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Lebanese Cannabis: Agronomic and Essential Oil Characteristics as Affected by Sowing Date and Irrigation Practice

Rhend Sleiman [1], Marie Therese Abi Saab [1,2,*], Jocelyne Adjizian Gerard [3], Salim Fahed [1], Ali Chehade [4], Abdel Kader Elhajj [1], Adla Jammoul [1], Georges Mansour [1], Mohamed Houssemeddine Sellami [5], Mladen Todorovic [6] and Rossella Albrizio [7]

1 Climate and Water Unit, Lebanese Agricultural Research Institute, Fanar 90-1965, Lebanon
2 Department of Agriculture, School of Engineering, The Holy Spirit University of Kaslik, Kaslik P.O. Box 446, Lebanon
3 Department of Geography, CREEMO (Centre de Recherche en Environnement-Espace Méditerrannée Orientale), Saint Joseph University, Beirut 17-5208, Lebanon
4 Department of Biotechnology, Lebanese Agricultural Research Institute, Tal Amara P.O. Box 287, Lebanon
5 ARCA 2010 Contrada Varignano, 7, 81100 Acerra, Italy
6 CIHEAM—Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano, Italy
7 National Research Council of Italy, Institute for Mediterranean Agricultural and Forestry Systems (CNR–I.S.A.FO.M.), P.le Enrico Fermi 1–Loc. Granatello, 80055 Portici, Italy
* Correspondence: mtabisaab@lari.gov.lb; Tel.: +961-3-902-994

Abstract: A field experiment was carried out in Lebanon to assess the agronomic and essential oil characteristics of cannabis as affected by sowing date and irrigation practice. The experiment consisted of a split-plot design with the water regime being the main factor (I_{opt}-irrigated when the readily available soil water is depleted; I_{50}- receiving 50% of the irrigation amounts in I_{opt} treatments) and sowing date as the sub-plot factor (mid-April; end of April; mid-May). Biometric and seed quality parameters of the cannabis crop were determined. The essential oils (EO) of the inflorescence were subjected to a multivariate analysis such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). The obtained results revealed that the aboveground fresh biomass, the dry matter, and the plant height were 55.08%, 59.62%, and 43.11% higher in I_{opt} than in I_{50}, respectively. However, the EO content was neither statistically affected by the irrigation regime nor by the sowing date. Under early sowing, both the water-use efficiency (WUE) for biomass and the EO production reached their highest values. All treatments presented a similar seed composition except that the crude fat and crude protein content were more elevated in I_{opt} than in I_{50} treatments. The main extracted essential oils in cannabis inflorescence corresponded to twenty-six identified compounds representing 79.34% of the monoterpenes and 81.25% of the sesquiterpenes. The monoterpenes were highly correlated with the irrigation treatment and early-April sowing while the sesquiterpenes were better enhanced under I_{50} and end of April to mid-May sowing. The study reveals that agronomic practices lead to differential responses of pharmacologically useful plant compounds for improved health benefits. Further research is required to clarify the potential for cannabis cultivation in Lebanon.

Keywords: *Cannabis sativa* L.; agronomical practices; terpenoids; essential oil

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1. Introduction

Cannabis (*Cannabis sativa* L.) is an important herbaceous species native to Central Asia [1] and its use by humans as a food source [2], bioenergy [3], fiber production [4], cosmetics [5], and medicinal preparations [6] has spread around the world for millennia. Cannabis is an annual, dioecious plant [7]. Three hemp species are widely recognized:

Cannabis sativa, *Cannabis indica,* and *Cannabis ruderalis* [8]. An alternative point of view is that cannabis is monotypic, while the subpopulations are subspecies of *Cannabis sativa* L. [9].

To date, more than 500 phytochemicals have been identified in cannabis strains [10]. Amino acids, fatty acids, and steroids belong to primary metabolism. Cannabinoids, terpenoids, and flavonoids belong to secondary metabolites [11]. Of the cannabis compounds, cannabinoids have been the best studied for their potential therapeutic value. A total of 113 cannabinoids have been identified [10]. They are accumulated mainly in the inflorescences and the leaves of the female flowers [12]. Cannabis drugs (marijuana) contain large amounts of the psychoactive cannabinoid Δ9-tetrahydrocannabinol (THC), which has medicinal and recreational uses. Hemp strains grown for seed and fiber production contain low levels of THC [13]. Another group of compounds in cannabis is the terpenes [14]. These are the main contributors to the plant's unique aroma [15]. The correlation between cannabinoids and terpenoids could contribute to the advantageous medical effect of various cannabis strains [16–18]. Cannabis can grow under a variety of agroecological conditions [19], requires low input of water and fertilizers [20], and its cultivation becomes possible without the use of agrochemicals to control weeds, pests, and diseases [2]. This culture has a positive impact on the environment [21] and is an excellent cover crop that, thanks to its extensive root system, can improve soil structure [22] and its ability to adapt to different situations of abiotic stress [23]. In addition, there is growing interest in cultivating cannabis for other purposes, such as using its inflorescences to extract essential oils (EO) [24,25].

The optimum selection of the most adapted cultivars concerning the growing area and respective agronomic practices such as sowing dates, irrigation regime, planting density, and nitrogen fertilization should require special attention [26–29]. Particularly, the effects of the sowing date and irrigation regime on cannabis essential oil have not been properly addressed so far. Some research studies indicated a significant improvement when the plant is sown by the end of April in semi-arid agricultural areas [30]. Moreover, some studies observed that the optimal sowing date from the last week of April to mid-May and water stress in late spring had reduced the radiation use efficiency, the leaf area index, and therefore, the aboveground biomass and yield of cannabis in a Mediterranean environment [26–31]. Another study conducted in Iran showed that water stress had reduced the cannabis yield of different screened ecotypes [32]. The effect of agronomic practices on the differential responses of pharmacologically useful plant compounds is becoming an important topic nowadays. Some of the recent studies that highlighted the effect of water irrigation levels on different essential oil compounds were presented by [33–35].

