

Article

Inflorescence Yield, Essential Oil Composition and Antioxidant Activity of *Cannabis sativa* L. cv ‘Futura 75’ in a Multilocation and On-Farm Study

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Abstract: Industrial hemp (*Cannabis sativa* L.), being a multiharvest crop, can increase farm profitability and cropping system diversification, including in marginal areas. Since inflorescence essential oil (EO) represents a valuable co-product for cosmetics and pharmaceutical sectors, the aim of this study was to assess the effect of pedoclimatic conditions on the inflorescence yield. EO content, composition and antioxidant capacity of the monoecious variety ‘Futura 75’. So, on-farm trials were performed in central Italy at three sites (SL, LA and SPG), which differed in terms of soil (taxonomic classes; chemical and physical characteristics), microclimate conditions (rainfall and air temperatures) and agricultural value. The results highlighted how location specificities significantly influence crop performance. Strong differences in productive parameters were observed among the farms, with inflorescence yields ranging from 1.3 to 4.9 Mg ha^{−1}, mainly depending on the differences between the maximum and minimum air temperatures (ΔT) since negative correlations were found. Similarly, the concentration of monoterpene and sesquiterpene hydrocarbons showed a reduction when ΔT during the studied period was higher; conversely, oxygenated sesquiterpenes showed an increment due to a higher ΔT . Only phenylpropanoids were affected by rainfall, showing a positive correlation. All these findings confirm that in hemp, satisfactory productions can be reached only by matching territorial suitability. The variability in EO’s characteristics suggests, in fact, that the “uniqueness” of the EOs derives from a combination of the pedological, climatic and agronomic variables of each site.

Keywords: hemp; cultivation site; agronomic management; environmental conditions; crop performance



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1. Introduction

‘Futura 75’ is a French monoecious cultivar of *Cannabis sativa* L. commonly grown in Europe for fibre and seed production, as its Δ^9 -tetrahydrocannabinol (THC) content complies with the EU-imposed threshold (0.2% *w/w*) for industrial hemp. *C. sativa* is an annual crop characterised by broad soil and climate adaptation due to its high disease- and pest-resistance and low water and fertiliser requirements [1–5]. The high diversity of the obtainable final products characterises hemp as an ecologically friendly, multipurpose crop exploitable in a large number of agro-industrial sectors, ranging from textiles, modern bio-composites for automotive and construction [6,7] and biofuels to functional foods, cosmetics and biomedical/pharmaceutical purposes.

The monoecious cultivar ‘Futura 75’ has been mainly developed to produce seeds and fibres, but the exploitation of inflorescences and their bioactive compounds could further contribute to the versatility of this variety. Hemp essential oil (EO) is a value-added

by-product obtainable from female inflorescences; its hydrodistillation might represent a viable utilisation of a material commonly regarded as a crop residue in fibre production [8]. It is mainly composed of mono- and sesqui-terpenes, volatile constituents biosynthesised by numerous terpene synthases and stored in the glandular trichomes of *C. sativa* flowers, where they co-exist with cannabinoids [9–11]. Geranyl pyrophosphate is a common bio-precursor for both cannabinoids and terpenes; over 200 different compounds belonging to these chemical classes have been identified in hemp so far, evidencing their importance in the secondary metabolism of this species [12–15]. Since hemp has long-established uses dating back to as much as 5000 years ago [16], its numerous cultivars, mainly developed through non-protocolled, informal breeding practices, display a high phytochemical diversity [8,17,18]. Hemp EO, obtained from different hemp cultivars, including ‘Futura 75’, exhibits a wide range of possible applications due to its numerous properties tested, such as antimicrobial on bacteria and yeasts [19,20] and antibiotic-enhancing [20] to pest repellents [21–24]. These properties are, obviously, linked to EO’s composition, which ultimately varies based on numerous factors which make up the terroir of hemp. It is known, in fact, that the genotype (dioecious or monoecious varieties), environmental conditions, agronomic management, and phenological stage at harvest significantly affect the volatile profile of EO hemp composition and its extraction yield [25–27]. In general, for the optimal growth and development of ‘Futura 75’, the optimal climatic conditions include at least 250 mm of water under a Mediterranean climate [28]. However, it is known that depending on the genotype, hemp behaviour against drought can be either isohydric or anisohydric, and the different strategies have repercussions on the phytochemical composition of inflorescence [29]. The optimum temperature for monoecious hemp development varies according to different authors but is generally identified between 24 and 26 °C [28,30]. In a study conducted by Giupponi et al. [31], in which the characteristics of hemp essential oil were compared between mountain and plain environments, it emerged that plants grown in colder environments tend to have increased terpene concentrations. However, it is suggested that the effect of low temperatures on the chemical composition of an inflorescence may vary functionally according to the sowing time and the plant age of the plant [32].

So, hemp adaptability to a specific environment and to real operational conditions at the farm level is of paramount importance to the success of hemp cultivation. Giupponi et al. [33] underlined that ‘Futura 75’ is the most widespread monoecious variety among Italian farms that produce hemp. Consequently, the main purpose of this multilocation study was to assess the agronomic performances of ‘Futura 75’ through on-farm experiments carried out by farmers who were approaching this crop for the first time. The trials were performed at different sites across Northern Tuscany (Central Italy) and managed by adopting ordinary agrotechniques commonly used by local farmers to define hemp suitability to available equipment and practices. All trials were rainfed and carried out under low-input agronomic management. Although the sites chosen for the on-farm trials fall within the macro-area of the Mediterranean climate, they are characterised by different microclimatic conditions as well as by different agricultural values. So, the effect of these characteristics was evaluated on the main biometric and productive characteristics, with particular attention to the total biomass, inflorescence production, essential oil yield, composition and radical-scavenging activity.

2. Materials and Methods

2.1. Site Description

The industrial hemp variety ‘Futura 75’ was tested in three large field-scale experimental trials established at three different sites in Northern Tuscany (Central Italy) during the 2019 growing season. The trials were conducted at the Experimental Centre of the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa (San Piero a Grado—SPG, 43°40′31″ N, 10°18′40″ E, 1 m a.s.l., 4 km from the sea) and at two farms, attempting hemp cultivation for the 1st time, located in the hilly area of Pisa Province (Santa Luce—SL, 43°26′17″ N, 10°29′32″ E, 40 m a.s.l., 10 km from the sea) and

in the plain area of Pistoia Province (Larciano—LA, 43°47′49″ N, 10°49′30″ E, 17 m a.s.l., 50 km from the sea), respectively. All the trials were managed according to the standard practices applied by local farmers to obtain as much reliable data as possible on hemp suitability to the tested environments and according to standard low-input practices. In SL and LA, an organic farming system was adopted, while in SPG, hemp was cultivated according to an integrated management system. The main information regarding the adopted agronomic management (i.e., soil tillage and seedbed preparation, sowing method, fertilisation, weed control) is presented in Table 1.

Table 1. Hemp crop management adopted at the three sites (Northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano).

