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Specific Way of Controlling Composition of Cannabinoids and Essential Oil from *Cannabis sativa* var. Finola

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Abstract: Recently, a series of papers reported preparation and physicochemical properties of various kinds of water treated in contact with various gases with low-temperature, low-pressure glow plasma of low frequency (LPGP). Consecutive papers presented results of watering numerous herbal plants with those kinds of water in planting of numerous herbal plants. Always, the watering influenced the yield and quality of the crops and considerably changed the composition of the essential oils extracted from the watered plants. This paper provides the effects of watering of *Cannabis sativum* var. Finola with water LPGP-treated either in the air (LPGPA), under molecular nitrogen (LPGPN) or carbon dioxide (LPGPC). *Cannabis sativa*, particularly its botanic class called hemp, attracts great attention for its numerous applications. They include rope, textiles, clothing, shoes, food, paper, bioplastics, insulation, biofuel and industrial fibre. The watering was maintained for 12 weeks. Regardless of the kind of the used plasma-treated water, a considerable increase in the plant crop yield was noted for first 7 weeks. Further cultivation resulted in a minute increase in the yield. The watering with LPGPC offered the highest crop yield, followed by nontreated water, LPGPN and LPGPA. The yield of essential oil per 1 g of plant was independent of the used kind of plasma-treated water. Watering Finola with LPGPA resulted in a decrease in the level of cannabidivarin (CBD V) and considerable increase in the deal of Δ 9-tetrahydrocannabinol (Δ^9-THC). The levels of the remaining components of the essential oil slightly decreased with respect to that in the control sample. Almost identical trends in the influence of watering upon the composition of essential oil were observed in the case of LPGPN. However, an unusually strong decrease in the level of CBD V accompanied by a very high increase in the level of Δ^9-THC could be noted. The performed study provided strong evidence that watering seeds and plants of Finola with various kinds of the LPGP-treated water could modulate and even tailor the crop yield, functional properties of the plant and essential oils extracted from it. The composition of the essential oil isolated from the plant watered with LPGPN suggests its application as a substitute of medical marijuana (medical cannabis).

Keywords: cannabinoids; tetrahydrocannabinoids; water treated with low-temperature; low-pressure glow plasma

Citation: Ciesielski, W.; Domagała, I.; Garcia, B.; Girek, T.; Oszczęda, Z.; Szczuka, E.; Tomasik, P. Specific Way of Controlling Composition of Cannabinoids and Essential Oil from *Cannabis sativa* var. Finola. *Water* **2022**, *14*, 688. https://doi.org/10.3390/w14050688

Academic Editor: Carmen Teodosiu

Received: 13 January 2022
Accepted: 19 February 2022
Published: 22 February 2022

1. Introduction

Recently, a series of papers was published on the preparation and physicochemical properties of water treated either in the air, ammonia, nitrogen, hydrogen, methane or oxygen with a unique low-temperature, low-pressure glow plasma of low frequency (LPGP) [1]. In consecutive papers, the application of these kinds of water in the planting

of numerous herbal plants was presented [2–6]. Effects of watering of those plants with such kinds of water depended on the way of their preparation. With few minor exceptions, watering of those plants with processed water positively influenced the yield of crops. Such procedures provided also an increased yield of essential oils and remarkable changes of their composition. Choice of the water for watering provided controlling biological properties of the herbs and their applications.

This paper describes the effect of watering *Cannabis sativa* var. Finola with water treated with LPGP for 30 min either in the air, under nitrogen or under carbon dioxide upon the yield and quality of crops and composition of essential oil.

In Finola, male and female plants can be distinguished. The male plants are shorter than the female plants [7,8]. In this study, solely female plants were taken into consideration.

Cannabis sativa [9–12], particularly its botanic class called hemp, attracts a great attention for its numerous applications. They include rope, textiles, clothing, shoes, food, paper, bioplastics, insulation, biofuel [13] and industrial fibre [13,14]. Hemp seeds contain a high level of dietary fibre; B vitamins and bioelements such as manganese, phosphorus, magnesium, zinc and iron. Moreover, they are rich in fats that contain essential fatty acids [15], mainly polyunsaturated fatty acids and linoleic, oleic and alpha-linolenic acids [16–18]. They constitute highly appreciated edible seed oil. For its amino acid profile, hemp seeds resemble such protein-rich foods as meat, milk, eggs and soy [17,19]. However, the seeds also contain significant amounts of antinutritional compounds such as phytic acid, trypsin inhibitors and tannins [20,21].