In Lebanon, cannabis cultivation dates back to Roman times. Until April 2020, Lebanon banned the cultivation of cannabis. However, the plant has been cultivated illegally in the Beqaa Valley in eastern Lebanon for decades [36]. Currently, there is an increased interest in the cultivation of cannabis, especially since the government's legalization efforts are underway. Limited early studies on Lebanese cannabis have been reported [37–40]. The novelty of this study is to make deeper and larger the partial knowledge of local research on the adaptability of cannabis to diverse agroecological conditions and to investigate in which it can be grown.

Some crucial agronomical practices, such as the optimum sowing dates and the irrigation criteria requirements to optimize cannabis yield and seed quality have not yet been conducted. Consequently, this study aimed to assess the impact of water regimes and sowing dates on the biometric parameters and seed quality of a cannabis crop, as well as the EO composition of the cannabis inflorescence in Lebanon.

2. Materials and Methods

2.1. Experimental Site and Climatic Data

A trial was implemented in the experimental field of the Lebanese Agriculture Research Institute (LARI) located in the Bekaa valley, Lebanon (36°02′47″ N, 34°00′34″ E and 1049 m above sea level), specifically in Kferden village, during the summer season of 2020.

The soil has a loam texture, a mean pH of 7.39 ± 0.15, an EC of 0.12 ± 0.02 dS.m^{-1}, and an OM content of 2.14 ± 0.30%. The average soil water holding capacity is 127 mm/m.

The climate of the study area is characterized by a hot and dry season from April to October. The main weather parameters were collected from a standard agrometeorological station located at the experimental station of the Lebanese Agriculture Research Institute (LARI). Figure 1 shows the meteorological regimes for the 2020 season's baseline (ETo), (P), (Tmax), and (Tmin).

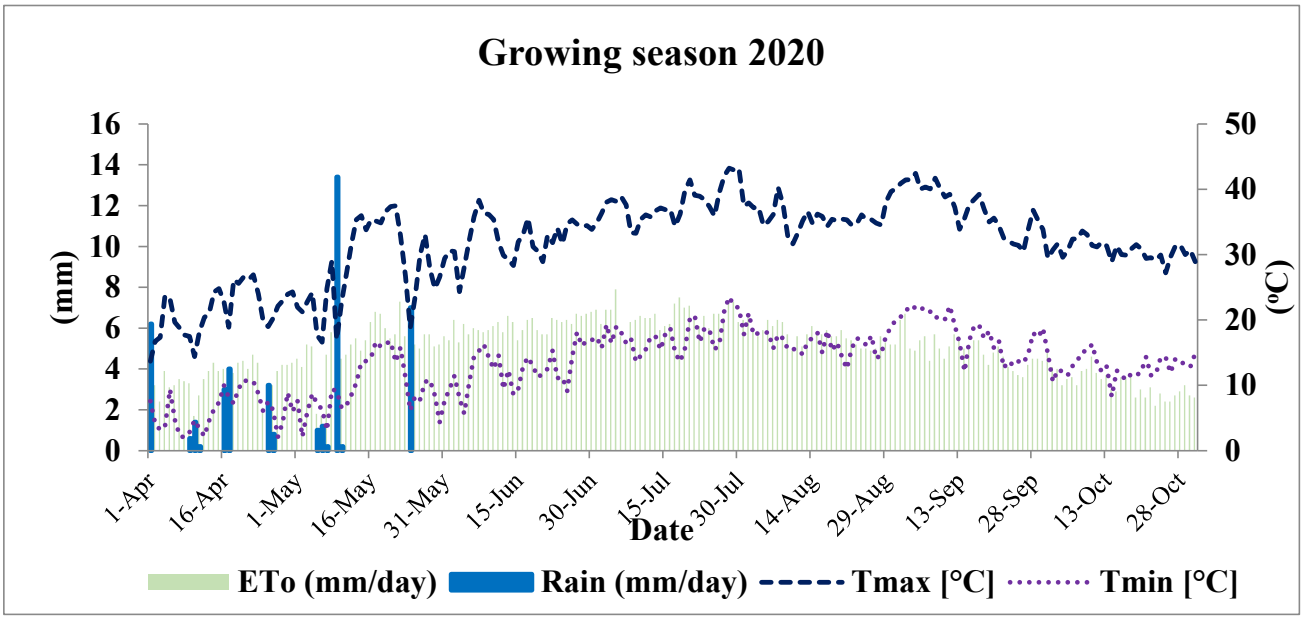

Figure 1. Rainfall (mm), maximum and minimum temperatures (°C), and reference evapotranspiration (ETo; mm) for the growing season of 2020.

In general, the average Tmax and Tmin from April to October 2020 were 31.9 and 13.4 °C, respectively. The total precipitation amount was 42.40 mm. These data are in agreement with the historical weather data for the study area as shown in the Supplementary Materials (Table S1).

2.2. Management of Crop and Experimental Design

The experiment was established as a split-plot design with the water regime as the main factor (I_{opt}—irrigated when the readily available soil water is depleted; I_{50}-receiving 50% of the irrigation amounts in I_{opt} treatments) and sowing date (SD) as the sub-plot factor (mid-April -SD1-; end of April -SD2-; mid-May -SD3). In total, there were six treatments and three replicates per treatment, which gave 18 plots. Each plot had a size of 2 m × 2 m, and cannabis seeds were sown in situ, 3 to 4 cm deep in rows 20 cm apart. Before sowing, the soil was prepared by following the local farming practices in the region. In particular, the soil was plowed at a depth of 30 cm in the autumn season, while in the spring, it was leveled once with a double disc halo and once with a cultivator just before planting. Then, triple superphosphate was applied at 60 kg ha^{-1}. Herbicides and pesticides were not applied during the growing season. Harvest took place in September.

All the plots were equipped with a drip irrigation system. The driplines were made using low polyethylene surface laterals with external diameters of 16 mm having inline drippers with a discharge rate of 4 L h^{-1}. The distance between the inline drippers was 20 cm. The spacing between laterals was 40 cm. Each plot had a separate shut-off valve.

