parameters were calculated, the total yield (kg ha^{-1}) and the EO production (L ha^{-1}).

2.5. Steam Distillation

About 300 g of fresh aerial parts of lavandin were extracted by steam distillation for 1 h by a stainless-steel distiller (Albrigi Luigi s.r.l., Stallavena, VR, Italy), according to the European Pharmacopoeia X Ed. The EOs and the hydrosols were collected in a Clevenger-type apparatus (Albrigi Luigi s.r.l.), and the EOs were measured on an analytical scale. The yield % of the EO was calculated as weight of the oil per weight of fresh aerial parts of lavender. The EOs were stored at 4 °C until analysis.

2.6. GC-MS and GC-FID Analyses

Prior to the analyses in GC, the EOs and the mixture of aliphatic hydrocarbons (C8-C40) were diluted 1:20 (*v/v*) with n-hexane. The chemical composition was determined on a 7890A gas chromatograph coupled with a 5975C network mass spectrometer (GC-MS) (Agilent Technologies, Milan, Italy). Compounds were separated on an Agilent Technologies HP-5 MS cross-linked poly–5% diphenyl–95% dimethyl polysiloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column, with a gradient temperature program to achieve a better separation of the peaks and to elute all the components. The column temperature was initially set at 45 °C, then increased at a rate of 2 °C min^{-1} up to 100 °C, then raised to 250 °C at a rate of 5 °C min^{-1}, and finally held for 5 min. The injection volume was 0.1 μL, with a split ratio 1:20. Helium was employed as carrier gas at a flow rate of 0.7 mL min^{-1}. The injector, transfer line, and ion-source temperatures were 250, 280, and 230 °C, respectively. MS detection was performed with electron ionization (EI) at 70 eV, operating in the full-scan acquisition mode in the *m/z* range 40–400.

The abundance percentage of the chemical constituents was determined on a 7820-gas chromatograph (Agilent Technologies, Milan, Italy) coupled with a flame ionization detector (FID). The compounds of the EOs were separated on an Agilent Technologies HP-5 crosslinked poly–5% diphenyl–95% dimethylsiloxane (30 m × 0.32 mm i.d., 0.25 mm film thickness) capillary column. The column temperature was set as described above. The injection volume was 1 μL, with a split ratio 1:20. The flow of the carrier gas helium was 1 mL min^{-1}.

The compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic reference standards run under the same conditions described above. Furthermore, the compounds were assigned by comparing the linear retention indices (LRIs) relative to C8-C40 n-alkanes obtained on the HP-5 column under the above-mentioned conditions with the literature [33]. Peak enrichment by co-injection with authentic reference compounds was also performed. The MS-fragmentation patterns of the target compounds were compared with those of pure components by using the National Institute of Standards and Technology (NIST version 2.0d, 2005) mass-spectral database.

The percentage relative number of chemical components was expressed as the percent peak area relative to the total peak area obtained by GC-FID analysis. Semi-quantitative data were expressed as the mean of two analyses. The percentage of each component was expressed as the mean ± standard deviation of the three replicates for each kind of treatment.

2.7. Statistical Analyses

The effects of plant biostimulants and locations were tested using analysis of variance (ANOVA) followed by Duncan post hoc test (p value < 0.05) in GENSTAT 17th software (VSN International, Hemel Hempstead, UK). The semi-quantitative results of the EOs obtained from steam distillation after the crop treatments during the years 2020 and 2021 were pretreated by means of auto-scale. All the PCAs were performed using PLS-Toolbox 8.9.2 software (Eigenvector Research Inc., Manson, WA, USA) for MATLAB®, using standard assumptions about normality, equal variances, and independence.

3. Results

3.1. Weather Conditions

The two growing seasons were quite different in terms of major meteorological parameters (Figure 1). In the first year, there was a higher rainfall in comparison with the second one (776.3 and 652.5 mm, respectively). In the winter period, during the flower formation (from December to February), the average temperature was milder (6 °C) in 2019/2020 compared to average temperature (4 °C) in 2020/2021. In June, a critical month for the EO synthesis and accumulation, the average temperature in 2020/2021 was higher compared to the average temperature in 2019/2020 (21 °C and 19 °C, respectively), and the amount of rainfall was much lower in 2020/2021 (24 mm) compared to 2019/2020 (110.6 mm).

3.2. Morphological Data

Data recorded in the two growing seasons are summarized in Tables 1 and 2. In 2019/2020, the PR farm showed the highest values for all the morphological parameters assessed. Conversely, the CA farm scored the highest values for all morphological parameters measured in 2020/2021. The lowest values were obtained by the PE farm in both the growing seasons.

Considering the plant biostimulant treatments, in the first year, the use of FITOSTIM ALGA® increased the fresh weight (FW) of spikelets per plant (+35%) compared to the control treatment. Both the plant biostimulant treatments improved the FW of stems per plant (+23%) and total FW per plant (+20%) in comparison with the control treatment. On the other hand, no significant effects were found in the dry weight (DW) values for both treatments.

Data measured in the second year were almost comparable with results found in the first year. The FITOSTIM® and FITOSTIM ALGA® treatments showed the highest values for FW of spikelets per plant, total FW per plant, and DW of stems per plant. In addition, FITOSTIM ALGA® treatment had the highest values for plant DW compared to FITOSTIM® and control treatments. Concerning the number of spikelets per plant, no significant results were obtained in both the growing seasons.

Considering farm x treatment interactions (Table 2), they were significant in 2020 for all the parameters except the number of spikelets plant^{-1}. On the other hand, except for DW stem per plant and DW plant, all the parameters were significant in 2021. In the first growing season, PR and FITOSTIM® treatment showed the best interaction. The analysis of the number of spikelets per plant produced no significant results. In the second growing season, the interactions between farm and treatment showed that PR and FITOSTIM ALGA® treatment had the highest values for the FW spikelets per plant. In the PR farm, both plant biostimulant treatments showed the highest values compared to the control treatment for FW stem per plant (+54%), for FW per plant (+58%), and for the number of spikelets per plant (+47%).