Growing interest in the use of *Cannabis sativa* in medicine and recreation results from a range of cannabinoids that the plant produces [22,23]. Among them, Δ 9-tetrahydrocannabinol ($^{9}\Delta$-THC) and cannabidiol (CBD) are the main components. Apart from cannabinoids, there are also about 120 compounds responsible for its characteristic aroma. These are mainly volatile terpenes and sesquiterpenes, such as α-pinene, myrcene, linalool, limonene, trans-β-ocimene, α-terpinolene, trans-caryophyllene, α-humulene and caryophyllene [15].

In this study, the application of LPGP-treated kinds of water in the plantation of *Cannabis sativa* var. Finola was checked. The experiments performed under strictly identical conditions provided strong evidence that watering seeds and plants of Finola with various kinds of the LPGP-treated water could modulate and even tailor the crop yield and functional properties of the plant and compositions of their essential oils.

2. Materials and Methods

2.1. Materials

2.1.1. *Cannabis sativa* L. var. Finola

Seeds offered by Hemp it Coop. (Beaufort en Valle, France) were purchased from Agro Solution Sp. z o.o. in Siemianice (Poland).

2.1.2. Substrate

A medium size turf fraction Florabalt® Pot Medium-Coarse (Floragard, Oldenburg, Germany) of pH = 5.6, total salt 1.2 g/L, 210-mg N/L, 120-mg P_2O_5/L and 260-mg K_2O/L was used. It was blended with multicomponent PG-Mix 18-10-20 fertilizer (1.20 kg/m^3) (Yara, Oslo, Norway).

2.1.3. Water

Tap water from Bolesławiec of total hardness 129-mg/dm^3 $CaCO_3$, pH 7.1, conductivity 334 μS/cm, Fe < 50 μg/dm^3, Mn < 5 μg/dm^3 and 6.93-mg/dm^3 dissolved oxygen was LPGP-treated for 30 min either in contact with the air following Białopiotrowicz et al. [24] and providing LPGPA or, alternatively, treated for the same time with LGPG under nitrogen as described by Chwastowski et al. [25], providing LPGPN. LPGPC was prepared

following the methods described by Ciesielska et al. [26]. LPGP of 38 °C was generated at 5 × 10^{-3} mbar, 800 V, 50 mA and 10-KHz frequency in a plasmothrone patented by Oszczęda et al. [27] and Reszke et al. [28]. The produced water was stored at ambient temperature in 1-L hermetically closed Teflon containers. The water was stored for no longer than 2 weeks.

2.1.4. Trays

Multiplate long life trays model QP 24RW (Herkuplast Kubern GmbH, Ering/Inn, Germany) were composed of 24 trays of 230 cm^3 capacity (=140 trays per 1 m^2).

2.2. Methods

2.2.1. Cannabis Plantation

The monofactorial experiment was carried out from May 28th (sowing) for 12 weeks in a greenhouse at NGO Zielony Dom in Paniowice. The experiment involved four sets of trays with 5 pots each. Each pot was filled with the substrate (230 cm^3). Three seeds of Cannabis Finola were sown into every pot. The trays with germinated plants contained initially male and female Finola plants. In the fourth week, the male plants were discarded. The plants were watered manually to avoid any casual contact of water with the leaves. Initially, in the 5-day period until 24th June, plants consumed 3 L of water in total; that is, 1 L per each replication. Subsequently, for 1 month, the watering was intensified, and the same amount of water was administered to the plants in 3-day periods. In the final period, the grown plants were watered, consuming daily the same amount of water. In such a manner, the daily watering contained 40 mL each kind of water. The experiment terminated on the 15th of September. The plants were collected and separated into leaves and stems. The plant samples were then dried at 105 °C for 4 h to determine the dry mass of the crops.

2.2.2. Estimation of the Crop Yield

Estimation of the crop yield was performed on freshly collected plants deprived of their roots.