Irrigation management was applied by checking the conditions of the weather. The soil moisture balance of the active root zone (40 cm) was considered. Therefore, an Excel-based irrigation tool was used to calculate irrigation volumes [41]. The tool considers weather, soil, and crop data for a daily estimation of the soil water balance.

First, it calculates the reference evapotranspiration daily from weather data using the Penman—Monteith Equation (ETo) [42]. Then, the crop evapotranspiration (ET) was calculated on daily basis by multiplying the ETo with the crop coefficient (Kc) values of 0.5 from sowing to 3–4 pairs of leaves, 0.9 from 3–4 pairs of leaves to the appearance of male flowers, and 1.1 from the appearance of male flowers to the fruit ripening stage, as also adopted by [27–41] with hemp plants. The depletion factor of the readily available water was set up as 0.66 [31]. Irrigation began when the readily available water in the 40 cm soil layer was completely depleted. I_{opt} plots were replenished to the field capacity level while I_{50} plots received half of the water supplied to I_{opt}.

Irrigation was stopped at the end of the fruit ripening stage. The total net irrigation amounts for $I_{opt\ mid\text{-}April}$, $I_{opt\ end\ of\ April}$, and $I_{opt\ mid\text{-}May}$ were 623, 644, and 681 mm, respectively. I_{50} treatments received 50% of those quantities.

2.3. Collection of Data and Analysis

2.3.1. Field Measurements and Sample Collection

In the field, the plants inside a 1 m × 1 m frame were manually cut at the base of the stem from each plot and placed in paper bags. In the laboratory, the plant height and fresh weight were determined. Then, some plants were oven-dried at 70 °C to a constant weight to measure dry weight, while the inflorescences of other plants were dried in the shade at room temperature (25 °C) for 3 days and powdered before hydrodistillation for essential oil extraction. Seeds were also separated from some inflorescence and then dried (at 25 °C) and cleaned.

The water-use efficiency (WUE) in terms of biomass and also essential oil production of the flowers were calculated by considering the ratio of those parameters over the total net irrigation amount.

2.3.2. Hydrodistillation for Essential Oil Extraction

EO was obtained by the method of hydrodistillation from dried inflorescence samples (60–80 g) using standard Clevenger-type apparatus. Hydrodistillation continued for about 2.5 h. EO was dried with anhydrous NA_2SO_4 and stored in dark glass bottles at 4 °C until analysis [43], as recommended by [25–44].

2.3.3. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

1. Chromatographic conditions

Analyzes were performed on Agilent Technologies, Inc. 19091S-433, with a helium flow rate of 1 mL/min, an inlet temperature of 250 °C, an injection volume of 2 µL, an oven temperature program of 50 °C, then 85 °C, then 165 °C, with the after-run temperature set at 280 °C for 10 min.

2. Mass spectrometric conditions

The MS (Agilent 5975B) was set from 40 to 450 amu; the ionization energy was set to 70 eV; the ion source temperature was set to 230 °C; the quadrupole temperature was set to 150 °C; the solvent delay was set to 4 min and the transfer line temperature was set to 280 °C. Software (NIST 2.0d; National Institute of Standards and Technology Standard Reference Data Program distributed by Agilent Technologies Germany) was used to help identify compounds.

3. Identification of constituents

The identification of constituents was performed based on retention indices (RI) determined from a homologous series of n-alkanes (C4–C30) under identical experimental conditions, with co-injection with either standard (Sigma-Aldrich, St. Louis, MO, USA) or known essential oil constituents, MS library search (NIST 05), and by comparing with MS literature data [45]. The used retention indices are shown in the Supplementary Materials (Table S2).

2.3.4. Seed Composition

Seed samples collected from each plot were used for the determination of the dry matter content, the ash content, the crude fat, the crude protein, and the crude fiber. All the analysis methods were according to AOAC [46].

1. The dry matter and ash content

The dry matter was determined by drying 1 g of the ground sample in the oven at 105 °C until reaching a constant weight. The ash content was analyzed in a 3 g seed sample that was dried in the oven at 500 °C for 3 h.

2. The crude protein

The crude protein was determined by weighing 0.5 g of the seed sample. 3.5 g of the catalytic mix and 8.5 mL of H_2SO_4 were added and heated for about 90 min. 30 mL of H_3BO_3 and 2 drops of the indicator were also added. The sample was transferred into the distiller and NaOH was poured into the boiling chamber. The beaker was held under the distiller and collected not less than 20 mL. Then, 0.1 N HCl was poured into the sample. The spent amount of 0.1 N HCl was noted.

3. The crude fat

The crude fat was determined by weighing 5 g of the sample. 70 mL of petroleum ether was added, then put into the digester extractor and heated up until the sample started boiling. The beaker was left to evaporate at room temperature, then placed in the oven. Finally, it was left in a desiccator until a constant weight was achieved.

4. The Crude fiber

Two grams of the defatted sample were treated sequentially with boiling 0.26 N H_2SO_4 solution and 0.23 N KOH. The residue was then filtered off, washed, and transferred to a crucible, then posed in a controlled oven at 105 °C for 24 h. The crucible with the sample was weighed and calcined in a muffle at 500 °C and weighed.

2.4. Statistical Analysis

According to the Kolmogorov–Smirnov test, all dependent variables were provisionally evaluated for a normal distribution. The field experimental plot was set up in a whole-plot factor split-plot design with three replicates in a randomized complete block.

The least significant difference (LSD) was calculated to test the significance of the difference between the means. Analysis of variance was performed using SAS University Edition (Cary, NC, USA). Essential oil compositions (monoterpenes and sesquiterpenes) underwent principal component analysis (PCA) to examine relationships between variables and treatments. The PCA was based on the Pearson correlation matrix. The PCA results included the factor loading of a variable and a given principal component (PC). This analysis was performed using the software package FactoMineR [47] in the software R Studio [48].