Site	SPG (Plain Area, Pisa Province)	SL (Hilly Area, Pisa Province)	LA (Plain Area, Pistoia Province)
Primary tillage	Shallow ploughing (30 cm) at the end of August	Shallow ploughing (30 cm) at the end of September	Shallow ploughing (30 cm) associated with subsoiling at the beginning of March
Seedbed preparation	Disk harrow (2 passes)	Disk harrow (three passes)	Spring-tooth harrow combined with a rotary harrow
Sowing method	25 kg ha ^{−1} on 15 cm-spaced rows using a plot drill for wheat	30 kg ha ^{−1} on 14 cm-spaced rows using a plot drill for wheat	25 kg ha ^{−1} on 25 cm-spaced rows using a plot drill for wheat
Fertilisation	80 kg ha ^{−1} of P ₂ O ₅ (triple superphosphate) and 80 kg ha ^{−1} of K ₂ O (potassium sulphate) for pre-sowing; 40 kg N ha ^{−1} (ammonium nitrate) as topdressing distribution	20 Mg ha ^{−1} of digestate applied in March	0.5 Mg ha ^{−1} of commercial organic fertiliser (12% Norg)
Weed and Pest Control	No chemical applications or manual weeding Weeds are controlled by stale seedbed technique before sowing		

Among the study areas, SL can be defined as “marginal” according to Elbersen et al. [34] since it was a sloppy site with approximately a 15% slope. At each study site, meteorological data (minimum (Tmin), maximum (Tmax) and mean (Tmean) air temperature, total rainfall, and thermal excursion (as the difference between Tmax and Tmin; ΔT) were recorded by automated weather stations located nearby each area. Soil physical and chemical characteristics were assessed at the beginning of the experiment, with the soil samples collected at a depth of 0.3 m in each field.

The soil pH was measured on a 1:2.5 soil-to-water suspension [35]. Total nitrogen was evaluated by the macro-Kjeldahl digestion procedure [36]; the available phosphorus was determined by the Olsen method [37], while the Thomas method [38] was used for exchangeable potassium determination. Soil organic carbon (SOC) was assessed using the modified Walkley–Black wet combustion method [39], and the soil organic matter (SOM) content was estimated by multiplying a SOC concentration of 1.724 [39]. In SPG, the soil was classified as Typic Xerofluvents according to USDA soil taxonomy with loam texture (45.9% sand, 39.0% silt and 15.1% clay), an alkaline reaction (pH = 8.0), low available phosphorus (9.1 mg kg^{−1}) and a medium content of both soil organic matter (SOM = 19.8 g kg^{−1}) and total nitrogen (1.3‰). The soil in SL was Calcic Udic Haplustepts clay–loam (26.9% sand, 37.3% silt and 35.8% clay), with a sub-alkaline reaction (pH = 7.6), a low content of both soil organic matter (12.9 g kg^{−1}) and available phosphorus (6.1 mg kg^{−1}) and a medium level of total nitrogen (1.1‰). In LA, the soil was Fluventic Haploxerepts, with a sandy loam texture (57.9% sand, 26.5% silt and 15.6% clay), a neutral reaction (pH = 6.9) and low levels of organic matter (SOM = 11.8 g kg^{−1}), available phosphorus (5.9 mg kg^{−1}) and total nitrogen (0.2 ‰). Based on the soil’s chemical features, LA is characterised by low fertility in terms of organic matter, available phosphorus and total nitrogen.

2.2. Plant Material, Field Experiment and Sampling

The monoecious hemp variety 'Futura 75', generally cultivated for seed production, was used in this experiment. Crop techniques were implemented differently at the three sites according to the standard practices applied by farmers (Table 1). At each site, hemp was cultivated in strips ranging in size from 500 to 1000 m². The previous crops were *Camelina sativa* (L.) Crantz in SPG; common wheat (*Triticum aestivum* L.) in SL and dwarf bean (*Phaseolus vulgaris* L.) in LA. Sowing was performed during the 2019 growing season on 19 April in SL, 14 May in SPG and 20 April in LA. The cultivation was carried out under rainfed conditions. The harvest of inflorescences was carried out at the beginning of seed maturity, corresponding to the phenological code 2305 [40] for monoecious varieties on 12 August, 31 July and 4 August in SPG, SL and LA, respectively.

The inflorescences were manually sampled by cutting the 30 cm upper part of the stem from 8 sampling areas of 1 m² each, randomly chosen within the strips. The plant height was measured from the base of the stem to the top of the inflorescence. After the harvest, the inflorescences were air-dried to a constant weight at room temperature to avoid the degradation of phytochemicals and volatile compounds. Afterwards, dry weight determinations were detected, and dried inflorescences were placed in plastic bags and stored in the dark in a cool room until the analysis (EO yield and composition and antiradical activity evaluation). A sub-sample of inflorescences and vegetative biomass was oven-dried at 60° to a constant weight to obtain the inflorescence and vegetative yields on a dry matter basis. The inflorescence harvest index was obtained as the ratio between inflorescence and the whole aboveground biomass dry yields.

2.3. Hydrodistillation of the Essential Oil (EO)

For all the accessions, 70 g of representative sub-samples of dried plant material were roughly cut and inserted in an amber glass maceration bottle with 100 mL of distilled water; the maceration was performed at controlled room temperature in a roller agitator for 24 h at 150 rpm prior to hydrodistillation. The maceration was performed to increase the extraction yield, as experimentally verified and reported in the published literature [41,42]. Then, the contents of the maceration bottle were transferred into a 2000 mL flask; 900 mL of distilled water was added to reach the appropriate hydrodistillation volume inside the flask. The flask was then connected to a standard Clevenger apparatus and inserted into an electric mantle heater. The hydrodistillation was performed for 2 h; this duration was experimentally evaluated since, after 2 h, no increment in the quantity of the hydrodistilled EO was obtained. The EO was withdrawn from the apparatus with a metal needle, dried over anhydrous magnesium sulphate, stored in amber glass vials and kept refrigerated until the analysis. For each accession, three hydrodistillation replicates were performed.

2.4. GC-MS Analyses and Peak Identification

The hydrodistilled essential oils were diluted to 0.5% in HPLC-grade *n*-hexane and then injected into a GC-MS apparatus. Gas chromatography–electron impact mass spectrometry (GC-EIMS) analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5MS (Agilent Technologies Inc., Santa Clara, CA, USA) capillary column (30 m × 0.25 mm; coating thickness: 0.25 µm) and an Agilent 5977B single-quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA, USA). The analytical conditions were as reported by Ascrizzi et al. [43]: injector and transfer line temperatures of 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; an injection of 1 µL; and a split ratio of 1:25. The acquisition parameters were as follows: full scan; scan range: 30–300 *m/z*; scan time: 1.0 s. The identification of the constituents was based on a comparison of their retention times with those of authentic samples (when available), comparing their linear retention indices relative to a series of *n*-hydrocarbons (C6–C25). Computer matching was also used against a commercial [44] and a laboratory-developed mass spectra library built up from pure substances and components of commercial essential oils of known composition and MS

literature data [45]. All the pure analytical standards were purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA).

2.5. Antiradical Activity Evaluation through DPPH Assay

The DPPH free radical-scavenging activity of the essential oils was evaluated according to Pistelli et al. [46]. The results were expressed as mmol Trolox equivalents (TE) per gram of sample using a standard curve of Trolox in the range of 0–200 $\mu\text{mol L}^{-1}$. The inhibition percentage of free radicals in each sample was also evaluated using the following formula: $(\%I) = [(A_0 - A_t)/A_0] \times 100$. Here, A_0 is the absorbance of the DPPH solution at 0 min and A_t is the absorbance in the presence of an essential oil after the incubation time ($t = 30$ min).

2.6. Statistical Analyses

The agronomic parameters (plant height, inflorescence yield, harvest index, essential oil yield) and anti-radical activity of the EOs were subjected to a one-way ANOVA using the statistical software CO-STAT Cohort, version 6.451 (CoHort Software, Monterey, CA, USA), with the environment as a variability factor. Means were compared by Tukey's HSD post hoc test when the ANOVA F-test was significant at the 0.05 probability level.