Table 1. Result of the ANOVA test performed on the morphological parameters from 2019/2020. a–e means followed by different letters are statistically significant at $p < 0.05$; ns, not significant. FW, fresh weight; DW dry weight; F * T, farm * treatment interaction. CA, Campazzo; PE, Pedroni; PR, Preci; CTRL, control with foliar application of tap water; T1, foliar application of FITOSTIM; T2, foliar application of T2.

	Number of Spikelets Plant^{-1}		**FW Spikelets Plant^{-1} (g)**		**FW Stem Plant^{-1} (g)**		**FW Plant (g)**		**DW Spikelets Plant^{-1} (g)**		**DW Stem Plant^{-1} (g)**		**DW Plant (g)**	
Farm														
CA	112.0	b	137.9	b	331.1	b	468.9	b	60.7	b	61.4	b	117.4	b
PE	105.4	b	58.7	c	121.7	c	180.5	c	24.7	c	19.1	c	43.2	c
PR	282.1	a	251.0	a	478.3	a	729.2	a	87.3	a	73.9	a	148.4	a
Treatment														
CTRL	161.4	ns	131.4	b	268.9	b	400.3	b	55.3	ns	44.0	ns	99.3	ns
T1	179.6	ns	138.7	b	334.5	a	473.3	a	55.8	ns	58.9	ns	114.7	ns
T2	175.2	ns	177.4	a	327.6	a	505.0	a	61.6	ns	51.4	ns	113.0	ns
F * T														
CA CTRL	136.3	ns	149.8	cd	347.2	b	497.0	c	75.1	bc	59.9	bcd	135.0	b
CA T2	100.1	ns	117.0	d	347.5	b	464.5	c	50.7	d	74.4	abc	125.0	b
CA T1	85.4	ns	146.8	d	298.6	b	445.3	c	56.3	cd	50.0	d	106.3	b
PE CTRL	107.2	ns	49.9	e	116.8	c	166.7	d	24.4	e	18.2	e	42.6	c
PE T2	113.6	ns	69.2	e	135.8	c	205.0	d	27.5	e	21.3	e	48.8	c
PE T1	111.6	ns	57.1	e	112.6	c	169.7	d	22.2	e	17.9	e	40.1	c
PR CTRL	240.7	ns	194.6	bc	342.7	b	537.3	c	66.4	cd	54.0	cd	120.4	b
PR T2	325.2	ns	230.0	b	520.4	a	750.3	b	89.3	ab	81.2	ab	170.4	a
PR T1	328.6	ns	328.4	a	571.6	a	900.0	a	106.4	a	86.4	a	192.7	a
Farm		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Treatment		0.732		0.004		0.016		0.006		0.388		0.055		0.212
F * T		0.196		0.001		<0.001		<0.001		0.001		0.048		0.005

Table 2. Result of the ANOVA test performed on the morphological parameters from 2020/2021. a–d means followed by different letters are statistically significant at $p < 0.05$; ns, not significant. FW, fresh weight; DW dry weight; F * T, farm * treatment interaction. CA, Campazzo; PE, Pedroni; PR, Preci; CTRL, control with foliar application of tap water; T1, foliar application of FITOSTIM; T2, foliar application of FITOSTIM ALGA.

	Number of Spikelets Plant^{-1}		**FW Spikelets Plant^{-1} (g)**		**FW Stem Plant^{-1} (g)**		**FW Plant (g)**		**DW Spikelets Plant^{-1} (g)**		**DW Stem Plant^{-1} (g)**		**DW Plant (g)**	
Farm														
CA	827.3	a	204.6	a	321.7	a	526.3	a	101.8	a	159.5	a	261.3	a
PE	787.3	ab	113.4	b	111.6	c	225.0	c	71.6	b	70.3	b	141.8	c
PR	648.7	b	217.5	a	200.8	b	418.3	b	95.6	a	89.5	b	185	b
Treatment														
CTRL	701.6	ns	136.0	b	185.1	ns	321.1	b	71.5	b	94.5	ns	166	b
T1	778.9	ns	199.7	a	219	ns	418.7	a	94.8	a	104.7	ns	199.5	ab
T2	782.8	ns	199.7	a	230	ns	429.8	a	102.7	a	120.1	ns	222.8	a
F * T														
CA CTRL	877	a	185.2	bc	322.7	a	507.9	a	84.8	abcd	147.7	ns	232.5	ns
CA T2	778.6	a	214.6	b	316.6	a	531.2	a	105.3	abc	153.5	ns	258.8	ns
CA T1	826.4	a	214	b	325.8	a	539.7	a	115.4	ab	177.3	ns	292.8	ns
PE CTRL	819	a	117.9	cd	118.3	c	236.2	b	74.0	cd	74.3	ns	148.4	ns
PE T2	687	a	88.9	d	88.6	c	177.5	b	57.0	d	56	ns	113	ns
PE T1	855.8	a	133.3	cd	128	c	261.2	b	83.7	bcd	80.4	ns	164.1	ns
PR CTRL	408.8	b	104.9	cd	114.3	c	219.2	b	55.6	d	61.5	ns	117.1	ns
PR T2	871	a	295.6	a	251.9	ab	547.5	a	122.1	a	104.5	ns	226.6	ns
PR T1	666.2	a	251.9	ab	236.4	b	488.3	a	109.0	abc	102.4	ns	211.5	ns
Farm		0.043		<0.001		<0.001		<0.001		0.016		<0.001		<0.001
Treatment		0.388		0.012		0.084		0.021		0.015		0.129		0.04
F * T		0.021		0.011		0.027		0.01		0.044		0.344		0.112

3.3. Agronomical Data

Although CA and PE were transplanted in 2013 and 2009, respectively, lavandin plants in both farms were still in the productive phase.

A significant difference between the two years was found in the agronomical parameters. As reported in Table 3, in 2020, the yield (kg of FW per plant ha^{-1}) and EO production values were higher than those obtained in the second year (+41% and +55%, respectively).

Table 3. Result of the ANOVA test performed on the agronomical parameters from 2019/2020 and 2020/2021. a–c means followed by different letters are statistically significant at $p < 0.05$; ns, not significant. F * T, farm * treatment interaction.