The hierarchical cluster analysis (HCA) with a single linkage method that used Euclidean distances for the essential oil compositions (monoterpenes and sesquiterpenes) was performed using the clValid package [49] in the R studio software [47]. All packages used in the statistical analysis are available through the Comprehensive R Archive Network (CRAN, https://cran.r-project.org, accessed on 1 October 2020).

3. Results

3.1. Biometric, Productive Parameters and WUE

Table 1 shows the means of the biometric, quality parameters, and WUE of cannabis as affected by water regime and sowing date during the growing season.

Table 1. The biometric and productive characteristics of cannabis as affected by water regime and sowing date.

Source of Variation	Aboveground Fresh Biomass ($t.ha^{-1}$)		Aboveground Dry Biomass ($t.ha^{-1}$)		Plant Height (cm)		Essential Oil Yield (%)		WUE- Fresh Biomass (Kg/m^3)		WUE- Dry Biomass (Kg/m^3)		WUE-Essential Oil Extracted From Flowers (Kg/m^3)	
	Pr.	**Means**	**Pr.**	**Means**	**Pr.**	**Means**	**Pr.**	**Means**	**Pr.**	**Means**	**Pr.**	**Means**	**Pr.**	**Means**
Water regime (Wr)	***		*		**		ns		***		*		ns	
I_{50}		34.22 ± 11.37 b		19.02 ± 8.41 b		92.78 ± 17.70 b		0.27 ± 0.09		10.67 ± 3.85 a		5.95 ± 2.81 a		0.59 ± 0.37
Iopt		53.07 ± 10.05 a		30.36 ± 7.48 a		132.78 ± 22.65 a		0.21 ± 0.05		8.23 ± 1.84 b		4.72 ± 1.31 b		0.68 ± 0.20
Sowing date (SD)	****		***		****		ns		****		****		**	
SD1		53.60 ± 10.92 a		33.07 ± 5.15 a		129.17 ± 26.16 a		0.25 ± 0.10		12.19 ± 2.59 a		7.63 ± 1.9 a		0.90 ± 0.24 a
SD2		46.63 ± 9.70 b		24.93 ± 8.38 b		120.83 ± 24.38 a		0.24 ± 0.09		10.19 ± 1.83 b		5.25 ± 0.83 b		0.61 ± 0.19 b
SD3		30.70 ± 11.99 c		16.07 ± 6.93 c		88.33 ± 19.15 b		0.22 ± 0.04		5.98 ± 0.56 c		3.12 ± 0.51 c		0.39 ± 0.22 b
Wr × SD	ns		ns		ns		*		*		*		ns	

ns, *, **, ***, **** indicate no significant difference or significant difference at $p \leq 0.05$, 0.01, 0.001 and 0.0001, respectively. The mean value followed by different characters in each column varies significantly depending on the LSD test ($p = 0.05$). SD1: mid-April, SD2: end of April, SD3: mid-May.

If we consider the source of variance is the water regime, there was a significant difference among the treatments in terms of aboveground biomass production and plant height, with higher values in the fully irrigated treatments (I_{opt}) compared to those that received 50% levels of irrigation water (I_{50}). The aboveground fresh biomass, the dry biomass, and the plant height were 55.08%, 59.62%, and 43.11% higher in I_{opt} than in I_{50}, respectively. Biomass-WUE was the highest under I_{50}. However, there was no statistical significance between I_{opt} and I_{50} for the essential oil content, although the treatments under deficit irrigation showed a 22.20% higher oil yield than those under full irrigation. The WUE for oil production was also not dependent upon the water regime.

If we consider the source of variance as the sowing date, there was a significant difference among the treatments in terms of aboveground biomass production and plant height, with the highest values in the treatments established in mid-April and the lowest values in those sown in mid-May. The essential oil content was not statistically influenced by the sowing date but the WUE for oil production was significantly higher under early sowing.

3.2. Essential Oil Composition of the Inflorescence

Table 2 shows the means of the main essential oils extracted from the cannabis inflorescence as affected by the water regime and sowing date during the trial period in season 2020.

The main extracted essential oils in cannabis inflorescence corresponded to twenty-six identified compounds representing 79.34% of the monoterpenes and 81.25% of the sesquiterpenes. The main monoterpenes were the β-pinene, (8.87% in I_{opt} and 14.2% in I_{50}), β-myrcene (9.96% in I_{opt} and 14.81% in I_{50}), and D-limonene (8.46% in I_{opt} and 10.86% in I_{50}). It should be noted that most of the identified compounds were not affected by the irrigation regime. Only the B-pinene and the β-caryophyllene resulted in significantly higher in the I_{50} treatment than in the I_{opt}, while the 1.8-cineol and the cis-α-bergamotene were significantly higher in the I_{opt} treatment than in the $I_{50.}$

Considering the sowing date as the source of variance, results revealed that B-pinene, D-limonene, β-caryophyllene levels were significantly higher in the treatments sown by the end of April (12.19, 10.86 and 16.45%, respectively), while the β-myrcene, α-phelandrene, borneol, and γ-cadinene were significantly higher in the treatments sown by mid-April (14.83, 2.69, 1.65 and 0.7%, respectively), and the β-ocimene, γ-terpinene,α-caryophyllene were significantly higher in the mid-May-sown treatments (7.45, 1.6 and 13.25%, respectively). Finally, a multivariate statistical analysis was conducted to better understand the combined effect of the water regime and sowing date.

3.3. Multivariate Statistical Analysis

The entire data set was analyzed by multivariate statistical analysis (PCA and HCA) to provide a thorough overview of the essential oil composition of the cannabis, notably the monoterpenes and sesquiterpenes, in response to the water regime and sowing time. The first three principal components (PCs) had eigenvalues greater than one which explained 81.25% and 79.34% of the cumulative variance for monoterpenes and sesquiterpenes compositions of the essential oils extracted, respectively (Tables 3 and 4). PC1 (first component) accounted for 45.84% and 36.70%, while PC2 (second component) accounted for 21.13% and 24.65% of the cumulative variance of the monoterpenes and sesquiterpenes compositions, respectively (Figures 2b and 3b). For monoterpenes compositions, PC1 was positively and strongly correlated (>0.6) with increased α-thujene %, α-fenchol, camphene %, borneol %, 1,8-cineol %, and terpinolene %. PC2 was positively correlated with increased δ-3-carene% and β-ocimene % (Table 3). In sesquiterpenes, PC1 was positively correlated with aromadandrene %, α-bisabolol %, caryophyllene oxide %, and γ-selinene %, whereas PC2 was significantly correlated with only α-farnescene % and γ-cadinene % (Table 4).