All the analyses regarding EO composition were performed with the JMP® Pro 13.2.1 (SAS Institute Inc., Cary, NC, USA) software. For the statistical evaluation of the composition of all the EOs, an 83×3 correlation matrix (83 individual compounds $\times$ 3 samples = 249 data) was used. To perform the PCA, linear regressions were operated on mean-centred, unscaled data to select the two highest principal components (PCs). This unsupervised method reduced the dimensionality of the multivariate data in the matrix whilst preserving most of the variance [47]. The chosen PC1 and PC2 cover 63.00% and 37.00% of the variance, respectively, for a total explained variance of 100.00%. The HCA was performed using Ward's method, with Euclidean distances as a measure of similarity. The observation of the groups of samples performed with the HCA and the PCA unsupervised methods can be applied even when there are no reference samples that can be used as a training set to establish a model. The linear discriminant analysis (LDA) used the relative abundances detected for each EO compound in all the samples as continuous independent variables (whose distribution is reasonably assumed to be non-normal) and the geographical area of origin (*a priori* known grouping) as the categorical dependent variable to find the linear combination of features separating the samples according to their growth area.

For the evaluation of the correlation between (i) the chemical classes identified in the EO compositions, the hydrodistillation yield (% w/w), the plant height (cm), the inflorescence yield (Mg ha^{-1}), the harvest index, the EO yield (kg ha^{-1}) and the meteorological data from plant sowing to collection (total rainfall, average T_{max} , average T_{min} , average T_{mean} , average ΔT , defined as the difference between T_{max} and T_{min}) and (ii) the compound relative abundances and the measured antioxidant activity, the Spearman's ρ non-parametric correlation matrix was used since the variables did not show Gaussian distributions. Spearman's correlation coefficient's statistical significance was tested by setting $\alpha = 0.05$.

3. Results

3.1. Weather Conditions

'Futura 75' showed good environmental plasticity and suitability to be grown in the tested areas of Central Italy, even on marginal land.

The climate of the three areas is classified as Mediterranean, with a mild winter, a hot summer and rainfall mainly concentrated in the autumn. The temperature patterns registered during the cultivation trials were quite similar in the three locations, while strong variations in rainfall and thermal excursion were observed among the sites. The total rainfall during the period from sowing to inflorescence harvest was 133.2 mm in SPG, 278.0 mm in SL and 195.0 mm in LA (Figure 1). In both SPG and SL, the precipitations were

mainly concentrated in July, while in LA, they were concentrated in April and May. From sowing to harvest, the average maximum and minimum air temperatures were (i) 27.7 °C and 13.6 °C with a ΔT equal to 14.1 °C in LA; (ii) 26.1 °C and 17.9 °C with a $\Delta T = 8.1$ °C in SPG; and (iii) 25.8 °C and 14.2 °C with a ΔT of 11.7 °C in SL. SPG was characterised by higher mean air temperatures (22.0 °C) from sowing to harvest compared to LA (20.6 °C) and SL (20.0 °C). Furthermore, from June to July, during the flowering period, in LA, the greatest mean (24.2 °C vs. 23.3 °C in SPG and 23.7 °C in SL) and maximum temperatures (32.3 °C vs. 27.5 °C in SPG and 30.4 °C in SL) and the lowest minimum temperatures (16.2 °C vs. 19.1 °C in SPG and 17.1 °C in SL) were registered, with consequently the highest thermal excursion (16.1 °C vs. 8.4 °C in SPG and 13.3 °C in SL). The LA site also showed the lowest amount of rainfall during the flowering period (65.6 mm vs. 91.2 mm in SPG and 142.2 mm in SL).

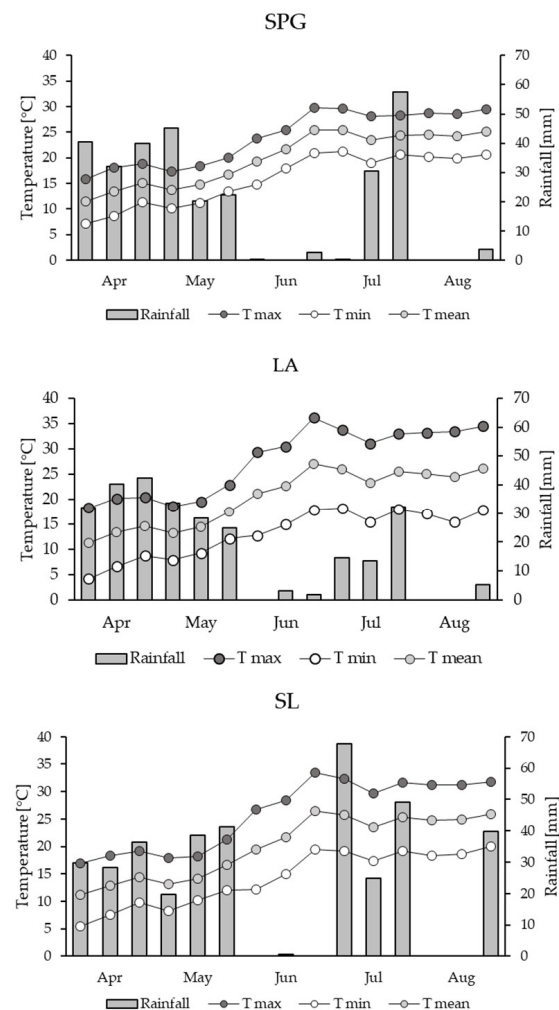


Figure 1. Monthly decade mean maximum temperature (Tmax), minimum temperature (Tmin), mean temperature (Tmean) and total rainfall during the growing seasons in SPG, LA and SL (Northern Tuscany, Italy; SPG = San Piero a Grado; LA = Larciano; SL = Santa Luce) during the 2019 growing season.

3.2. Inflorescence and EO Yield

In Table 2, the main biometric and productive parameters are shown. Among the environments, smaller plants were measured in LA, with plant height values 37.4% lower compared with those measured in SPG and SL.

Table 2. Plant height, inflorescence yield, harvest index and essential oil yield of hemp cv ‘Futura 75’ at the three sites (northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano).

	Plant Height (cm)	Inflorescence Dry Yield (Mg ha ^{−1})	Harvest Index	Hydrodistillation Yield (% w w ^{−1})	EO Yield (kg ha ^{−1})
SPG	207.0 ± 18.9 a	4.9 ± 0.5 a	42.8 ± 2.0 a	0.15 ± 0.04 a	7.1 ± 1.8 a
SL	218.2 ± 7.3 a	3.4 ± 0.5 b	26.1 ± 2.8 b	0.22 ± 0.08 a	7.5 ± 2.5 a
LA	133.2 ± 5.5 b	1.3 ± 0.2 c	21.1 ± 5.5 b	0.20 ± 0.01 a	2.6 ± 0.1 b

Means were subjected to Tukey’s HSD test; different letters indicate significant differences ($p < 0.05$) according to the one-way ANOVA test, with the environmental site as a variability factor. Harvest index: dry inflorescence yield-to-total above-ground dry biomass ratio.

The highest inflorescence yield was obtained in SPG, where the hemp produced +44.4 and +265.3%, compared to SL and LA, respectively. On the contrary, in LA, the lowest yields were obtained. A higher HI was calculated in SPG, with values of +21.7 and +16.8%, compared to LA and SL.

No difference in the EO yield was observed between SPG and SL, for which values slightly higher than 7 kg EO ha^{−1} were obtained, while the lowest EO yield (−64.1% compared to SPG and SL) was found in LA. Lastly, no differences in the EO hydrodistillation yield were measured among the three environments.

3.3. Essential Oil (EO) Compositions

The complete composition and extraction yields of the EOs obtained from the three accession sites are reported in Table 3.

Table 3. Complete compositions of the essential oils hydrodistilled from female flowers of *C. sativa* L. ‘Futura 75’ at the three sites (Northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano).

