# Specific modulation of flavor and aroma of Greek oregano (Origanum vulgare var. hirtum) and its essential oil

Specyficzna modulacja smaku i aromatu greckiego oregano (Origanum vulgare var. hirtum) i jego olejku eterycznego

Wojciech Ciesielski<sup>1</sup>, Maciej Gąstoł<sup>2</sup>, Tomasz Girek<sup>1</sup>, Damian Kulawik<sup>1</sup>, Zdzisław Oszczęda<sup>3</sup>, Elżbieta Pisulewska<sup>4</sup>, Piotr Tomasik<sup>3</sup>

- <sup>1</sup> Institute of Chemistry, Jan Długosz University, 42-201 Częstochowa, Poland
- <sup>2</sup> Faculty of Biotechnology and Horticulture, University of Agriculture, Krakow, Poland
- 3 Nantes Nanotechnological Systems, 59-700 Bolesławiec, Poland
- State University of Applied Sciences in Krosno, 38-400 Krosno, Poland, e-mail: elzbieta. pisulewska@gmail.com

Keywords: carvacrol, glow plasma, glow plasma treated water, thymol Słowa kluczowe: karwakrol, plazma jarzeniowa, tymol, woda plazmowana

## Summary

Plantations of Greek oregano (*Origanum vulgare var. hirtum*) were watered with water treated for 30 min with low-temperature, low-pressure glow plasma of low frequency either in the air, under nitrogen, carbon dioxide, methane or molecular oxygen.

The kind of the water influenced the yield and quality of crops in terms of a number of plants, their height, total biomass, total number and mass of leaves. Watering the plants with every kind of the plasma treated water provided essential changes in the composition of isolated essential oil. In such manner the composition of the essential oil could be tailored following the consumer demands, particularly in cases of designing better cure potential for herbal medicine and aromatherapy. The selection of particular kind of the plasma treated water for watering provides essential oil of diverse suitability as the spice and flavouring agent. Regarding the kind of water taken for watering always carvacrol was a dominating component of the essential oil. Application of particular kind of treated water always changed the yield of particular components of the oil up to a total inhibition of its formation but never resulted in formation of novel components.

#### Streszczenie

Plantacje greckiego oregano (*Origanum vulgare var. hirtum*) podlewano wodą traktowaną przez 30 min niskotemperaturową, niskociśnieniową plazmą jarzeniową o niskiej częstości pod powietrzem, azotem ditlenkiem węgla, metanem lub cząsteczkowym tlenem.

Rodzaj wody miał wpływ na wydajność i jakość plonów wyrażoną liczbą roślin, ich wysokością, całkowitą biomasą oraz liczbą i masą liści. Podlewanie roślin każdym rodzajem plazmowanej wody prowadziło do istotnych różnic w składzie wydzielanego olejku eterycznego. Dzięki temu można będzie regulować jakość olejku stosownie do żądań konsumentów, co jest szczególnie istotne w przypadku ziołolecznictwa i aromatoterapii. Wybór rodzaju wody do podlewania pozwalał na pozyskiwanie olejku eterycznego o odmiennych właściwościach przyprawowych i nawaniających. Bez względu na rodzaj wody stosowanej do podlewania karwakrol był zawsze dominującym składnikiem olejku. Stosowanie poszczególnych rodzajów wody odmiennie wpływało też na wydajność poszczególnych składników olejku. Podlewanie plazmowaną wodą pozwalało eliminować niektóre składniki olejku, ale nigdy nie prowadziło do pojawiania się nowych.

### Introduction

Invention of low-temperature low pressure glow plasma of low frequency (LPGP) and equipment for its development [1, 2] induced a series of studies on physical, physicochemical and chemical structure of water treated with that plasma. At temperature, pressure and frequency maintained constant on the plasma generation the time of exposure of water to glow plasma and atmosphere under which the treatment was performed appeared crucial for the properties of resulting water. Thus, series of papers was published on the structure and physical, physicochemical and chemical properties of water treated with LPGP in the air (LPGPA) [3], under nitrogen (LPGPN) [4], ammonia (LPGPAM) [5], carbon dioxide (LPGPC) [6], methane (LPGPM) [7] and molecular oxygen (LPGPO) [8].