Table 2. The main essential oils extracted from the cannabis inflorescence as affected by the water regime and sowing date.

Source of Variation			Water Regime (Wr)	Sowing Date (SD)					Wr × SD
				I_{50}	Iopt		SD1	SD2	SD3	
Monoterpenes	β-pinene %	Pr.	0.023 *			0.032 *				<0.0001 ****
		Means		14.32 ± 2.03 a	8.87 ± 1.88 b		11.62 ± 4.93 ab	12.19 ± 3.21 a	10.99 ± 1.86 b	
	β-myrcene %	Pr.	0.053			0.006 **				0.020 *
		Means		14.81 ± 2.97	9.96 ± 2.58		14.83 ± 3.94 a	10.9 ± 4.18 b	11.42 ± 1.44 b	
	D-Limonene %	Pr.	0.532			0.024 *				0.034 *
		Means		9.69 ± 1.5	8.99 ± 2.49		8.46 ± 1.35 b	10.86 ± 0.99 a	8.71 ± 2.64 b	
	β-ocimene %	Pr.	0.721			0.015 *				0.414
		Means		6.33 ± 1.65	6.44 ± 1.35		4.94 ± 0.48 b	6.77 ± 1.07 a	7.45 ± 1.36 a	
	δ-3-carene %	Pr.	0.273			0.224				0.197
		Means		3.13 ± 0.76	2.88 ± 0.33		2.72 ± 0.45	2.99 ± 0.56	3.32 ± 0.65	
	4-Carene %	Pr.	0.729			0.164				0.406
		Means		4.98 ± 1.46	5.05 ± 1.42		5.52 ± 1.46	3.97 ± 1.06	5.55 ± 1.21	
	α-phelandrene %	Pr.	0.566			0.005 **				0.151
		Means		2.49 ± 0.73	2.09 ± 0.97		2.69 ± 0.38 a	2.65 ± 0.71 a	1.52 ± 0.88 b	
	α-thujene %	Pr.	0.864			0.511				0.315
		Means		1.7 ± 0.53	1.75 ± 0.45		1.89 ± 0.47	1.73 ± 0.55	1.56 ± 0.44	
	Borneol %	Pr.	0.164			0.022 *				0.161
		Means		1.42 ± 0.30	1.63 ± 0.26		1.65 ± 0.23 a	1.64 ± 0.37 a	1.29 ± 0.05 b	
	α-fenchol	Pr.	0.463			0.343				0.260
		Means		1.5 ± 0.40	1.73 ± 0.51		1.84 ± 0.48	1.55 ± 0.59	1.46 ± 0.18	
	Camphene %	Pr.	0.475			0.189				0.290
		Means		1.36 ± 0.54	1.73 ± 0.44		1.80 ± 0.47	1.46 ± 0.48	1.38 ± 0.57	

Table 2. *Cont.*

	Source of Variation		Water Regime (Wr)	Sowing Date (SD)				Wr × SD
				I_{50}	Iopt	SD1	SD2	SD3	
	γ-terpinene %	Pr.	0.104		0.001 **				0.147
		Means		1.46 ± 0.25	1.3 ± 0.22	1.16 ± 0.05 c	1.38 ± 0.21 b	1.60 ± 0.20 a	
	Terpinolene %	Pr.	0.245		0.142				0.383
		Means		0.48 ± 0.27	0.6 ± 0.28	0.74 ± 0.29	0.47 ± 0.27	0.42 ± 0.15	
	1.8-cineol %	Pr.	0.012		0.003				0.477
		Means		0.32 ± 0.19 b	0.52 ± 0.33 a	0.59 ± 0.33 a	0.48 ± 0.23 a	0.19 ± 0.05 b	
Sesquiterpenes	B-Caryophyllene %	Pr.	0.010 *		0.002 **				0.001 **
		Means		9.53 ± 1.26 a	19.01 ± 3.76 b	13.07 ± 5.44 b	16.45 ± 7.67 a	13.3 ± 2.99 b	
	α-Caryophyllene %	Pr.	0.221		0.0001 ***				0.007 **
		Means		9.86 ± 1.65	10.88 ± 3.61	9.84 ± 1.34 b	8.01 ± 1.37 c	13.25 ± 2.35 a	
	Caryophyllene oxide %	Pr.	0.425		0.073				0.0004 ***
		Means		5.25 ± 1.23	4.42 ± 1.38	4.57 ± 1.45	4.6 ± 1.27	5.32 ± 1.4	
	Aromadandrene %	Pr.	0.535		0.920				0.071
		Means		2.44 ± 0.46	2.27 ± 0.55	2.41 ± 0.46	2.31 ± 0.69	2.34 ± 0.41	
	α-farnescene %	Pr.	0.567		0.516				0.625
		Means		1.80 ± 0.42	1.86 ± 0.52	2.05 ± 0.4	1.74 ± 0.49	1.69 ± 0.48	
	α-bisabolol %	Pr.	0.938		0.952				0.095
		Means		1.5 ± 0.23	1.48 ± 0.3	1.47 ± 0.27	1.51 ± 0.27	1.49 ± 0.30	
	β-bisabolene %	Pr.	0.383		0.585				0.322
		Means		1.54 ± 0.42	1.64 ± 0.35	1.46 ± 0.36	1.61 ± 0.39	1.7 ± 0.40	
	Guaiol %	Pr.	0.417		0.166				0.324
		Means		1.19 ± 0.57	1.41 ± 0.25	1.23 ± 0.48	1.57 ± 0.24	1.11 ± 0.49	
	γ-selinene %	Pr.	0.417		0.407				0.446
		Means		1.41 ± 0.26	1.27 ± 0.13	1.26 ± 0.19	1.34 ± 0.29	1.42 ± 0.12	