Compounds	Experimental l.r.i. ¹	Literature l.r.i. ²	Relative Abundance ± SD		
			LA	SPG	SL
α-pinene *	941	939	4.7 ± 1.04 ^B	8.3 ± 0.79 ^A	9.4 ± 1.20 ^A
camphene *	954	954	- ³	0.1 ± 0.02	0.2 ± 0.06
β-pinene *	982	981	1.2 ± 0.27 ^B	2.6 ± 0.30 ^A	2.4 ± 0.46 ^A
myrcene *	993	992	1.4 ± 0.34 ^C	6.4 ± 0.41 ^A	2.8 ± 0.24 ^B
α-phellandrene *	1005	1005	-	0.2 ± 0.01	-
δ-3-carene *	1011	1011	0.1 ± 0.09	0.2 ± 0.02	-
α-terpinene *	1018	1018	-	0.2 ± 0.01	-
limonene *	1032	1031	0.5 ± 0.10	0.9 ± 0.08	0.8 ± 0.10
1,8-cineole *	1034	1033	0.2 ± 0.06	0.1 ± 0.01	0.3 ± 0.05
(Z)-β-ocimene	1042	1043	-	0.2 ± 0.01	0.1 ± 0.03
(E)-β-ocimene	1052	1052	0.4 ± 0.15 ^C	2.1 ± 0.03 ^A	0.7 ± 0.12 ^B
γ-terpinene *	1062	1062	-	0.2 ± 0.02	0.0 ± 0.06
terpinolene *	1088	1088	0.8 ± 0.26 ^B	7.3 ± 0.19 ^A	0.6 ± 0.36 ^B
linalool *	1101	1099	-	0.1 ± 0.03	-
trans-pinocarveol	1139	1140	0.0 ± 0.06	-	0.0 ± 0.08
trans-verbenol	1144	1145	-	0.2 ± 0.01	-
borneol *	1165	1168	0.0 ± 0.06	0.1 ± 0.06	0 ± 0.06
lavandulol *	1168	1170	0.2 ± 0.06	-	-
4-terpineol *	1179	1177	0.2 ± 0.06	0.3 ± 0.03	0.1 ± 0.09
α-terpineol *	1189	1189	0.0 ± 0.06	0.2 ± 0.0	-
eugenol *	1358	1358	0.0 ± 0.06	-	0.3 ± 0.03
1-hexyl hexanoate *	1387	1386	-	0.1 ± 0.06	-
(Z)-caryophyllene	1405	1403	0.4 ± 0.05	0.3 ± 0.07	0.5 ± 0.08
α-gurjunene	1410	1410	-	-	0.0 ± 0.06
cis-α-bergamotene	1416	1415	0.2 ± 0.04	0.2 ± 0.19	0.1 ± 0.13
β-caryophyllene *	1420	1418	19.1 ± 0.47 ^B	27.1 ± 3.49 ^A	22.9 ± 1.24 ^{A,B}

Table 3. Cont.

Compounds	Experimental l.r.i. ¹	Literature l.r.i. ²	Relative Abundance $\pm$ SD		
			LA	SPG	SL
<i>trans</i> - α -bergamotene	1438	1438	1.3 $\pm$ 0.13 ^B	2.3 $\pm$ 0.57 ^A	1.7 $\pm$ 0.19 ^{A,B}
α -guaiene	1440	1440	-	-	0.1 $\pm$ 0.13
aromadendrene *	1445	1443	0.1 $\pm$ 0.02	-	-
α -humulene *	1456	1455	7.8 $\pm$ 0.11 ^C	11.3 $\pm$ 0.29 ^A	9.0 $\pm$ 0.64 ^B
(<i>E</i>)- β -farnesene *	1460	1459	1.6 $\pm$ 0.15 ^B	3.1 $\pm$ 0.93 ^A	2.3 $\pm$ 0.22 ^{A,B}
alloaromadendrene	1461	1461	1.1 $\pm$ 0.02 ^{A,B}	0.7 $\pm$ 0.34 ^B	1.5 $\pm$ 0.14 ^A
β -chamigrene	1473	1472	-	0.1 $\pm$ 0.06	-
γ -gurjunene	1474	1474	0.2 $\pm$ 0.10	0.2 $\pm$ 0.00	0.5 $\pm$ 0.05
γ -muurolene	1477	1477	0.1 $\pm$ 0.11	0.1 $\pm$ 0.07	0.2 $\pm$ 0.05
γ -selinene	1482	1481	0.5 $\pm$ 0.04	0.3 $\pm$ 0.29	0.7 $\pm$ 0.07
β -selinene	1485	1486	2.3 $\pm$ 0.07 ^A	2.3 $\pm$ 0.75 ^A	2.4 $\pm$ 0.43 ^A
valencene *	1492	1491	0.3 $\pm$ 0.06	-	-
α -selinene	1494	1497	1.5 $\pm$ 0.09 ^A	1.7 $\pm$ 0.71 ^A	2.3 $\pm$ 0.27 ^A
germacrene A	1503	1506	-	0.1 $\pm$ 0.07	-
α -bulnesene	1504	1505	-	-	0.1 $\pm$ 0.09
β -himachalene	1505	1503	0.1 $\pm$ 0.01	-	-
(<i>E,E</i>)- α -farnesene	1507	1508	0.2 $\pm$ 0.06	-	-
β -bisabolene	1509	1509	-	0.5 $\pm$ 0.10	0.3 $\pm$ 0.04
β -curcumene	1512	1510	0.1 $\pm$ 0.04	0.2 $\pm$ 0.05	0.2 $\pm$ 0.01
<i>trans</i> - γ -cadinene	1513	1513	0.1 $\pm$ 0.09	0.1 $\pm$ 0.10	0.2 $\pm$ 0.03
sesquicineole	1514	1513	0.2 $\pm$ 0.03	-	-
(<i>Z</i>)- γ -bisabolene	1515	1515	0.2 $\pm$ 0.18	-	-
7- <i>epi</i> - α -selinene	1517	1517	0.4 $\pm$ 0.02	0.2 $\pm$ 0.16	0.5 $\pm$ 0.07
β -cadinene	1520	1520	0.1 $\pm$ 0.11	0.1 $\pm$ 0.05	0.3 $\pm$ 0.16
δ -cadinene	1524	1524	0.4 $\pm$ 0.06	0.4 $\pm$ 0.13	0.5 $\pm$ 0.05
selina-3,7(11)-diene	1542	1542	2.3 $\pm$ 0.38 ^{A,B}	1.0 $\pm$ 0.99 ^B	3.8 $\pm$ 1.57 ^A
<i>cis</i> -sesquisabinene hydrate	1545	1544	0.1 $\pm$ 0.20	0.5 $\pm$ 0.25	0.3 $\pm$ 0.06
elemol	1549	1549	0.5 $\pm$ 0.04	0.1 $\pm$ 0.06	0.3 $\pm$ 0.07
germacrene B	1557	1556	-	0.1 $\pm$ 0.08	-
(<i>E</i>)-nerolidol *	1565	1566	1.1 $\pm$ 0.12 ^{A,B}	0.9 $\pm$ 0.10 ^B	1.2 $\pm$ 0.17 ^A
palustrol	1568	1571	0.5 $\pm$ 0.07	0.1 $\pm$ 0.07	0.4 $\pm$ 0.07
caryophyllene oxide *	1581	1578	12.1 $\pm$ 0.57 ^A	3.9 $\pm$ 0.83 ^C	6.7 $\pm$ 0.87 ^B
isoaromadendrene epoxide	1589	1590	-	-	0.3 $\pm$ 0.04
<i>epi</i> -globulol	1590	1587	0.1 $\pm$ 0.07	0.2 $\pm$ 0.04	-
viridiflorol *	1591	1589	0.5 $\pm$ 0.04	0.2 $\pm$ 0.19	-
guaiol *	1595	1597	0.5 $\pm$ 0.04	-	-
humulene epoxide II	1608	1605	4.3 $\pm$ 0.23 ^A	1.5 $\pm$ 0.24 ^C	2.4 $\pm$ 0.21 ^B
humulane-1,6-dien-3-ol	1613	1619	0.1 $\pm$ 0.13	0.3 $\pm$ 0.11	1.4 $\pm$ 0.20
selin-6-en-4-ol	1618	1624	1.8 $\pm$ 0.13	-	-
(<i>E</i>)-longipinocarveol	1624		0.5 $\pm$ 0.10	-	-
1- <i>epi</i> -cubenol	1628	1629	0.6 $\pm$ 0.21	-	0.9 $\pm$ 0.38
caryophylla-4(14),8(15)-dien-5-ol	1637	1636	6.9 $\pm$ 0.39 ^A	1.6 $\pm$ 0.45 ^C	4.8 $\pm$ 0.80 ^B
<i>epi</i> - α -cadinol	1641	1640	0.2 $\pm$ 0.03	-	-
cubenol	1643	1642	-	0.1 $\pm$ 0.07	-
β -eudesmol	1645	1645	-	0.1 $\pm$ 0.09	-
himachalol	1646		0.8 $\pm$ 0.07	0.7 $\pm$ 0.13	0.6 $\pm$ 0.23
neointermedeol	1660	1662	3.0 $\pm$ 0.11 ^A	0.9 $\pm$ 0.18 ^C	2.2 $\pm$ 0.26 ^B
14-hydroxy-9- <i>epi</i> -(<i>E</i>)-caryophyllene	1664	1665	3.0 $\pm$ 0.18 ^A	- ^C	1.8 $\pm$ 0.29 ^B