These kinds of LPGP treated water distinguished from one another with their macrostructure and, hence, with their functional properties. For instance, LPGPAM was suggested as an efficient nitrogen fertilizer [5]. Macrostructure dependent functional properties of LPGP-treated water were particularly extensively studied in case of plant breeding cultivation. Wolski et al. [9] presented beneficial effect of LPGPA on the quality and yield of crops of fodder grass. Pisulewska et al. [10] demonstrated that watering peppermint with LPGPA considerably changed the composition of extracted essential oil. The water macrostructure dependent effect of various kinds of LPGP treated water was well demonstrated in case of watering cress [11].

Subsequently, relevant studies focused on herbs that is, on lavender [12] and basil [13]. The results confirmed pronounced effect of watering on the composition of essential oil and, to a lesser extent, on the yield and quality of the crops. In this study, Greek oregano (*Origanum vulgare var. hirtum*) was taken under consideration to extend the understanding links between the macrostructure of water used for watering plants and the yield as well as quality of the crops. Such studies also contribute to the knowledge of possible mechanisms of the influence of particular kinds of LPGP treated water upon biosynthesis of the components of its essential oil. In this paper effect of the specific modulation of the flavor is signalized. It is based on known [14, 15] effect of associations of sensory impressions. Results of those associations depend on the temperament and personality of individual consumers.

Greek oregano [16] is one of many species of flowering plants in the mint family (Lamiaceae). It originates from Western and Southwestern Eurasia and the Mediterranean region but its plantations spread all over the World. It prefers a hot, relatively dry climate, but does well in other environments.

The soil pH of 6.0 to 8.0 is beneficial [17]. Oregano is used chiefly as a culinary herb. Its taste and aroma depend on climate, season and soil composition and even on the drying method [18]. Over 60 different compounds have been identified in the essential oil either or distilled from the plant.

Among them dominate carvacrol and thymol ranging to over 80% of the total content, while p-cymene,  $\gamma$ -terpinene, caryophyllene, spathulenol, germacrene-D,  $\beta$ -fenchyl alcohol,  $\delta$ -terpineol limonene, pinenes and ocimene are less abundant components [19–22].

These components are responsible for flavour and aroma of the plant. Frequently, cold pressed oil is in use. Apart from flavouring compounds it contains also fatty acids such as linoleic, stearic and palmitic acids in form of glycerides responsible for the taste of the product and tocopherols acting as antioxidants [23]. The commercial cold pressed oils from seeds undergo standardization [24]. Although Greek oregano essential oil was traditionally used in folks therapy any clinical evidence of its beneficial influence on the human health is lacking [25]. Instead, there are warnings on its anti-disease effects [26–28].

## Materials and methods

#### Materials

Greek oregano (Origanum vulgare var. hirtum) seeds were provided by Novisem vegetable seed company (Baarlo, The Netherlands). It is perennial, white flowering and extremely aromatic plant.

Specific modulation of flavor and aroma of Greek oregano...

#### Substrate

Substrate was composed of medium size turf fraction Florabalt\* Pot Medium-Coarse (Floragard, Oldenburg, Germany). The medium had pH = 5.6, total salt 1.2 g/L, 210 mg N/L, 120 mg  $P_2O_5/L$ , 260 mg  $K_2O/L$ . It was supplemented with multicomponent PG-Mix 18-10-20 fertilizer (1.20 kg/m<sup>3</sup>) (Yara, Oslo, Norway).