Table 2. *Cont.*

Source of Variation		Water Regime (Wr)	Sowing Date (SD)					Wr × SD
			I_{50}	Iopt		SD1	SD2	SD3	
δ-guaiene %	Pr.	0.219			0.411				0.027 *
	Means		0.67 ± 0.4	0.98 ± 0.41		0.94 ± 0.56	0.68 ± 0.19	0.85 ± 0.47	
cis-α-bergamotene %	Pr.	0.022 *			0.571				0.225
	Means		0.37 ± 0.15 b	0.61 ± 0.24 a		0.51 ± 0.19	0.55 ± 0.28	0.42 ± 0.24	
γ-cadinene %	Pr.	0.055			<0.0001 ****				<0.0001 ****
	Means		0.48 ± 0.1	0.54 ± 0.26		0.70 ± 0.15 a	0.54 ± 0.03 b	0.30 ± 0.06 c	

*, **, ***, **** significant differences at $p \leq 0.05$, 0.01, 0.001 and 0.0001, respectively. The mean value followed by different characters in each column varies significantly depending on the LSD test ($p = 0.05$). SD1: mid-April, SD2: end of April, SD3: mid-May.

Table 3. Eigenvalues, the relative and cumulative percentage of the total variance, and correlation coefficient for monoterpenes compositions of the extracted essential oils with respect to the three principal components (PC1, PC2, and PC3).

Principal Components	PC1	PC2	PC3
Eigenvalue	6.42	2.96	1.99
Relative variance (%)	45.84	21.13	14.28
Cumulative variance (%)	45.84	66.97	81.25
Eigenvectors			
β-myrcene %	−0.114	**−0.830**	−0.297
D-Limonene %	0.037	0.393	**−0.801**
δ-3-carene %	−0.424	**0.702**	−0.031
4-Carene %	0.237	0.415	**−0.789**
α-phelandrene %	0.558	-0.399	0.367
α-thujene %	**0.823**	0.298	0.102
α-fenchol	**0.908**	0.328	−0.161
Camphene %	**0.834**	0.165	−0.044
γ-terpinene %	**−0.835**	0.355	−0.125
β-pinene %	−0.483	**−0.695**	0.210
β-ocimene %	−0.662	**0.675**	0.273
borneol %	**0.787**	−0.035	0.484
1,8-cineol %	**0.902**	0.026	0.096
terpinolene %	**0.926**	0.048	−0.309

The factor load in bold shows the characters that are most relevant to each principal component.

Table 4. Eigenvalues, the relative and cumulative percentage of the total variance, and correlation coefficient for sesquiterpenes compositions of the essential oils extracted with respect to the three principal components (PC1, PC2, and PC3).

Principal Components	PC1	PC2	PC3
Eigenvalue	4.40	2.96	2.16
Relative variance (%)	36.70	24.65	17.99
Cumulative variance (%)	36.70	61.35	79.34
Eigenvectors			
Aromadandrene %	0.739	0.662	−0.013
α-farnescene %	−0.107	0.923	0.075
γ-cadinene %	−0.512	0.756	−0.122
α-bisabolol %	0.708	0.482	−0.290
β-bisabolene %	−0.524	−0.296	0.712
β-Caryophyllene %	−0.833	−0.048	−0.154
α-Caryophyllene %	0.502	−0.096	0.295
Caryophyllene oxide %	0.842	−0.345	−0.393
cis-α-bergamotene %	−0.642	−0.229	−0.657
Guaiol %	−0.182	0.582	−0.342
γ-selinene %	0.678	0.013	0.624
δ-guaiene %	−0.499	0.504	0.603

The factor load in bold shows the characters that are most relevant to each principal component.

In the current study, the positive side of PC1 for monoterpenes (Figure 2b), in particular the upper right quadrant (A), included the combined treatment water supply regime (I_{opt}) with the sowing date SD1 (mid-April) and SD2 (end of April) and was characterized by high α-thujene %, α-fenchol, camphene %, borneol %,1,8-cineol %, terpinolene %, δ-3-carene %, and β-ocimene %. The I_{50}-SD1 (I_{50} grown in mid-April) treatment from the lower right quadrant (D) was characterized by high α-thujene %, α-fenchol, camphene %, borneol %, 1,8-cineol %, terpinolene %, β-myrcene %, and β-pinene %. The treatments coming from the third sowing time (SD3: mid-May) for both water regimes (I_{50} and I_{opt}) from the upper left quadrant (B) were characterized by high γ-terpinene %, δ-3-carene %, and β-ocimene %. Finally, the combined treatment I_{50}-SD2 (Io grown at the end of April) from the lower left quadrant (C) was characterized by high γ-terpinene %, β-myrcene %, and β-pinene %.