Table 3. Cont.

Compounds	Experimental I.r.i. ¹	Literature I.r.i. ²	Relative Abundance $\pm$ SD		
			LA	SPG	SL
aromadendrene oxide II	1678	1678	0.3 $\pm$ 0.02	0.5 $\pm$ 0.18	0.1 $\pm$ 0.09
α -bisabolol *	1683	1685	0.7 $\pm$ 0.03	0.1 $\pm$ 0.13	0.5 $\pm$ 0.13
juniper camphor	1692	1700	0.6 $\pm$ 0.1	-	0.6 $\pm$ 0.15
(Z,E)-farnesol	1697	1697	0.0 $\pm$ 0.08	-	-
(E,E)-farnesyl acetone	1920	1921	0.0 $\pm$ 0.08	-	-
2-methyl tricosane	2365	2365	0.4 $\pm$ 0.74	-	-
cannabidiol	2419		8.8 $\pm$ 0.95 ^A	6.1 $\pm$ 0.70 ^B	5.7 $\pm$ 1.20 ^B
cannabichromene	2427		0.1 $\pm$ 0.01	0.2 $\pm$ 0.01	0.2 $\pm$ 0.01
dronabinol (= THC)	2468		tr ⁴	tr	tr
Monoterpene hydrocarbons			9.1 $\pm$ 2.22 ^C	28.8 $\pm$ 1.00 ^A	17.2 $\pm$ 1.98 ^B
Oxygenated monoterpenes			0.5 $\pm$ 0.30 ^A	0.9 $\pm$ 0.06 ^A	0.4 $\pm$ 0.28 ^A
Sesquiterpene hydrocarbons			40.3 $\pm$ 0.48 ^B	52.0 $\pm$ 1.50 ^A	50.0 $\pm$ 2.30 ^A
Oxygenated sesquiterpenes			37.8 $\pm$ 1.58 ^A	11.7 $\pm$ 1.18 ^C	24.6 $\pm$ 3.10 ^B
Cannabinoids			9.0 $\pm$ 0.95 ^A	6.2 $\pm$ 0.71 ^B	5.9 $\pm$ 1.21 ^B
Apocarotenoids			0.0 $\pm$ 0.08 ^A	- ^A	- ^A
Phenylpropanoids			0.0 $\pm$ 0.06 ^A	- ^B	0.3 $\pm$ 0.03 ^B
Other non-terpene derivatives			0.4 $\pm$ 0.74 ^A	0.1 $\pm$ 0.06 ^A	- ^A
Total identified (%):			97.2 $\pm$ 0.76	99.6 $\pm$ 0.09	98.4 $\pm$ 0.64

¹ linear retention indices on a HP-5MS capillary column; ² NIST Chemistry WebBook (<https://webbook.nist.gov/>, accessed on 24 January 2024) and citations therein; ³ not detected; ⁴ traces, <0.1%; * identification confirmed by direct comparison with a pure analytical sample. Compounds, chemical classes, and extraction yields, reported in **bold**, were subjected to Tukey's HSD test; different superscript uppercase letters (A,B,C) indicate significant differences ($p < 0.05$) among the samples along the same row.

A total of 83 compounds were identified in the EOs, whose profiles exhibited more quantitative than qualitative differences. The EOs were mainly composed of sesquiterpenes, of which hydrocarbons were more represented, ranging from 40.3% in the LA sample to over 50% in the SPG and SL samples (52.0 and 50.0%, respectively). Among this chemical class, β -caryophyllene was detected as the most represented as well as the most abundant compound in all the compositions; its highest (27.1%) relative abundance was identified in the SPG essential oil, closely followed by the SL essential oil (22.9%). α -Humulene followed among the sesquiterpene hydrocarbons; like β -caryophyllene, it was more represented in the SPG sample (11.3%). Oxygenated sesquiterpenes followed as the second most abundant chemical class of compounds in the LA and SL samples, with relative concentrations of 37.8 and 24.6%, respectively. Among this class, caryophyllene oxide, caryophylla-4(14),8(15)-dien-5-ol and humulene epoxide II were the most represented; their relative presences were significantly lower in the SPG EO. The latter, indeed, showed a significantly higher abundance of monoterpene hydrocarbons compared to the other two accessions. Among this class, α -pinene, myrcene and terpinolene were the most represented, exhibiting a significantly higher relative presence in the SPG EO. Cannabinoids were more abundant in the LA EO, where they accounted for up to 9.0%. Among them, cannabidiol exhibited the highest relative concentration in all the samples, with a significantly higher presence in the LA EO. Cannabichromene and Δ^9 -tetrahydrocannabinol were, instead, found with relative contents of around 0.1% in all the samples.

3.4. Statistical Evaluation of the EO Compositions

The two-way dendrogram of the hierarchical cluster analysis (HCA) performed on the complete compositions of the hydrodistilled EOs reported in Figure 2 shows the distribution of the samples in two clusters.

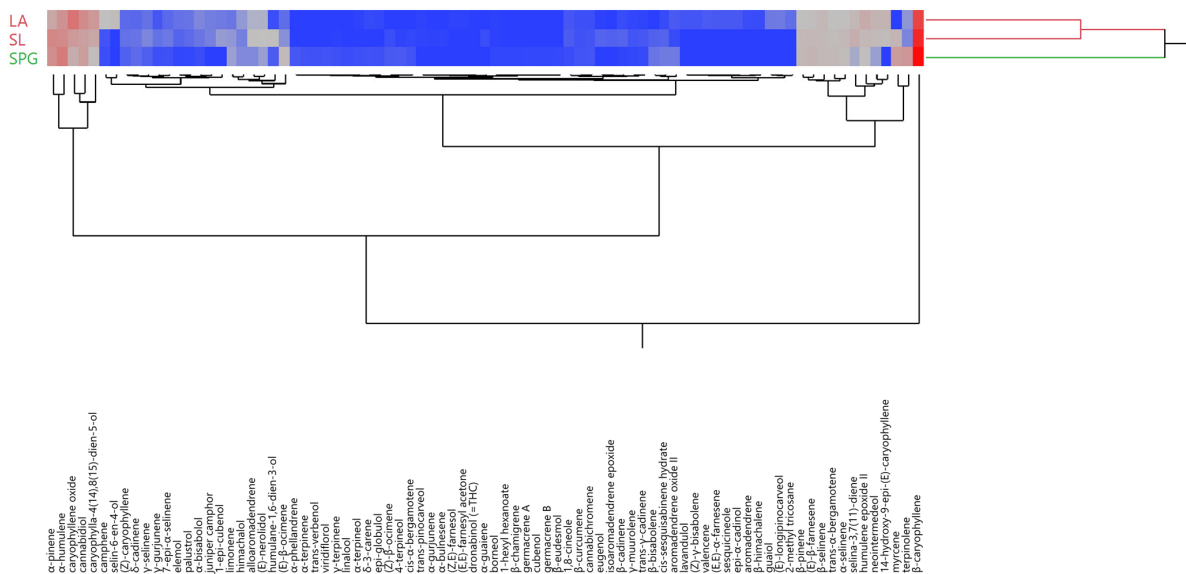


Figure 2. Two-way dendrogram of the hierarchical cluster analysis (HCA) of the complete composition of the EOs hydrodistilled from the three sites (northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano). Red colour indicates higher relative concentrations; blue colour indicated lower relative concentrations.