	EO L ha^{-1}		**Flower Yield kg ha^{-1}**	
Year				
2020	93.6	a	4717	a
2021	43	b	2785	b
p value	<0.001		0.002	

2020	**EO L ha^{-1}**		**Flower Yield kg ha^{-1}**		**2021**	**EO L ha^{-1}**		**Flower Yield kg ha^{-1}**	
Farm					**Farm**				
CA	66.2	b	5517	b	CA	38.8	b	4099	ns
PE	52.5	b	2123	c	PE	23.9	b	1765	ns
PR	162.3	a	6511	a	PR	66.3	a	2490	ns
p value	<0.001		<0.001			<0.001		0.097	
2020	**EO L ha^{-1}**		**Flower Yield kg ha^{-1}**		**2021**	**EO L ha^{-1}**		**Flower Yield kg ha^{-1}**	
Treatment					**Treatment**				
CTRL	85.1	ns	4202	ns	CTRL	36.6	ns	2181	ns
T1	95.3	ns	4859	ns	T1	45.3	ns	3321	ns
T2	100.6	ns	5091	ns	T2	47.1	ns	2851	ns
p value	0.265		0.161			0.336		0.553	
2020	**EO L ha^{-1}**		**Flower Yield kg ha^{-1}**		**2021**	**EO L ha^{-1}**		**Flower Yield kg ha^{-1}**	
F * T					**F * T**				
CA CTRL	75.	c	5.9	bc	CA CTRL	39	ns	3386	ns
CA T1	61.4	c	5.5	c	CA T1	37	ns	5312	ns
CA T2	62.2	c	5.2	c	CA T2	40.3	ns	3598	ns
PE CTRL	45.8	c	2	d	PE CTRL	18.1	ns	1853	ns
PE T1	59.9	c	2.4	d	PE T1	26.9	ns	1392	ns
PE T2	51.7	c	2	d	PE T2	26.9	ns	2049	ns
PR CTRL	134.3	b	4.8	c	PR CTRL	52.7	ns	1305	ns
PR T1	164.7	a	6.7	b	PR T1	72	ns	3259	ns
PR T2	187.8	a	8.	a	PR T2	74.1	ns	2907	ns
p value	0.026		0.001			0.811		0.797	

Lavandin EO production was significantly higher in PR (+74% in the first year, +53% in the second) compared to PE and CA. In 2020, PR obtained the highest values for total yield production, while in the second year, no significant results were found. In the present study, neither the total yield nor EO production were affected by the treatments in either 2020 or 2021.

Based on the data reported in Table 3, in the first year, the use of both plant biostimulants in PR was the best sustainable management for EO production. On the other hand, only the use of FITOSTIM ALGA® in the PR farm obtained the best results for fresh weight per plant per hectare.

3.4. Chemical Composition of the EOs Extracted

For the first year of the trial (summer 2020), the effects of the treatment with biostimulants were analyzed in depth in our previous work [21]. The chemical composition

of the EOs extracted by steam distillation from the aerial parts of lavandin crops treated with biostimulants during summer 2021 is displayed in Table 4. As observed during the previous year, CA-EOs appeared completely different from PE and PR-EOs, even though the agro-climatic conditions were the same, except for the beginning of summer. Indeed, among the 43 mono- and sesquiterpenes identified by gas chromatography, significant differences ($p < 0.01$) were noticed in the concentration of 41 compounds of the CA-EOs compared to the other two farms (Table S2). Regarding the effects induced by the treatments with biostimulants, no relevant changes were observed within the EOs of the same farm in terms of percentages of chemical compounds ($p > 0.05$). This evidence was in agreement with the results of the previous year.

Table 4. Chemical composition % of the EOs expressed as mean ± standard deviation (n = 3). CA, Campazzo; PE, Pedroni; PR, Preci. CTRL, control with foliar application of tap water; T1, foliar application of FITOSTIM; T2, foliar application of FITOSTIM ALGA.