#### Water

Tap water from Bolesławiec of total hardness 129 mg/dm³ CaCO₃, pH 7.1, conductivity 334 mS/cm, Fe < 50 mg/dm³, Mn < 5 mg/dm³ and 6.93 mg/dm³ dissolved oxygen was used as the standard. That water was LPGP treated for 30 min in contact with the air following Białopiotrowicz et al. [3] providing LPGPA and, alternatively, treated for the same time with LGPG under nitrogen as described by Chwastowski et al. [4], providing LPGPN. LPGPC, LPGPM and LPGPO were prepared following methods described by Ciesielska et al. [5–7], and Chwastowski et al. [8], respectively. LPGP of 38°C was generated at 5x10⁻³ mbar, 800 V, 50 mA and 10 KHz frequency in a plasmothrone patented by Oszczęda et al. [1] and Reszke et al. [2]. The produced water was stored at ambient temperature in 1L closed teflon containers. Water used in particular experiments was stored for no more than 2 weeks.

#### Trays

Multiplate long life trays model QP 24RW (Herkuplast Kubern GmbH, Ering/ Inn, Germany) were used. Each of them contained was composed of 24 trays. and 24 pots, each of 230 cm<sup>3</sup> capacity (= 140 trays per 1m<sup>2</sup>).

#### Methods

## Greek oregano plantation

The monofactorial experiment was carried out from January 24th (sowing) till May 18th (harvesting) 2019 (harvesting) in a greenhouse at the University of Agriculture in Cracow. The greenhouse was set for 22/18°C (day/night) temperature. The automatic additional 16h illumination with sodium lamps early spring was used when natural light intensity decreased below 100 W/m². The experiment involved three sets of trays with 24 pots each. Ten seeds of *origanum* were sown into every pot. The watering was adjusted according to tensiometer readings (Irrometer model SR 150 mm) when soil water tension was < -40 kPa. The plants were watered by hand manually to avoid the accidental contact of water with leaves. Initially, plants consumed totally 3L water, that is 1L per each replication in the 5 day period until March 24th. In the subsequent 1 month period the watering was intensified and the same

amount of water was administered to the plants in 3 day periods. In the final period of breading the grown plants were watered daily consuming the same amount of water. In such manner the watering consumed totally 40 mL each kind water daily. The experiment terminated on May 18<sup>th</sup> 2019 when the plants were collected and separated into leaves and stems. The plants were then dried at 105°C for 4 hours to determine dry mass of the crops.

#### Preparation of extracts

Extracts were prepared on 30 min. grounding of the plant material (leaves and stems) in a mortar (20 g) with 96% ethanol (100 cm<sup>3</sup>) added. Composition of extracts was studied with gas chromatography

## Separation of essential oils by distillation of dry mass of plants

Samples of the plant (leaves and stems) dried at 35°C to constant weight (1 g) were steam distilled for 2 h in a Deryng apparatus with a closed water circulation. The collected oils were transferred to a closed vial and stored in dry ice until analyzed. Gas chromatographic analysis was performed within three days.

## Gas chromatographic analyses

Sample of essential oil (5  $\mu$ L) was transferred to closed chromatographic viol and evaporated on a heating plate. Using gas-tight syringe gaseous sample (10  $\mu$ L) was analysed using a Bruker 436-GC gas chromatograph coupled with Bruker SCION SQ (single quadruple, electron ionization) mass spectrometer (Durham, UK). The estimations were duplicated.

The instrument was equipped with BR-5ms; 0.25 mm x 30 m, df = 0.25  $\mu$ m. The column operated at the following temperature programme: 50°C (2 min) at the temperature rate increase 10°C/min up to 170°C (0 min), then at 25°C to 280°C (5 min). Dispenser, transfer line and the ion-source temperature was 300, 280 and 200°C, respectively. Sample separation was set for 1:20, helium was used as the carrier gas. The flow of the mobile phase was 1,0 mL/min, and ionization energy was 70 eV. Scanning was performed in the 50–500 m/z range.

Chromatographic signals were identified by comparison with mass spectra available in the NIST 11 library [29]. Area under particular chromatographic peaks were calculated involving CompassCDS software installed in the chromatograph.

#### Statistics

Average values of number of plants, height of plants, total mass of plants, total number of leaves, number of leaves per plant, mass of stems, total mass of foliage and mass of one leaf and corresponding standard deviations (Table 1) were calculated for results of plantations grouped in 6 sets, each containing 24 pots.