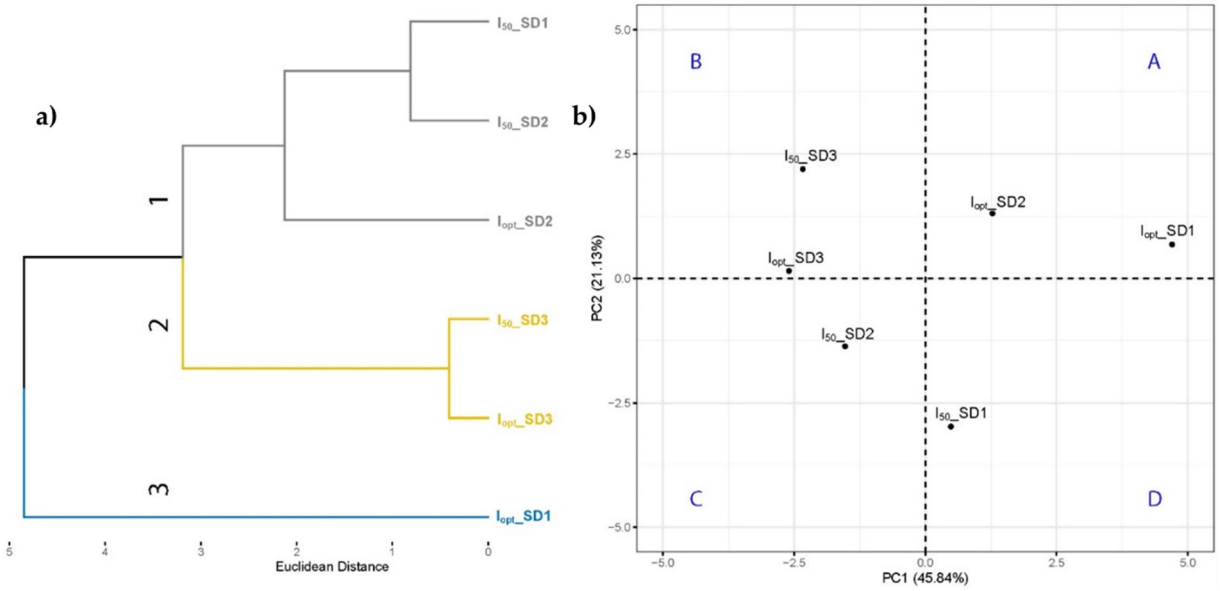

Figure 2. (**a**) Hierarchical cluster analysis (HCA) dendrogram for the monoterpenes compositions of the essential oils extracted from all cannabis accessions. Each joining (fusion) of two clusters is represented on the graph by splitting a horizontal line into two horizontal lines. The horizontal position of the split, shown by the short vertical bar, gives the distance (dissimilarity) between the two clusters. The outlier, Iopt_SD1, was fused in rather arbitrarily at a much higher distance. Two major coupled treatment groups are identified by this analysis and their numbers are presented above the differentiating node. (**b**) Principal component analysis (PCA) score plot for the monoterpenes compositions of the essential oils extracted from all the samples.

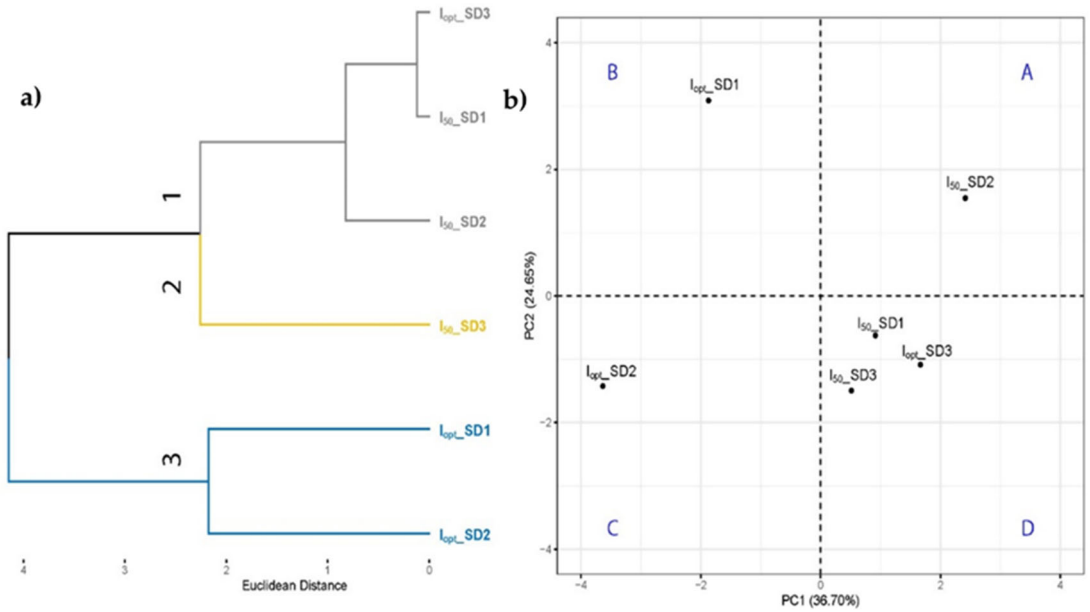

Figure 3. (**a**) Hierarchical cluster analysis (HCA) dendrogram for the sesquiterpenes compositions of the essential oils extracted from all cannabis accessions. Each connection (merge) of the two clusters is represented in the figure by splitting one horizon into two horizons. The horizontal position of the split, represented by a short vertical bar, indicates the distance (difference) between the two clusters. This analysis identifies two large pairs of treatment groups and their numbers are shown above the identification node. (**b**) Principal component analysis (PCA) score plot for the sesquiterpenes compositions of the essential oils extracted from all the samples.

Figure 2a shows the dendrogram generated by the HCA of dissimilarities among the combined treatment on their Euclidean distances for the monoterpenes compositions of the extracted essential oils. The HCA revealed three major treatment groups (Figure 2a).

The three combined treatments (I_{50}-SD1, I_{50}-SD2 and I_{opt}-SD2) caused similar responses in the monoterpene's quality parameters, and were, therefore, grouped into one cluster: Group 1. Group 2 included two combined treatments (I_{50}-SD3 and I_{opt}-SD3). The combined treatment I_{opt}-SD1 was the most distinctive, creating a group of its own, i.e., Group 3.

The positive side of PC1 for sesquiterpenes (Figure 3b), in particular the upper right quadrant (A), included the combined treatment water supply regime (I_{50}) with the sowing date SD2 (end of April) and was characterized by high aromadandrene %, α-bisabolol %, caryophyllene oxide %, γ-selinene %, α-fremescence %, and γ-cadinene %. The combined treatments I_{50}-SD1, I_{50}-SD3, and I_{opt}-SD3 from the lower right quadrant (D) were characterized by high aromadandrene %, α-bisabolol %, caryophyllene oxide %, and γ-selinene %. The water supply regime (I_{opt}) with the sowing date SD1 (mid-April) treatment from the upper left quadrant (B) was characterized by high β-caryophyllene %, α-fremescence %, and γ-cadinene %. Finally, the combined treatment I_{opt}-SD2 (I_{opt} grown at the end of April) from the lower left quadrant (C) was characterized by high β-caryophyllene %.