		CA			PE			PR		
	LRI	**CTRL**	**T1**	**T2**	**CTRL**	**T1**	**T2**	**CTRL**	**T1**	**T2**
2-hexenal	863	0.24 ± 0.02	0.24 ± 0.04	0.28 ± 0.03	–	–	–	–	–	–
α-thujene	924	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.02	–	–	–	–	–	–
α-pinene	930	0.61 ± 0.04	0.61 ± 0.04	0.66 ± 0.04	0.56 ± 0.01	0.55 ± 0.05	0.58 ± 0.01	0.64 ± 0.06	0.64 ± 0.03	0.62 ± 0.02
camphene	944	0.52 ± 0.02	0.53 ± 0.03	0.51 ± 0.04	0.32 ± 0.02	0.31 ± 0.03	0.33 ± 0.01	0.35 ± 0.01	0.36 ± 0.03	0.35 ± 0.01
sabinene	970	0.22 ± 0.03	0.21 ± 0.02	0.28 ± 0.05	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.23 ± 0.02	0.23 ± 0.01	0.23 ± 0.01
β-pinene	973	0.29 ± 0.04	0.26 ± 0.03	0.34 ± 0.05	0.67 ± 0.04	0.68 ± 0.03	0.71 ± 0.01	0.75 ± 0.09	0.76 ± 0.02	0.72 ± 0.03
Oct-1-en-3-ol	977	0.65 ± 0.04	0.66 ± 0.05	0.64 ± 0.10	–	–	–	–	–	–
myrcene	989	1.06 ± 0.08	1.03 ± 0.07	1.23 ± 0.16	1.40 ± 0.22	1.26 ± 0.02	1.27 ± 0.03	1.48 ± 0.05	1.35 ± 0.07	1.27 ± 0.08
α-phellandrene	1002	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.02	–	–	–	–	–	–
3-carene	1008	0.23 ± 0.01	0.22 ± 0.03	0.33 ± 0.08	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
α- terpinene	1013	–	–	–	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.12 ± 0.03	0.13 ± 0.01	0.12 ± 0.02
limonene	1027	4.62 ± 0.52	4.68 ± 0.43	6.22 ± 1.19	0.62 ± 0.11	0.53 ± 0.08	0.52 ± 0.04	0.62 ± 0.11	0.60 ± 0.13	0.62 ± 0.06
1,8-cineole	1029	4.75 ± 0.56	4.27 ± 0.28	4.90 ± 0.65	6.99 ± 0.57	7.48 ± 0.43	7.49 ± 0.26	7.26 ± 0.91	7.68 ± 0.72	7.19 ± 0.63
cis-ocimene	1037	3.25 ± 0.37	3.23 ± 0.37	4.22 ± 0.91	1.30 ± 0.11	1.38 ± 0.19	1.33 ± 0.10	1.51 ± 0.19	1.65 ± 0.26	1.36 ± 0.22
trans-ocimene	1046	1.05 ± 0.12	1.02 ± 0.13	1.26 ± 0.30	0.65 ± 0.11	0.59 ± 0.03	0.59 ± 0.03	0.67 ± 0.04	0.62 ± 0.04	0.56 ± 0.07
γ-terpinene	1059	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.13 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.18 ± 0.02	0.17 ± 0.01	0.20 ± 0.03
cis linalool oxide	1072	0.19 ± 0.03	0.20 ± 0.01	0.24 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
trans linalool oxide	1086	0.47 ± 0.02	0.48 ± 0.02	0.47 ± 0.01	0.32 ± 0.02	0.32 ± 0.01	0.32 ± 0.02	0.34 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
linalool	1107	50.97 ± 0.40	50.70 ± 1.90	45.47 ± 4.03	27.85 ± 0.76	27.48 ± 1.23	27.74 ± 0.62	28.87 ± 1.77	29.23 ± 2.13	29.47 ± 1.18
fenchol	1113	–	–	–	0.28 ± 0.04	0.28 ± 0.03	0.30 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.02
trans rose oxide	1129	0.12 ± 0.02	0.13 ± 0.01	0.17 ± 0.04	–	–	–	–	–	–
camphor	1143	2.91 ± 0.03	3.06 ± 0.26	3.40 ± 0.58	6.46 ± 0.42	6.91 ± 0.42	6.83 ± 0.13	6.85 ± 0.10	7.26 ± 0.39	7.06 ± 0.25
isopulegol	1149	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	–	–	–
borneol	1167	11.12 ± 0.65	11.75 ± 0.76	11.15 ± 1.27	2.78 ± 0.43	2.48 ± 0.36	2.39 ± 0.30	2.01 ± 0.22	2.08 ± 0.22	2.22 ± 0.39
lavandulol	1168	–	–	–	0.31 ± 0.03	0.30 ± 0.03	0.03 ± 0.04	0.32 ± 0.08	0.33 ± 0.06	0.33 ± 0.06
terpinen-4-ol	1178	3.48 ± 0.32	3.71 ± 0.13	4.18 ± 0.28	1.25 ± 0.17	1.17 ± 0.05	1.20 ± 0.05	1.17 ± 0.12	1.21 ± 0.15	1.33 ± 0.20
p cymen-8-ol	1185	0.33 ± 0.04	0.36 ± 0.04	0.41 ± 0.03	–	–	–	–	–	–
α-terpineol	1190	0.28 ± 0.01	0.27 ± 0.03	0.32 ± 0.05	0.60 ± 0.03	0.60 ± 0.01	0.60 ± 0.04	0.58 ± 0.04	0.64 ± 0.03	0.60 ± 0.05
myrtenal	1193	0.51 ± 0.03	0.52 ± 0.03	0.48 ± 0.04	0.24 ± 0.01	0.26 ± 0.03	0.24 ± 0.01	0.19 ± 0.01	0.19 ± 0.02	0.19 ± 0.01
nerol	1229	0.19 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	–	–	–	–	–	–
thymol methyl ether	1241	0.30 ± 0.02	0.30 ± 0.02	0.34 ± 0.03	–	–	–	–	–	–
pulegone	1246	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.17 ± 0.03	0.15 ± 0.01	0.15 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
linalyl acetate	1261	5.56 ± 0.58	5.11 ± 0.67	5.33 ± 0.73	35.31 ± 0.93	35.48 ± 1.07	35.35 ± 0.91	34.52 ± 1.57	33.65 ± 1.87	33.90 ± 1.50
lavandulyl acetate	1292	0.89 ± 0.13	0.96 ± 0.10	1.09 ± 0.18	3.13 ± 0.01	3.06 ± 0.08	3.10 ± 0.11	3.03 ± 0.17	3.07 ± 0.24	3.12 ± 0.09
neryl acetate	1366	–	–	–	0.28 ± 0.06	0.24 ± 0.01	0.25 ±0.01	0.28 ± 0.01	0.25 ± 0.01	0.24 ± 0.02
β-cubebene	1385	–	–	–	0.56 ± 0.12	0.50 ± 0.01	0.49 ± 0.03	0.58 ± 0.02	0.51 ± 0.01	0.50 ± 0.04
β-caryophyllene	1422	0.25 ± 0.22	0.39 ± 0.03	0.44 ± 0.02	1.75 ± 0.15	1.63 ± 0.07	1.64 ± 0.08	1.63 ± 0.13	1.61 ± 0.12	1.61 ± 0.08
α-bergamotene	1439	–	–	–	0.11 ± 0.01	0.11 ± 0.01	–	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.02
α-humulene	1459	1.75 ± 0.08	1.82 ± 0.17	2.11 ± 0.20	1.40 ± 0.03	1.35 ± 0.07	1.32 ± 0.07	1.27 ± 0.03	1.21 ± 0.06	1.23 ± 0.04
alloaromadendrene	1467	–	–	–	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.03
ar curcumene	1485	–	–	–	0.50 ± 0.01	0.49 ± 0.03	0.48 ± 0.05	0.66 ± 0.12	0.64 ± 0.08	0.70 ± 0.12
γ-cadinene	1520	–	–	–	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.04	0.32 ± 0.02	0.30 ± 0.02	0.32 ± 0.06
δ-cadinene	1528	–	–	–	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	–	0.14 ± 0.01
total		97.61 ± 0.32	97.58 ± 0.32	97.51 ± 0.23	98.28 ± 0.28	98.07 ± 0.11	98.09 ± 0.1	98.27 ± 0.12	98.25 ± 0.09	98.14 ± 0.18

3.5. Principal Component Analysis

Measured data were analyzed using PCA to define the association between parameters, treatments, and farms. All the results for a single year were organized in an ordination biplot.