2.2.3. Preparation of Samples for Analyses (Extraction)

Extractions were performed with Automated Solvent Extraction System EDGE, CEM Corp. Matthews, NC, USA. Three and a half grams of each sample were placed in an extraction tube with a S1 Q-Disc (The S1 Q-Disc is a preassembled sandwich of the G1 Q-Disc between two C9 Q-Discs). Each sample was secured by Q-Screen. The extraction program: methanol; top volume: 10 mL, bottom volume 5 mL, temp. 35 °C, hold time 3 min. Rinse with methanol 5 mL. Wash program: first cycle: isopropanol 30 mL, hold time 15 s, temp. 50 °C; Second cycle: methanol 30 mL. Each sample was filtered over a 0.22 μm PTFE filter and subjected to HPLC analysis.

2.2.4. High Pressure Liquid Chromatography (HPLC)

Chromatography was run using an Agilent LC 1260 Infinity II (Santa Clara, CA, USA) HPLC system with Agilent DAD detector. For peak integration, Agilent EZChrom Elite was used. The final liquid chromatography analysis was performed on a RP Infinity Lab Poroshell 120 EC-C18 column (100 × 4.6 mm, 4 μm) applying gradient elution and using pure water (with 0.1% FA) (phase A) and acetonitrile (with 0.1% FA) (phase B) as the organic phase. The injection volume was 5 μL. The column oven temperature was set at 50 °C, and the flow rate was 0.8 mL/min. The procedure described by Zivovinovic et al. [29] was followed. All cannabinoids were monitored at λ = 230 nm. Gradient of the elution at that frequency is given in Table 1.

Table 1. Gradient elution at 230 nm.

Time (min)	Mobile Phase	
	A	**B**
0.0	30	70
2.0	30	70
4.5	28.7	71.3
6.0	5	95
8.6	5	95
9.4	5	95
10.0	5	95

Chromatographic signals were identified by comparison with several CBDs and THC standards of analytical grade purchased from Lgcstandards Polska (Warsaw, Poland). Areas under particular chromatographic peaks were calculated involving OpenLAB CDS LC ChemStation computer software (Agilent, Santa Clara CF, USA).

3. Results and Discussion

Watering seeds of *Cannabis Finola* with various kinds of LPGP-treated water revealed its tremendous influence upon the crop yield of the female variety. The average mass of the plant after 12 weeks of cultivation reached 461, 321, 259 and 123 g after watering with LPGPC, nontreated water, LPGPN and LPGPA, respectively. Figure 1 visualizes the examples of those plants.

(**a**) (**b**) (**c**) (**d**)

Figure 1. Female variety of *Cannabis sativa* L. Finola after 12 weeks of watering with LPGPC (**a**), LPGPN (**b**), LPGPA (**c**) and nontreated water (**d**).

Thus, taking under consideration the hemp-dependent applications of the plant [13,14], the watering with LPGPN seemed to be the most lucrative.

Identical volumes of each kind of water were used for watering 25 seeds distributed in five trays with the same support. As shown in Table 2, regardless of the kind of water, the watering provided fast growth for the first 7 weeks, then slowed down up to the 12th week. Watering with nontreated water, LPGPA and LPGPC also resulted in the germination of seven seeds from a total of 25, whereas only five seeds germinated from watering with LPGPN. The watering with LPGPC offered the best crops in terms of plant height.

The watering with LPGPN, LPGPA and nontreated water produced shorter plants (Table 2).

Table 2. Results of watering *Cannabis sativa* L. var. Finola with nontreated water (control) and with LPGPA, LPGPN and LPGPC for 12 weeks.