The results were subjected to statistical interpretation, mean values and standard errors were calculated, and the significance of the variables was determined. Statistically significant differences between means (p < 0.05) were evaluated using one-way analysis of variance (ANOVA) with a post hoc multiply Duncan's range test [30]. Moreover, the Pearson product-moment correlation coefficients between analyzed variables were calculated. The significance level for correlation coefficient was p = 0.05, and the number of pairs for the calculations was N = 216. All statistical analyses were calculated using Statistica 13.3 software (Tibco Software Inc., Palo Alto, USA).

Table 1. Quantitative characteristics of the oregano crops watered with various kinds of water treated with low-pressure glow plasma in contact with various gases.

	Plasmed watera								
Estimation	Non-plasmed (control)	LPGPA	LPGPN	LPGPC	LPGPM	LPGPO			
Number of plants	4.21 ± 0.15a	5.07 ± 0.14b	5.49 ± 0.12c	5.12 ± 0.23d	4.79 ± 0.31b	3.61 ± 0.34e			
Height of plants/1 pot [cm]	18.2 ± 3.1a	25.4 ± 3.4b	28.6 ± 3.4b	24.2 ± 1.8b	23.6 ± 2.5ab	20.6 ± 1.1a			
Total mass of plant [g]	8.32 ± 0.21a	9.17 ± 0.12b	10.04 ± 0.12c	9.12 ± 0.21b	9.23 ± 0.23b	7.84 ± 0.21d			
Total number of leaves	84.7 ± 1.6a	93.2 ± 3.2b	94.4 ± 1.7b	89.4 ± 2.4c	80.6 ± 1.2d	76.3 ± 1.4e			
Number of leaves per plant	11.39 ± 0.33a	13.27 ± 0.25b	13.94 ± 0.24c	12.82 ± 0.14d	12.45 ± 0.14e	11.23 ± 0.31a			
Mass of stems [g]	1.67 ± 0.13a	2.29 ± 0.21b	2.06 ± 0.22b	2.27 ± 0.11b	2.24 ± 0.11b	2.11 ± 0.12b			
Total mass of foliage [g]	6.33 ± 0.33a	7.51 ± 0.64b	7.88 ± 0.28b	7.13 ± 0.18b	6.98 ± 0.22b	5.51 ± 0.22c			
Mass of one leaf [g]	0.073 ± 0.007a	0.077 ± 0.009a	0.087 ± 0.005a	0.075 ± 0.008a	0.071 ± 0.008a	0.067 ± 0.009a			

<sup>\*</sup>Every experiment with particular kind of water employed 24 pots. Thus, the values presented in that Table are averages of 24 estimations. Differences in values in verses followed by identical letter differ from one another on statistically unessential manner.

Source: own research.

## Results and Discussion

Generally, watering O. vulgare with LPGP treated water considerably influenced measured parameters describing the plant (Table 1). Number of plants after watering with LPGPN increased by about 30% whereas watering with LPGPC and LGPGA provided about 20% increase. Watering with LPGPM resulted in solely about 13% increase in that parameter whereas watering with LPGPO reduced the number of plant by about 15% in respect to the result of watering with non-treated water. Watering with LPGP-treated water was beneficial for height of plants. It increased by over 55% in case of LPGPN in order to decline to about 18% in case of the appli-

cation of LPGPO. Total number of leaves increased by approximately to 10% to 5% after watering with LPGPN, LPGPA and LPGPC and watering with LPGPM and LPGP) reduced it by about 5 and 10% in respect to the result collected after watering with control water. Number of leaves per plant increased in all cases except watering with LPGPO by between about 20 to 10% but watering with LPGPO reduced it by about 15% in respect to control. An increase in the mass of stems, depending on the kind of treated water used balanced between 25 and 35%. LPGPN provided the lowest about 25% increase.

Total mass of foliage rose from about 10% for LPGPM to about 25% in case of LPGPN. Solely watering with LPGPO reduced that mass by about 10% in respect to the control. Only w Watering with LPGPN was beneficial in terms of all kinds of plasmed water had a little effect upon mass of one leaf providing about 20% increase whereas no effect on this property could be noted after watering with remained kinds of water.