As reported in Figure 2, for the first season, the two principal components (PCs) explained 96.4% of the total variance. Precisely, 81.79% and 14.56% of the variance were described by PC1 and PC2, respectively. The PC1 clearly separated the morphological and agronomical parameters and the harvest index; in addition, PC1 divided the PE treatments and CA FITOSTIM ALGA® treatment. The PR FITOSTIM ® treatment was associated with EO L ha^{-1}, FW per plant, and FW and DW of spikelets per plant. PE treatments were inversely associated with DW per plant, and DW and FW per steam.

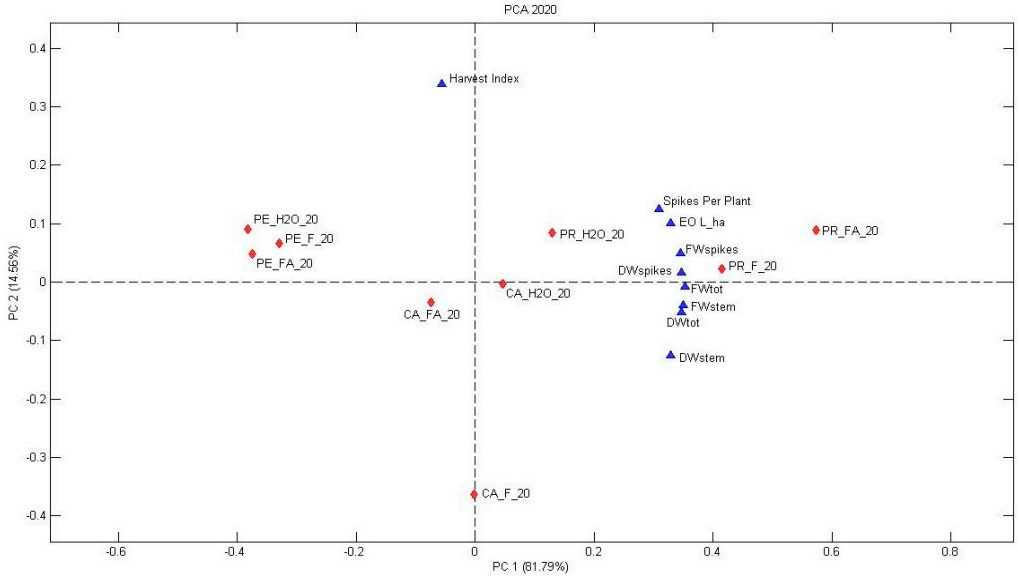


Figure 2. Principal component analysis (PCA) biplot of the morphological data from 2010/2020. CA, Campazzo; PE, Pedroni; PR, Preci; H2O, control with foliar application of tap water; F, foliar application of FITOSTIM; FA, foliar application of FITOSTIM ALGA; DW, dry weight; FW, fresh weight; EO L_ha, yield of essential oils (L ha^{-1}).

As shown in Figure 3, the two principal components for the second season extracted by PCA's algorithm explained 88.94% of the total variance. In particular, 66.03% and 22.15% of the variance were described by PC1 and PC2, respectively. PC1 clearly separated PR plant biostimulant treatments with respect to the PR control treatment. In addition, CA plant biostimulant treatments were associated with FW and DW stem per plants. It is noteworthy that PCA highlighted an effect of the plant's age: older plants (transplanted in 2009 in PE and in 2013 in CA) showed a limited reaction to the plant biostimulant treatments, while younger ones (transplanted in 2016 in PR) were more responsive.

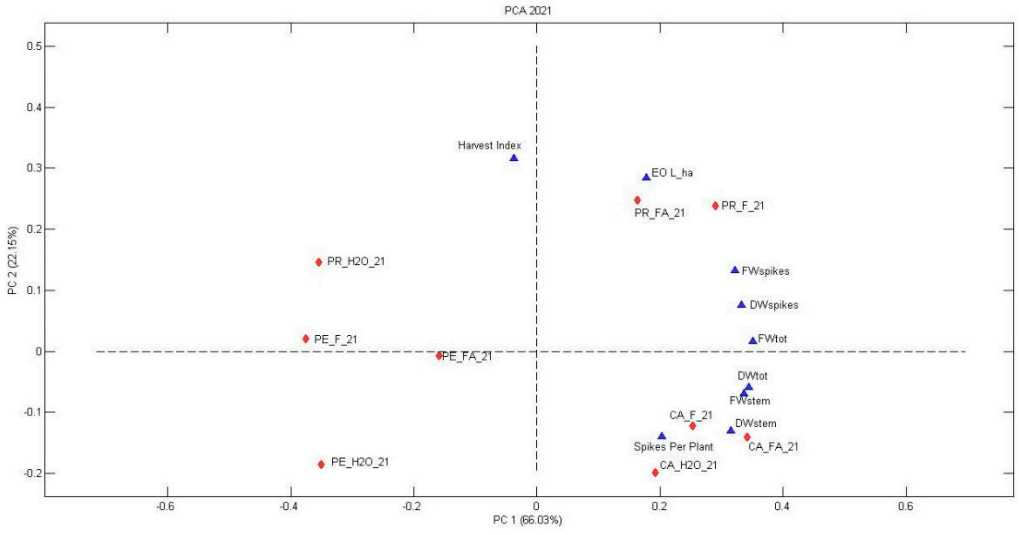


Figure 3. Principal component analysis (PCA) biplot of the morphological data from 2020/2021. CA, Campazzo; PE, Pedroni; PR, Preci; H2O, control with foliar application of tap water; F, foliar application of FITOSTIM; FA, foliar application of FITOSTIM ALGA; DW, dry weight; FW, fresh weight; EO L_ha, yield of essential oils (L ha^{-1}).

To summarize the differences in the chemical composition of the EOs, PCA was performed on the semi—quantitative results obtained by the GC analyses in the year 2021.

The dataset was composed of the percentages of abundances of the 43 terpenes identified and quantified. In Figure 4, the score plot and the loading plots of the extracted principal components are displayed.