The red one is composed of the LA and SL samples, while the green one consists of the SPG sample alone; higher similarities were, thus, evidenced among the former samples. The clustering of the identified constituents drove the first main division between β -caryophyllene and all the other compounds. Among the latter, a higher proximity was shown for the most abundant compounds (α -pinene, α -humulene, caryophyllene oxide, cannabidiol and caryophylla-4(14),8(15)-dien-5-ol) compared to all the others.

The distribution of the samples based on their EO chemical compositions was further explored by means of a principal component analysis (PCA), for which the score and loadings plots are reported in Figure 3 (left and right, respectively).

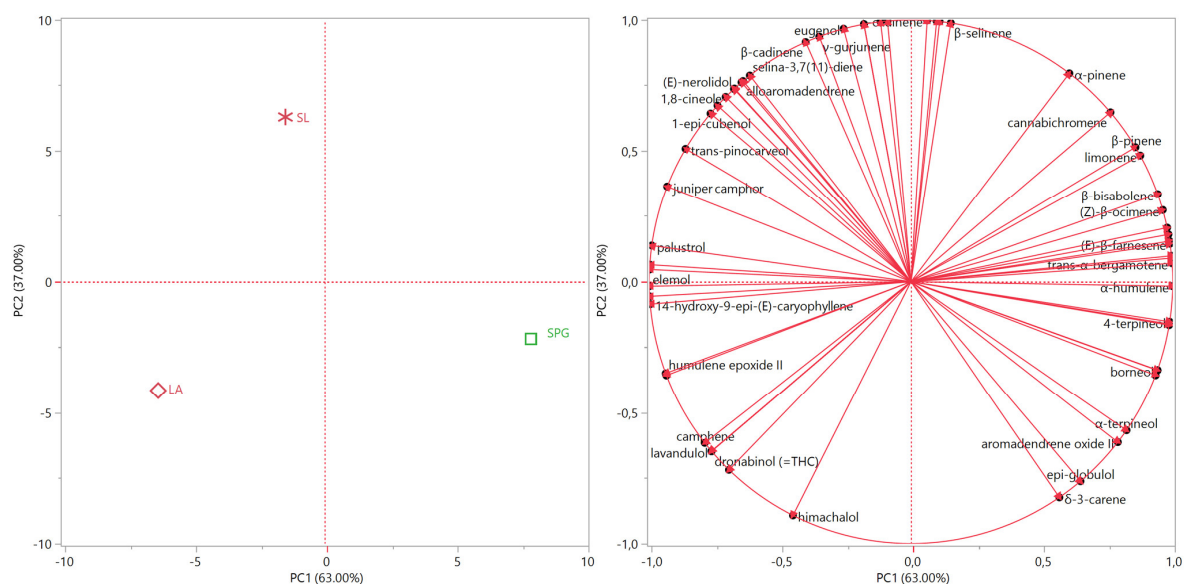


Figure 3. Score (left) and loadings (right) plots of the principal component analysis (PCA) of the complete compositions of the EOs hydrodistilled at the three sites (northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano).

The higher proximity pattern that emerged in the HCA for the LA and SL samples is confirmed by their being plotted in the left quadrants ($PC1 < 0$) of the score plot (Figure 3, left), although SL was positioned in the upper quadrant ($PC2 > 0$), while LA was in the lower one ($PC2 < 0$). As evidenced in the loadings plot (Figure 3, right), the position of the LA sample was due to its significantly higher content of the main oxygenated sesquiterpenes (caryophyllene oxide, 14-hydroxy-9-*epi*-(*E*)-caryophyllene and humulene epoxide II). The EO of the SL inflorescences, instead, was plotted in the upper quadrant due to its higher α -pinene content compared to the LA EO. The EO of SPG, instead, exhibited the rightmost positioning in the lower quadrant of the score plot (Figure 3, left), due to its significantly higher relative concentrations of β -caryophyllene, α -humulene and myrcene.

A linear discriminant analysis (LDA) was performed on the EOs to assess the compositional features separating the samples according to their growth area, taken as an *a priori* grouping. In the obtained canonical plot (Figure 4), the Euclidean distances among the samples are reported.

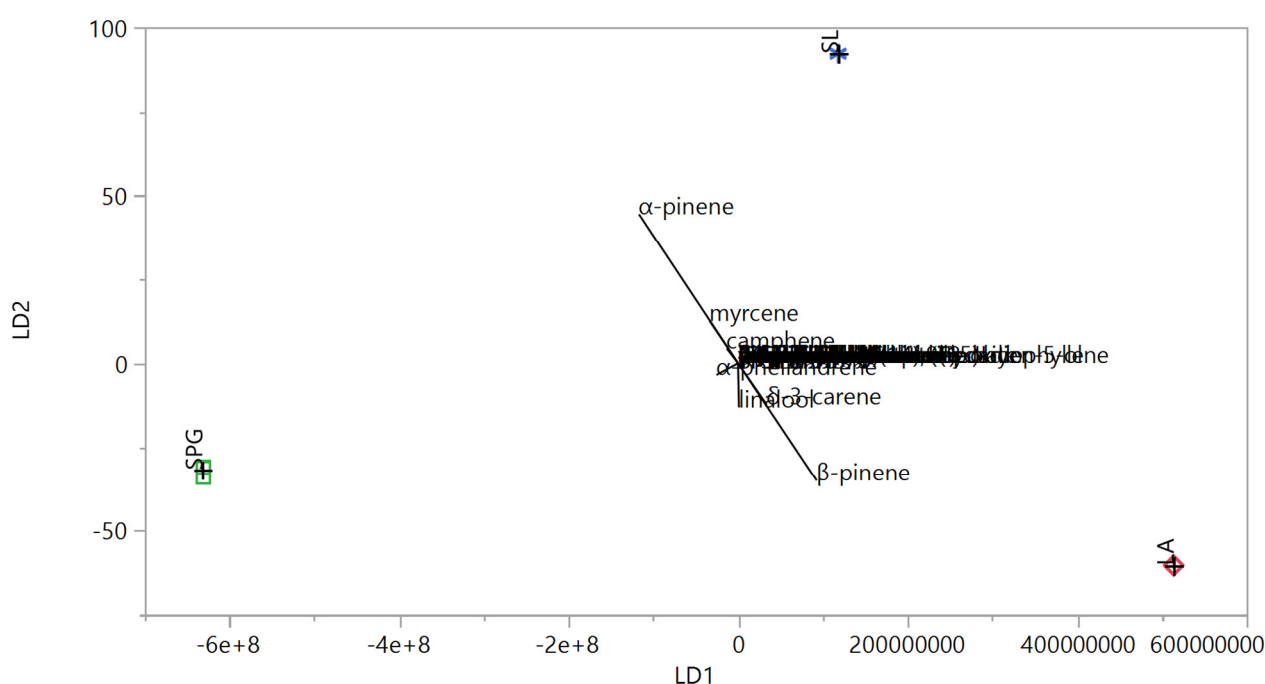


Figure 4. Canonical plot of the linear discriminant analysis (LDA) of the complete compositions of the EOs hydrodistilled at the three sites (northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano).