Figure 2a shows the dendrogram generated by the HCA of dissimilarities among the combined treatment on their Euclidean distances for the sesquiterpenes compositions of the extracted essential oils. The HCA showed three major treatment groups (Figure 3a). The three combined treatments (I_{50}-SD1, I_{50}-SD2, and I_{opt}-SD3) caused similar responses in the sesquiterpenes quality parameters and were, therefore, grouped into one cluster: Group 1. The combined treatment I_{50}-SD3 was the most distinctive, creating a group of its own, i.e., Group 2. Group 3 included two combined treatments (I_{opt}-SD1 and I_{opt}-SD2).

3.4. Seed Quality

Table 5 shows the means of the seed quality of cannabis as affected by the water regime and sowing date during the trial period. If we consider the source of the variance as the water regime, all treatments presented a similar seed composition except that the crude fat and crude protein content were higher in I_{opt} than in I_{50} treatments. If we consider the source of variance as the sowing date, only the crude fat was significantly higher in the treatments that were planted in mid-April.

Table 5. The cannabis seed composition is affected by the water regime and sowing date.

Source of Variation	Crude Fat (%)		Ash (%)		Crude Protein (%)		Dry Matter (%)		Crude Fiber (%)	
	Pr.	Means	Pr.	Means	Pr.	Means	Pr.	Means	Pr.	Means
Water regime (Wr)	*		ns		*		ns		ns	
I_{50}		5.04 ± 2.0 b		12.76 ± 2.77		20.54 ± 1.59 b		92.00 ± 1.24		27.51 ± 2.82
Iopt		7.03 ± 1.25 a		13.75 ± 2.55		24.63 ± 1.49 a		92.26 ± 1.71		28.55 ± 1.16
Sowing date (SD)	***		ns		ns		ns		ns	
SD1		7.18 ± 0.73 a		14.77 ± 2.33		22.93 ± 3.07		91.38 ± 1.73		28.43 ± 3.01
SD2		6.85 ± 1.21 a		13.64 ± 2.88		23.02 ± 1.88		92.58 ± 0.62		27.48 ± 2.21
SD3		4.07 ± 1.85 b		11.36 ± 1.6		21.81 ± 2.93		92.42 ± 1.68		28.18 ± 1.17
Wr × SD	ns		ns		ns		ns		ns	

*, *** significant difference at $p \leq 0.05$ and 0.001, respectively. The mean value followed by different characters in each column varies significantly depending on the LSD test ($p = 0.05$). SD1: mid-April, SD2: end of April, SD3: mid-May.

4. Discussion

The study showed that the biometric and oil characteristics of cannabis could be influenced by agronomic practices.

Irrigation practice, in particular, affected cannabis biomass, plant height, and WUE. The results agree with the findings of [31] who reported that water stress could reduce the aboveground biomass production of cannabis, and of [24] who showed that plant height is positively correlated to plant density. Other studies reported that irrigation significantly affected the yield of fresh stems, fresh leaves, flowers, and plant height [41].

Sowing timing also affected the biomass and essential oil WUEs that were the highest under early sowing. These results agree with the findings of [27] who evidenced that cannabis cultivars grown in April resulted in better biometrics, productive characteristics, and WUE. Other works similarly observed that the optimal sowing date was between the last week of April and mid-May [31]. The main compounds of the essential oil found in our study agreed with the findings of other authors in the literature. Specifically, β-pinene was the main extracted oil from cannabis [50]. For the sesquiterpenes, the main extracted compounds were β-caryophyllene (9.53% in Iopt and 19.01% in I50), α-caryophyllene (8.01% in Iopt and 13.25% in I50) and caryophyllene oxide (4.42% in Iopt and 5.32% in I50). Other studies [25] found also that β-caryophyllene was the main compound in cannabis. The results confirm that the terpenoids in the plant vary according to numerous parameters including the variety of cannabis, the plant part, the environmental conditions, and the maturity stage of the plant [51,52].

It is important to highlight that the considered agronomic practices, irrigation, and sowing time had mainly affected the terpenoid type rather than the essential oil yield. The monoterpenes were highly correlated with the irrigation treatment I_{opt} and early-April sowing, while the sesquiterpenes were better enhanced under I_{50} and end of April to mid-May sowing. In the literature, there is some evidence of the accumulation of terpenoids in response to drought conditions (e.g., [53]) in several medicinal plants. In fact, both the quantity and the quality of some specialized metabolites, such as terpenoids, can be strongly affected by environmental stresses [35]. Some studies showed that the selection of the appropriate irrigation water regime or drought stress could influence the levels of different plant compounds for improved health benefits. For example, the increase in drought stress duration was reported to enhance the concentrations of plant phenolic and flavonoid compounds [54]. The study of [33] showed that the total phenolic and flavonoid contents were elevated under drought stress treatment, while antioxidants responded inconsistently to stress. Moderate water stress, coupled with the use of biostimulants was reported to enhance specialized metabolites like EO components, but also yield [34]. Imposed water-limited stress has led to differential responses of pharmacologically useful diterpenoids for the obtention of the desired composition [35]. Finally, the results confirm that the interfaces in the production and synthesis of fatty acids in plants are influenced by variations in temperature, light, moisture amount, and farming conditions, as reported by [50–55].

5. Conclusions

This work presented the first evidence of the agronomic and essential oil characteristics of Lebanese cannabis as affected by the sowing date and irrigation regime. According to the findings, agronomic practices are closely linked to the quality characteristics of the main cannabis products. Within this context, further studies are needed to recommend the appropriate planting practices for the legalization framework of this crop in Lebanon.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14233842/s1, Table S1: The monthly maximum temperature (T max), minimum temperature (T min), rain and evapo-transpiration (ETo) for the Kferden region for the 2020 season (April to October) compared to the historical monthly data (2010–2020).; Table S2: The retention indices used for the identification of the essential oil components.

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