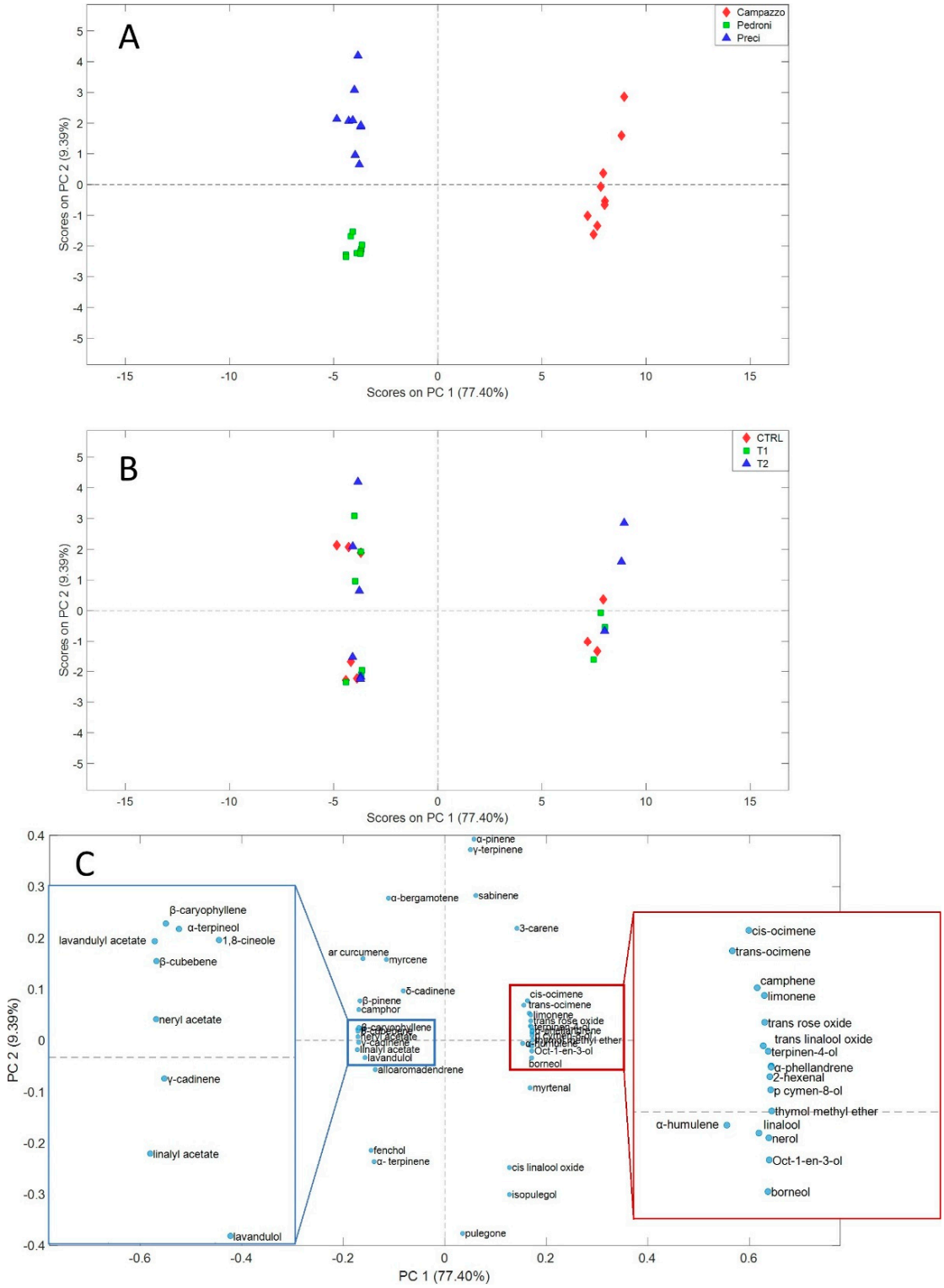


Figure 4. Principal component analysis (PCA) biplot for the chemical composition of EOs from 2020/2021. (**A**) Score plot labelling the farms and (**B**) the treatments; (**C**) loading plot. CA, Campazzo; PE, Pedroni; PR, Preci; CTRL, control with foliar application of tap water; T1, foliar application of FITOSTIM; T2, foliar application of FITOSTIM ALGA.

The raw data were pre-processed by autoscaling, and two PCs were extracted, explaining 86.79% of the total variance. Specifically, PC1 accounted for 77.40%, and PC2 for 9.39%. The PCs were able to efficiently cluster the EOs depending on the farm group. PC1 collocated in positive and in negative CA-EOs and PE-PR-EOs, respectively. On the other hand, PC2 separated PE and PR-Eos, which resulted in them being positively and negatively projected, respectively.

The fact that most of the variance was described by PC1 suggested that the variability among the samples relied on the differences between the CA group and the PE/PR group, as observed by the ANOVA analysis (Table S2). As far as the loading plots of PC1 are concerned, the most important chemical compounds for the discrimination of the samples can be highlighted. Indeed, the terpenes with the highest importance in disponing the Eos were those with the highest weights in positive and negative. The variables (terpenes) with opposite values were inversely correlated. CA-Eos were collocated on positive values of PC1, especially due to the higher content in limonene, *cis*—ocimene, linalool, borneol, and terpinene—4—ol. On the contrary, PE and PR-EOs were on negative values of the *x*—axis due to the high abundances of β—pinene, lavandulol, 1,8—cineole, neryl acetate, lavandulyl acetate, camphor, linalyl acetate, and the sesquiterpenes β—caryophyllene, alloaromandrene, γ—cadinene, and β—cubebene. PE— and PR—EOs were mainly clustered by the inversely correlated terpenes with the highest values in positive (α—bergamotene, α—pinene, γ—terpinene, sabinene) and negative (fenchol, α—terpinene, pulegone, isopulegol) on PC2.

As evident in Figure 4C, the EOs were not separated according to the treatment, and no trends were identified. Thus, we concluded that—as observed for the EOs collected during summer 2020—the plant biostimulants did not induce significant changes in the chemical composition of lavandin EOs.