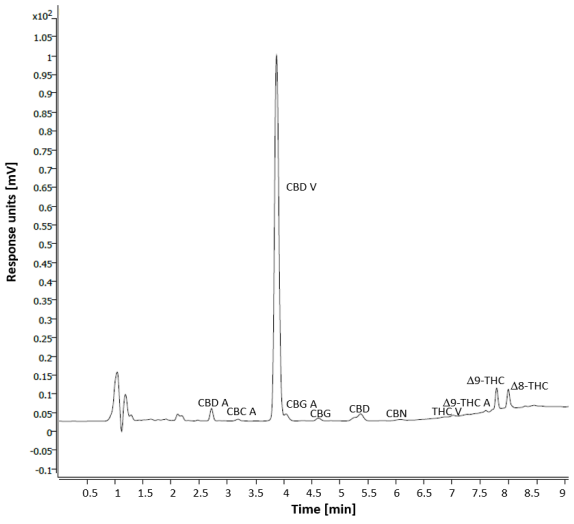
Water	No. of Germinated Seeds in 5 Trays	Average Finola Height [cm] [a]											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Nontreated	7	3.7	6.5	12.2	19.8	27.3	35.5	44.0	46.4	48.1	48.8	49.8	49.8
LPGPA	7	4.5	7.3	14.8	42	32	40	59	63	65	65	66	66
LPGPN	5	3.8	15.8	28.6	52	58	80	88	93	94	95	95	96
LPGPC	7	4	13.9	33.0	57.3	93.8	132.5	145.5	148	149.3	149.5	150	150.5

[a] After 4 weeks, the male variety (1 plant in every case) was discarded.

The kind of water used for the watering had a considerable influence upon the composition of essential oil extracted from the plant. The yield of essential oil per 1 g of plant was practically independent of the kind of the plasma-treated water.

The essential oil from the plants watered with nontreated water (control sample) (Figure 2) consisted of 11 components, among whose CBD V (cannabidivarin) constituted 54.95% of the total yield of the oil (Table 3).

The individual impacts of each of the remaining 10 components, i.e., cannabidiolic acid (CBD A), cannabichromenic acid (CBC A), cannabigerolic acid (CBG A), cannabigerol (CBG), cannabidiol (CDB), cannabinol (CBN), tetrahydrocannabivarin (THC V), Δ^9-tetrahydrocannabinolic acid (Δ^9-THC A), Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol (Δ^8-THC) did not exceed 10%. The biological functions of those components and therapeutic applications were characterized in several sources [12,29,30] (see also Figure 3).



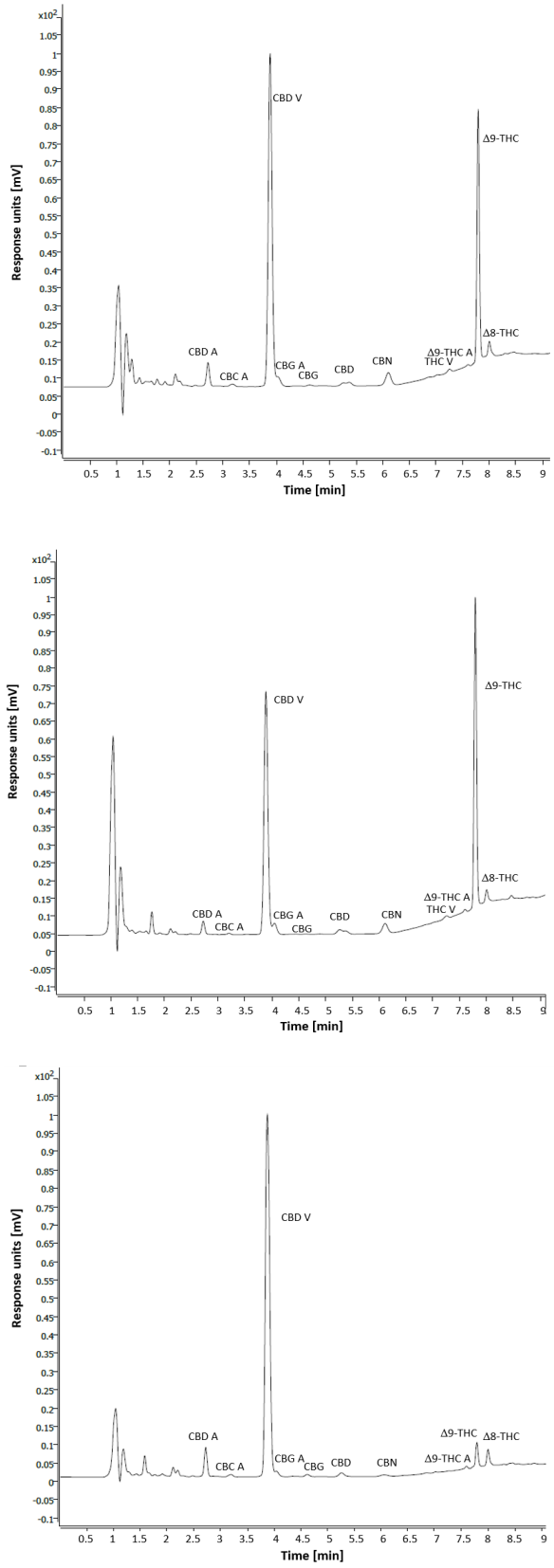


Figure 2. Chromatograms of essential oil extracted from *Cannabis sativa* L var. Finola watered for 12 weeks with, subsequently, from the top: nontreated water (control), LPGPA, LPGPN and LPGPC.