The length of each vector is directly proportional to the relative abundance of the corresponding compound, thus evidencing which compounds generated the most relevant differences among the samples. The highest proximity of the SL and LA samples was confirmed; their plotting was driven by the significantly more abundant content of α - and β -pinene, respectively. The SPG sample was positioned further away, but its higher degree of differentiation appeared to be due to mostly quantitative, rather than qualitative, differences in the most represented compounds, common to all the accessions.

3.5. Evaluation of the Correlation between Chemical and Agronomic Traits with the Meteorological Data

The agronomic traits and the chemical compositions of the hydrodistilled EOs were correlated with the meteorological data registered during the full plant cycle, from sowing to harvest. All the detected significant correlations according to Spearman's correlation coefficients and their significance levels are reported in Table 4.

Table 4. Spearman’s correlation coefficient (ρ) and statistical significance ($\text{Prob} > |\rho|$) of the correlation between the chemical classes (MH, monoterpene hydrocarbons; SH, sesquiterpene hydrocarbons; OS, oxygenated sesquiterpenes; PP, phenylpropanoids; CAN, cannabinoids; NT, non-terpene derivatives) identified in the EO compositions, the hydrodistillation yield (% *w/w*), plant height (cm), inflorescence yield (Mg ha^{-1}), harvest index, EO yield (kg ha^{-1}) and meteorological data from plant sowing to collection (total rainfall, average Tmax, average Tmin, average Tmean, average ΔT). Only the statistically significant correlations ($\alpha = 0.05$) are reported.

Variable	Variable by	Spearman’s ρ	Prob > $ \rho $
Significant negative correlations			
Tmin	OS	−0.9487	<0.0001
ΔT	Inflorescence yield	−0.9487	<0.0001
Tmean	PP	−0.866	0.0025
ΔT	SH	−0.8433	0.0043
ΔT	Harvest index	−0.8433	0.0043
Inflorescence yield	OS	−0.8333	0.0053
Harvest index	OS	−0.8167	0.0072
Plant height	OS	−0.7833	0.0125
Plant height	CAN	−0.75	0.0199
Tmax	Plant height	−0.7379	0.0232
ΔT	EO yield	−0.7379	0.0232
Tmax	PP	−0.6928	0.0386
Tmax	EO yield	−0.6852	0.0417
ΔT	MH	−0.6852	0.0417
ΔT	Plant height	−0.6852	0.0417
Tmean	Hydrodistillation yield	−0.6644	0.051
Tmean	Plant height	−0.0527	0.8929
Significant positive correlations			
EO yield	Hydrodistillation yield	0.0756	0.8467
Tmean	MH	0.1054	0.7872
Tmean	NT	0.2887	0.4512
Tmean	Inflorescence yield	0.4743	0.197
Plant height	SH	0.6667	0.0499
EO yield	Plant height	0.6667	0.0499
EO yield	Harvest index	0.6667	0.0499
Tmin	MH	0.6852	0.0417
Tmin	Plant height	0.6852	0.0417
EO yield	Inflorescence yield	0.7333	0.0246
Tmin	EO yield	0.7379	0.0232
Harvest index	SH	0.7833	0.0125
Inflorescence yield	SH	0.8333	0.0053
Harvest index	Inflorescence yield	0.8333	0.0053
Tmin	SH	0.8433	0.0043
Tmin	Harvest index	0.8433	0.0043
Plant height	MH	0.85	0.0037
Rainfall	PP	0.866	0.0025
Tmin	Inflorescence yield	0.9487	<0.0001
ΔT	OS	0.9487	<0.0001

The inflorescence yield, harvest index, plant height and EO yield were found to be negatively correlated with the ΔT , suggesting that the highest thermal excursion negatively affected these parameters. Furthermore, negative correlations were found between the EO yield and the Tmax and between the plant height and the Tmax and Tmean. The plant height, inflorescence yield, harvest index and EO yield were positively correlated with the Tmin, suggesting that milder minimum temperatures are favourable to optimal

hemp production. Finally, a positive correlation was observed between the Tmean and the inflorescence yield.

Monoterpene and sesquiterpene hydrocarbons (MHs and SHs, respectively) were both negatively correlated with the ΔT , thus showing a reduction when the difference in temperatures during the studied period was higher; conversely, they were both positively correlated with the plant height and Tmin. SHs were also positively correlated with the harvest index (HI) and the inflorescence yield, while the opposite was true for their oxygenated counterparts. Oxygenated sesquiterpenes (OSs), indeed, were negatively influenced by these factors as well as by plant height, while they showed an increment due to a higher ΔT . Cannabinoids (CAN), similar to Oss, had a negative correlation with the plant height. Phenylpropanoids (PPs) were the only compounds influenced by the rainfall parameter, as they showed a positive correlation.

3.6. EOs' Antioxidant Activity and Correlation with Their Compositions

The antioxidant activity of the hydrodistilled EOs was evaluated with a DPPH assay with the aim of evidencing the growth site effect on the radical-scavenging properties of the produced EOs. The results, expressed as mmol TE/g and inhibition %, are reported in Table 5.

Table 5. Antioxidant activity of the EOs hydrodistilled at the three sites (northern Tuscany, Italy; SPG = San Piero a Grado; SL = Santa Luce; LA = Larciano) tested by DPPH assay.

Site	DPPH mmol TE/g	Inhibition %
SPG	0.038 ± 0.0032 c	62.24 ± 5.25 c
SL	0.051 ± 0.0015 a	85.19 ± 2.56 a
LA	0.046 ± 0.0014 b	76.62 ± 2.34 b

Results were subjected to Tukey's HSD test; different letters indicate significant differences ($p < 0.05$) according to the one-way ANOVA test, with the environmental site as a variability factor.

All the tested EOs exerted antioxidant activity accounting for over 60% of inhibition: the least active was the SPG essential oil (62.24%), while the highest antioxidant capacity was found for the SL EO (85.19%). The Spearman's correlation coefficients and their significance levels for the antioxidant activity of all the EO compounds are reported in Table 6.

Table 6. Spearman's correlation coefficient (ρ) and statistical significance (Prob > $|\rho|$) of the correlation between each compound and the antioxidant activity shown by each EO in the DPPH assay. Only the statistically significant correlations ($\alpha = 0.05$) are reported.

Compounds	Spearman's ρ	Prob > $ \rho $
Significant negative correlations		
α -phellandrene	−0.69310328	0.038441
α -terpinene	−0.69310328	0.038441
γ -terpinene	−0.821583836	0.006603
terpinolene	−0.85	0.003705
linalool	−0.732709182	0.024739
<i>trans</i> -verbenol	−0.677296212	0.045043
4-terpineol	−0.821583836	0.006603
α -terpineol	−0.689454293	0.039906
Significant positive correlations		
7- <i>epi</i> - α -selinene	0.75	0.019942
β -cadinene	0.669461927	0.048568
selina-3,7(11)-diene	0.666666667	0.049867
palustrol	0.666666667	0.049867
1- <i>epi</i> -cubenol	0.91538573	0.000534

Table 6. Cont.

Compounds	Spearman's ρ	Prob > $ \rho $
caryophylla-4(14),8(15)-dien-5-ol	0.7	0.03577
14-hydroxy-9- <i>epi</i> -(<i>E</i>)-caryophyllene	0.678063504	0.044707
α -bisabolol	0.677830201	0.044809
juniper camphor	0.876794227	0.001912

Strong positive correlations were found between the antioxidant activity scored in this assay and 1-*epi*-cubenol and juniper camphor, while notable negative correlations were found with terpinolene, 4-terpineol and γ -terpinene.