Furthermore, PCA was performed on the chemical composition of all the EOs obtained from the two years of the trial (Figure 5). Accordingly, the datasets of the chemical compositions of the EOs collected during summer 2020 and 2021 were combined (samples number equal to 54). The PC1 and PC2 accounted for 63.02% and 12.00% of the variance, respectively, describing 75.02% overall. By examining the score plot (6A), all the groups of EOs were well—clustered. As previously observed, PC1 influenced the separation of CA—EOs, while PC2 played a central role in separating the EOs belonging to the different trial years. Specifically, the EOs produced during summer 2021 were grouped on negative values of PC2 due to the higher content of sabinene, α—pinene, 1,8—cineole, β—pinene, myrcene, 3—carene, and camphene, which exhibited the highest weights. On the contrary, these EOs showed lower concentrations, especially of lavandulol, α—terpinene, γ/δ—cadinene, fenchol, alloaromadendrene, and *trans*—linalool oxide compared to those obtained during summer 2020.

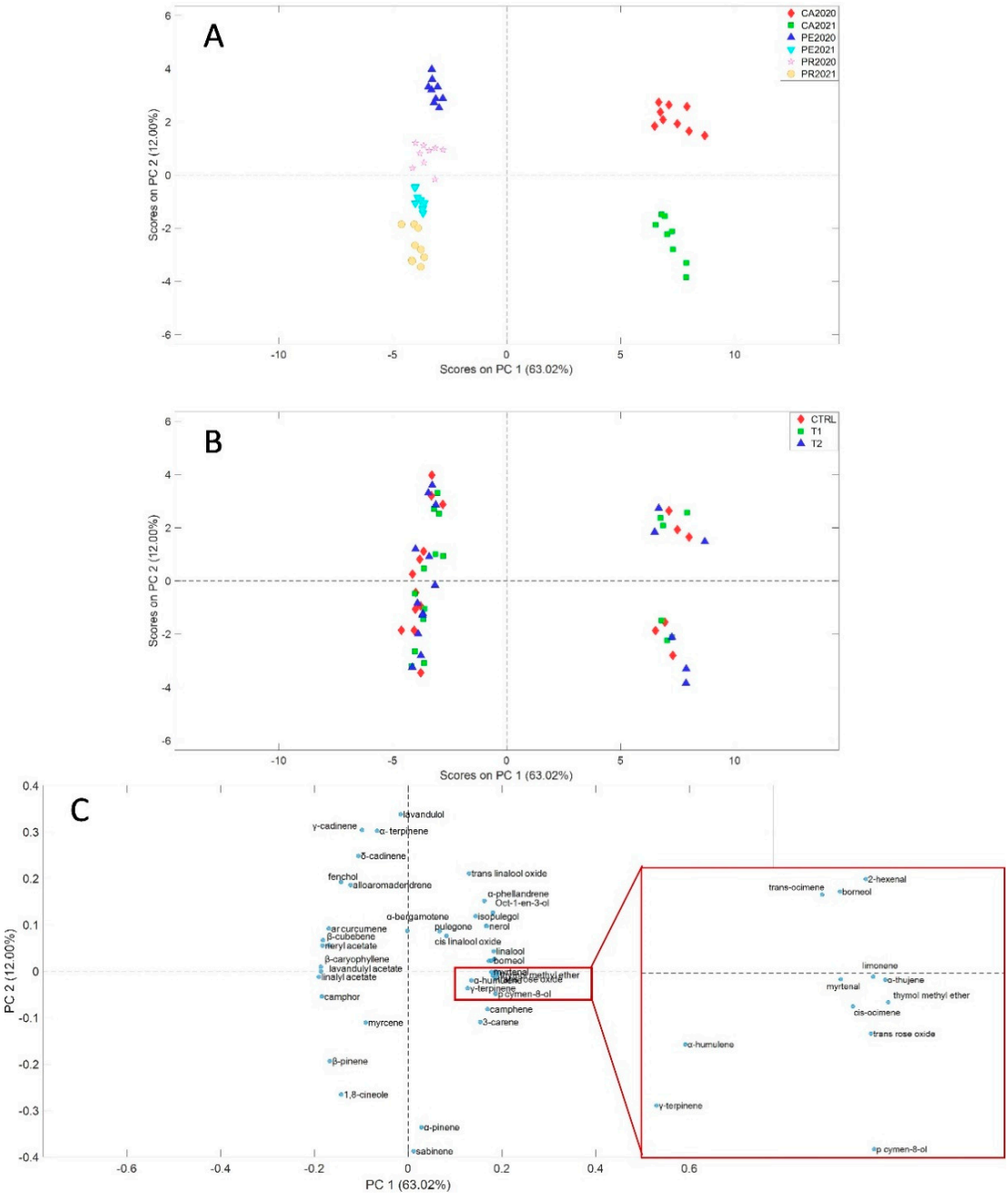


Figure 5. Principal component analysis (PCA) biplot for the chemical composition of EOs from 2019/2020 and 2020/2021. Score plots labelling the farms (**A**) and the treatments (**B**); (**C**) loading plot. CA, Campazzo; PE, Pedroni; PR, Preci; CTRL, control with foliar application of tap water; T1, foliar application of FITOSTIM; T2, foliar application of FITOSTIM ALGA.

4. Discussion

The present study was, to the authors' knowledge, one of the first reports to evaluate the effects of plant biostimulant treatments on the morphological and agronomical features of lavandin plants grown in a mountainous area. Nowadays, the market of products derived from local sustainable agriculture is rising, and consumers are more concerned with seeking out products with a lower impact on the environment [34]. Agronomical practices, such as the management of nutrients, mulching and tillage, and the use of plant biostimulants, may influence crop yield [35,36]. In this contest, plant biostimulants may be a useful tool to enhance EO production in lavandin and to make mountain agriculture more competitive in a sustainable way [15,37].

During the trial, a difference in biomass production was highlighted between the two cropping years. This could be partly explained by the contrasting weather conditions observed between the two growing seasons. Lavandin is a Mediterranean crop species, requiring mild winters and springs during flower formation. However, during late spring/early summer, when the EO synthesis and storage occur in the glandular trichomes, the amount of rain and atmospheric temperature are critical factors influencing the biosynthesis of essential oils [38–40]. Putatively, the combination of lower rain and stressful temperatures may have reduced the number of glandular trichomes, affecting the EO production and the formation of the flower shoots in the second year [41–43].