Table 3. The composition of the oil extracted from the plant watered with various kinds of water.

Peak Number	Retention Time (min)	Compound	Content 9%) of Component in the Oil from the Plant Watered with Water [a]			
			Nontreated	LPGPA	LPGPN	LPGPC
1	2.637	CBD A	9.61	6.27	5.53	7.34
2	3.173	CBD C	2.65	1.66	1.63	1.56
3	3.877	CBD V	54.95	51.68	26.59	60.95
4	3.973	CDG A	4.81	3.34	3.09	3.98
5	4.531	CBG	3.01	1.95	1.60	0.26
6	5.369	CBD	4.38	2.98	2.74	3.62
7	6.074	CBN	2.79	2.83	2.15	2.81
8	7.038	THC V	0.27	0.23	0.73	0.00
9	7.567	Δ9-THC A	0.34	0.29	0.58	0.66
10	7.870	Δ9-THC	9.36	23.22	48.89	10.65
11	8.132	Δ8-THC	7.84	5.5	6.48	8.16
Total numer of components			11	11	11	10

[a] Total yield of essential oil = 100%.

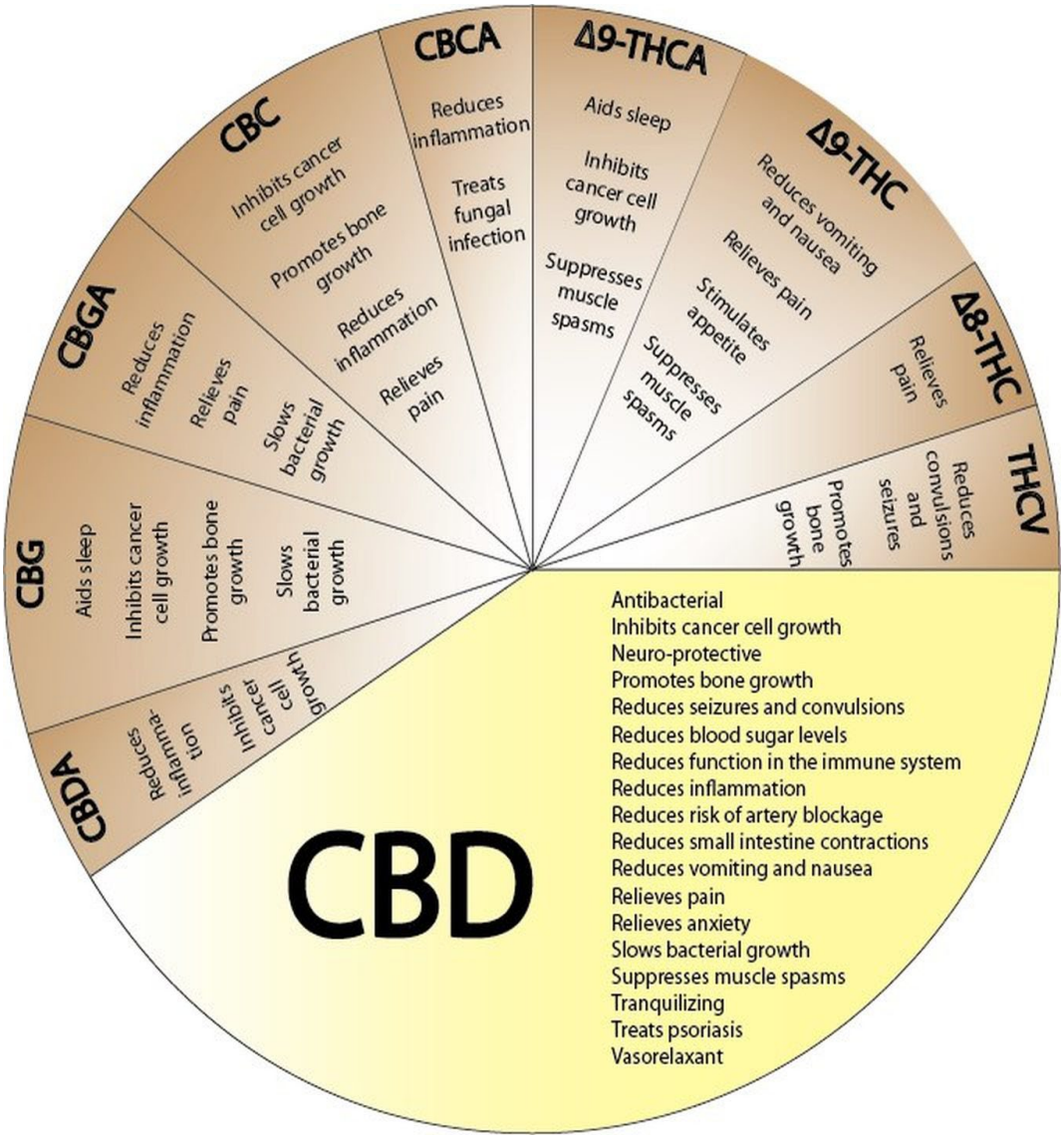


Figure 3. Therapeutic applications of cannabinoids [30].

THC V was recognized as a psychoactive compound and, therefore, requiring monitoring of its level in food [31]. The most recent studies [32] showed that it is deprived of psychoactivity and useful in controlling obesity and diabetes. However, undoubtedly, Δ^9-THC A [33,34] jointly with Δ^8-THC [35] is considered as the main psychoactive component of marihuana. Therefore, the presence of those three components of the essential oil from *Cannabis sativa*. L, var. Finola would be undesirable.

Watering Finola with LPGPA resulted in a decrease in the level of CBD V and in a considerably disappointing increase in the level of Δ^9-THC. The levels of the remaining components of the essential oil slightly decreased with respect to that in the control sample (Table 3). Almost identical trends in the influence of watering upon the composition of the essential oil were observed in the case of LPGPN. However, an unusually strong decrease in the level of CBD V accompanied by a very high increase in the level of Δ^9-THC should be noted. These results might suggest the application of that essential oil as a substitute for medical marijuana (medical cannabis). An increased level of CBD V and only a slight increase in the level of Δ^9-THC in the essential oil from Finola watered with LPGPC paid attention to such watering as promising in this respect.

4. Conclusions