4. Discussion

In the context of agroecological transition, cropping system diversification represents a win–win strategy for adaptation and mitigation to climate change and to increase sustainability. At the same time, farmers, by growing low-input and multipurpose crops such as hemp, can be key actors in producing high-added-value products, accessing new market opportunities and also exploiting marginal soils. In addition, local instances relating to the introduction of industrial hemp under real-farm conditions are very scarce. So, in this study, the potential to introduce industrial hemp at the farm level and in different pedo-climatic conditions was evaluated. Differences in productive parameters were observed among the farms, validating how location specificities, like agronomic management and environmental conditions, can significantly influence crop performance in terms of growth, development and yield. These findings underlined how weather conditions significantly affect hemp development and yields, despite its adaptability to various environments.

Several studies have demonstrated that the availability of nutrients and water (both received and groundwater), as well as photoperiod and day/night temperatures, are crucial factors in affecting flowering time, which, in turn, can influence biomass, inflorescence and seed yield [48,49]. In our study, in the period from sowing to harvest and during flowering, remarkable differences were registered among the three locations, with LA characterised by the lowest amount of rainfall and greater maximum air temperatures, together with the highest thermal excursion (ΔT). It is known that environmental conditions can significantly affect the flowering period. Some authors [1,50] have observed that, in hemp, drought combined with high temperatures can accelerate flowering development. This plant response can lead to a reduction in total biomass and inflorescence yield, as observed in LA. The correlation analyses carried out in our study confirmed these findings, demonstrating that, among all the weather variables considered, air temperatures were the most relevant in influencing the production parameters of Futura 75. In particular, a reduction in the inflorescence yield, harvest index, plant height and EO yield was observed in LA, which was characterised by the highest thermal excursion. These observations, together with the negative effect of the increases in maximum temperatures, suggest that these environmental variables constitute stress factors for hemp's reproductive development. On the contrary, the increase in the minimum daily temperature favoured the accumulation of biomass, with a consequent improvement in the productive parameters.

In addition, nutrient availability, such as nitrogen (N), is important for hemp growth and development as well as for the secondary metabolite content and inflorescence EO hydrodistillation yield [51–55]. Regarding nutrient availability, in the present study, significant variations in N availability were detected among the cultivation sites due to both the soil composition and cultivation practices adopted at the farm level (see Section 2.1). High inflorescence yields were observed in SPG due to both the favourable climatic conditions and the integrated management of hemp cultivation. In addition, an important role in defining hemp's response to the environment is played by soil water availability, especially during the initial stage of root establishment. As is known, soil water availability strongly depends on the weather conditions of a region as well as on the soil's physical and chemical

characteristics. Generally, well-drained soils with a loam texture that are rich in organic matter and have an adequate water-holding capacity and good aeration are preferable for hemp cultivation [56]. In general, the soil in SPG was characterised by these traits, and this probably contributed to the best performance of the hemp observed in this environment.

The EO yield per hectare is in line with that already observed in Futura in Italy by Vuerich et al. [57], where, similarly to what we observed, the EO yield per hectare depended mainly on the inflorescence yield rather than the hydrodistillation yield.

Overall, despite the environmental conditions (soil and climate) and agronomic management, this on-farm study showed a good agronomical adaptability of cv 'Futura 75' to the tested environments, confirming its feasibility to be introduced in organic and low-input farming systems and on marginal lands [33].

The yields and compositions of the essential oils reported in the present study were in good agreement with published reports on several hemp EOs, for which the general qualitative composition is consistent, while the most notable changes are quantitative [19,58]. An overall higher presence of sesquiterpene hydrocarbons compared to oxygenated compounds was also reported for 'Futura 75' accessions from Central Italy, although the latter were richer in monoterpene hydrocarbons compared to the samples in the present study [23].

Although compositional patterns mainly vary in quantitative terms, fragrance profiles are affected since each EO compound is characterised by an olfactive threshold. However, the characteristic aroma of an essential oil is a complex combination of the volatility, olfactive threshold, interaction and relative concentration of the involved constituents [59], as the whole volatile bouquet collectively determines the final perceived aroma [19,58,60]. The most abundant terpenes detected in the EOs in the present study, such as β -caryophyllene, myrcene, α -humulene and α - and β -pinene, are common to almost all hemp chemovars; they make up a typical hemp aroma bouquet, characterised by woody and hoppy odour notes [11,61]. The aroma contributions of the other compounds are considered cultivar-related undertones [11]. For example, fruity and sweet notes are attributed to caryophyllene oxide [61], while the odour of humulene epoxide II is described as woody and tea-like [62].

The variability observed in the EO compositions among the three sites was strictly correlated with the differences registered in the air temperature patterns. In this regard, Vuerich et al. [57] found a significant effect of the environment only on the inflorescence yield of Futura 75 but not on the composition of its EO, which is partially in contrast with our results. According to our correlation analyses, a higher ΔT reduced the MH and SH concentrations of EO, as observed in LA. OSs, instead, increased with a higher ΔT , as confirmed by the highest concentration of these compounds in the EO derived from LA inflorescences. These findings suggest that the increase in OSs and the reduction in MHs and SHs in hemp EO could be a plant response against thermal stress. To the best of our knowledge, no studies have been carried out aiming to evaluate the effect of weather conditions during plant growth on hemp EO. The only studies in this regard have been conducted on EOs extracted from other aromatic and medicinal plants [63,64], highlighting how air temperature and soil humidity can influence not only biomass yield but also the qualitative traits of EOs.

Although no statistical correlation was found between the β -caryophyllene and caryophyllene oxide contents and the antioxidant capacity of the studied EOs in the performed DPPH assay, the presence of these sesquiterpenes is reported as favourable for the radical-scavenging ability of EOs, not only for hemp [20] but also for other species [65–67]. Compared to the EO hydrodistilled from a 'Futura 75' accession from Central Italy reported by Benelli et al. [23], all the samples in the present study exerted a higher antioxidant activity on the DPPH assay; all of them, however, were less active than CBD, considering the data reported by Benelli et al. [23]. Moreover, the latter attributed to cannabidiol a significant contribution to the overall antioxidant power of the EO; in the present work, however, the highest cannabidiol relative abundance was reported for the LA accession, which did not exhibit the highest antioxidant activity. This is in accordance with the widely

accepted concept of the whole phytocomplex contributing to the overall antioxidant activity rather than a single or small handful of compounds detected in high concentrations [68].

5. Conclusions

Definitively, the present study, carried out under real on-farm conditions across different environments, demonstrated that industrial hemp could be an interesting opportunity for Mediterranean farmers who need to differentiate their rotations and their income.

Even in low-input and organic cropping systems and on marginal lands (LA and SL), promising productive results were achieved, suggesting that hemp is a versatile crop. These observations are particularly interesting since the study includes only on-farm trials carried out by farmers who were approaching this crop for the first time. Nevertheless, understanding the real influence of a specific environment on successfully introducing this crop plays a crucial role since our results underlined that pedoclimatic conditions can affect hemp yields despite its high rusticity and adaptability to various environments. Furthermore, the three sites did not induce a chemotype switch in *C. sativa* ‘Futura 75’; quantitative, rather than qualitative, differences were evidenced in the EO compositions. Similarly, the three EOs exhibited different antioxidant capacities, confirming the crucial role played by location specificities like agronomic management and environmental conditions.

The findings achieved in this study pointed out, for the first time, the important role of air temperature in defining hemp agronomic performance in terms of growth and inflorescence yield as well as EO composition. Further studies on the effect of environmental parameters on the quantitative–qualitative characteristics of hemp inflorescences will be necessary to confirm the important role played by climatic factors, such as air temperatures, including both multilocation and multiyear experimental trials.

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