Besides the difference registered between the two years, a marked difference was also found among farms, and the PR farm showed the best performance. Different management techniques on the field could have influenced the growth and development of the lavandin crop [44]. The PR farm was the only one to apply straw mulch on the row, and this aspect may have played a role to keep the moisture of the soil high during the summer. In fact, straw mulching can have several benefits [45], such as increased water retention, positively affecting soil moisture [46]; reduced soil temperature, controlling the evaporation lost [47]; and reduced disintegration of soil particles, avoiding the erosion and crevasse [48]. In addition, studies have reported an increase of growth in lavender plants and a better control of weeds in mulched plants [49,50]. Moreover, the straw mulching can reduce the soil erodibility on steep slopes and increase the water storage in clay soil [51,52]. Furthermore, the PR farm was also the farm that responded better to treatments: the year of the crop, the ageing of the leaves and green stems may have influenced the assimilation of the plant biostimulants and therefore the efficacy of the treatments [38]. The age of the plants and leaves can influence the intake of nutrients since the main channel for assimilation of organic compounds in the leaf or green stem is through external cuticula [53]. During the ageing of leaves and green stems, changes occur in the cuticle [54–56]: the thickness increases, and the composition changes, reducing the efficiency of foliar-applied fertilizers [57,58]. These characteristics were summarized by Baldoni [5], who reported that eight years after transplant, lavandin enters a phase of marked decrease in crop production. Putatively, the PR plants, transplanted in 2016, had higher leaves turnover and more efficient physiology compared to the PE and CA plants. On the other hand, the lavandin plants of the PE and CA farms, despite the year of transplanting (2009 and 2013 respectively), were still in a productive phase; however, the production was less efficient compared to the younger crop of PR.

In both years, FITOSIM® and FITOSIM ALGA® had a positive effect on biomass production of lavandin, confirming the data reported in other studies on lavender and petunia crops. Giannoulis et al. [59] tested two different brown algae extracts on lavender and obtained an increase of flower shoots production. Cristiano and De Lucia [60] evaluated animal-derived hydrolyzed proteins on petunia and discovered an increased number of flowers and leaves and an increase in leaf dimension. Several studies attribute the positive effect of hydrolyzed proteins to their composition based on soluble peptides, larger amount of free amino acids, organic nitrogen, iron, and potassium [61,62]. These molecules may be absorbed through the leaves and may enhance the nitrogen assimilation by stimulating carbon and nitrogen metabolism, thus increasing leaf biomass [63–65]. However, these data cannot be confirmed in the present study because plant nitrogen concentration was not evaluated. Concerning the brown algae (*Ascophyllum nodosum*) extracts, studies attributed the positive effects to the content of polysaccharides, such as betaine, and polyamines, phenolic compounds, mannitol, laminarin, and trace elements [24,66]. These molecules act on the plant's primary metabolism, enhancing the uptake of several elements, such as N, P, K, Mg, and Zn [67]; the photosynthetic efficiency; and the carbon assimilation [24]. In addition, the extracts of brown algae may either contain phytohormones such as auxins, cytokinins, and abscisic acid, or stimulate the hormone pathway [68–71].

Agricultural practices can affect the EO content and composition [72–74]. Tibaldi and colleagues [75] observed that manual weed control and mulching influenced the EO

quantity and quality. Minev [76] highlighted that the use of leaf fertilizer on lavender during the budding phase increased the yield of EOs. In our study, neither the agronomical management nor the effect of the plant biostimulants influenced the production of essential oils and the chemical composition of EOs, confirming the results obtained by Truzzi and colleagues [21]. The only variation in the composition of EOs measured was between 2020 and 2021, which might be due by the different weather conditions during the two experiments. This hypothesis could be partially confirmed by the work of Georgieva et al. [72], which observed a variation in the content of essential oils of coriander seeds correlated with different weather conditions during the trials. Owing to the treatment effects in each farm, the PR site had the best crop x treatment interaction in the morphological data for both years, and in yield and EO production in 2020. Nevertheless, variations in the treatment effects were measured in the farm between the two years. As previously mentioned, the age of the plant may have an impact on the efficiency of the treatments; however, it can be assumed that the different weather conditions might have also played a role [77]. Kolomazník [78] stated that during leaf penetration, the surface film of the plant biostimulant should remain liquid, since in case of rapid evaporation of the water, the penetration of cuticula is markedly reduced. The average air temperature during the treatment period was warmer in 2021 compared to 2020, leading us to speculate that a higher temperature—via faster leaf surface evaporation—may have reduced the amount of plant biostimulant adsorbed [79].

In conclusion, this work suggests that the use of plant biostimulants could be a tool to improve the sustainable cultivation of lavender in rural areas. However, further investigations should be carried out to evaluate the impact of interactions between the crop and the plant biostimulants to enhance the quality and the quantity of EOs, and to increase the efficiency of plant biostimulants depending on the seasonal variation.

5. Conclusions

Our results confirm the effectiveness of the hydrolyzed protein and products containing brown marine algae extracts in enhancing the biomass production of lavandin crop. The plant biostimulants evaluated in this study can be considered an innovative tool to develop new forms of sustainable agriculture of lavandin in the investigated areas. In this scenario, further works should be carried out to confirm our results for lavender, including in other geographical areas, and to investigate which physiological and biochemical mechanisms are influenced by the used plant biostimulants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092189/s1, Table S1: Soil analyses for Campazzo, Pedroni, and Preci farms; Table S2: One-way ANOVA results of significant differences between farms. The mean and the standard deviation (SD) of each terpene in each farm are reported, and distinct letters statistically differ according to Tukey's post hoc test ($p < 0.05$). Homogeneous subsets are indicated by the same letter.

Author Contributions: Conceptualization, E.F., D.R. and S.B.; investigation G.C., F.C., M.B.H., D.R. and E.T; data curation, G.C. and E.T.; writing—original draft preparation, G.C. and F.C.; writing—review and editing G.C., F.C., D.R., M.B.H., E.T., S.B. and E.F.; funding acquisition S.B. and E.F. All authors have read and agreed to the published version of the manuscript.

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