The watering of *Cannabis sativa L.* var. Finola with water treated with low-temperature, low-pressure glow plasma either in the air, under nitrogen or under carbon dioxide provided modulation and tailoring functional properties of crops and essential oil extracted from this plant. The effect considerably depended on the kind of used water. Regardless of the kind of applied plasma-treated water used, the considerable increase in the plant crop yield lasted up to the 7th week, and it was independent of the kind of the plasma-treated water. Further cultivation of the plant resulted in a minute increase of the yield.

Watering with the plasma-treated water under carbon dioxide offered the highest crop yield, followed by water treated under nitrogen and water treated in the air and nontreated water. The yield of essential oil per 1 g of plant was independent of the kind of plasma-treated water used.

Watering Finola with LPGPA resulted in a decrease in the level of CBD V and a considerable increase in the level of Δ^9-THC. The levels of the remaining components of the essential oil slightly decreased with respect to that in the control sample. Almost identical trends in the influence of watering upon the composition of the essential oil was observed in the case of LPGPN. However, an unusually strong decrease in the level of CBD V accompanied by a very high increase in the level of Δ^9-THC should be noted.

These results might suggest application of that essential oil as a substitute for medical marijuana (medical cannabis).

Author Contributions: E.S.: concept of the project and co-interpretation of the results, I.D.: funding the research and maintenance of the plant breeding; B.G.: maintenance of the breeding and documentation of that process; P.T.: methodology, writing the original draft, supervision and co-interpretation of the results; W.C.: methodology, investigation and co-interpretation of the results; T.G.: investigation and co-interpretation of the results and Z.O.: supplying the plasma-treated kinds of water and co-interpretation of the results. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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