The use of LPGP-treated water, except LPGPM had no effect on the yield of extracted essential oil. It was extracted from leaves and stems. The use of LPGPM resulted in 50% reduction of the yield of that oil in respect to its yield achieved from the material after watering with remaining kinds of water (Table 2). In that Table only components whose amount reaches 0.01% and above are characterized. Essential oil extracted from material from our plantation of Greek oregano consisted of 41 components. Among them definitely dominated carvacrol constituting over 56% of the total amount of oil. Subsequent 11 components residing in the oil in the amount 1.00% and above constituted hardly 32.88%. They were caryophyllene (8.25%),  $\gamma$ -terpinene (6.40%), thymol (4.43%), p-cymene (3.24%), 3-octanol (2.38%), cis- $\beta$ -terpineol (2.35%), sabinene (1.68%), trans-dihydrocarvone (1.61%), methyl thymyl ether (1.36%), thymoquinone (1.14%) and 3-carene (1.04%).

**Table 2.** Composition of essential oil collected from Greek oregano watered with non-treated water and water treated with glow plasma in the air (LPGPA), under nitrogen (LPGPN), carbon dioxide (LPGPC), methane (LPGPM and under molecular oxygen (LPGPO).

Peak position in chromato- gram	Retention time [min]	Component	Non-treated	LPGPA	LPGPN	LPGPC	LPGPO	LPGPM
1	2	3	4	5	6	7	8	9
1	7.08	β-Thujene	0.02	0.04	0.49	-	0.27	-
2	7.27	a-Pinene	0.01	0.03	0.16	1.83	0.09	1.02
3	7.68	Camphene	0.01	0.03	0.02	1.33	200	0.72
5	8.22	Sabinene	1.68	0.14	8.25	0.12	7.09	-

Specific modulation of flavor and aroma of Greek oregano...

1	2	3	4	5	6	7	8	9
6	8.36	1-Octen-3-ol	0.59	0.83	2.42	-	4.82	3.47
8	8.49	3-Octanone	0.69	0.35	0.01	-	-	-
9	8.60	β-Pinene	0.69	0.48	2.09	1.25	4.12	1.15
10	8.79	3-Octanol	2.38	0.08	-	-		1.05
12	9.05	α-Phellandrene	0.95	0.10	0.35	0.76	0.30	0.42
13	9.13	3-Carene	1.04	0.04	2.08	1.92	-	2.31
14	9.32	a-Terpinene	0.56	0.82	1.67	0.27	1.56	0.23
16	9.51	p-Cymene	3.24	1.21	0.05	0.28	0.06	0.79
17	9.63	D-Limonene	0.49	0.10	1.60	4.19	1.40	4.74
20	9.77	trans-β- Ocimene	0.34	0.20	0.08	2.94	0.08	-
21	10.04	β-Ocimene	0.23	0.20	0.46	1.62	0.06	_
22	10.37	y-Terpinene	6.40	4.60	2.13	0.35	2.00	0.37
23	10.67	cis-β-Terpineol	2.35	0.51	5.95	0.62	0.35	0.90
27	11.40	Linalool	0.06	0.53	<u>ū</u>	0.35	-	2.44
28	11.46	cis-4-Thujanol	0.20	0.19	1.35	0.35	6.20	0.97
30	12.11	Neo-allo- ocimene	0.19	0.03	0.14	0.15	0.13	0.13
39	13.19	1,3-Dimethyl- -1-cyclohexene	0.65	0.06	0.09	0.21	3.48	0.21
40	13,24	endo-Borneol- -dup1	0.32	0.57	0.07	1.28	0.07	4.54
44	13.50	Terpinen-4-ol	0.20	0.06	0.72	0.05	0.84	0.62
46	13.80	a-Terpineol	0.65	0.07	3.11	0.35	6.28	4.65
48	13.87	Dihydroca- rvone	0.04	0.06	0.14	-	0.15	1.24
50	14.04	trans-Dihydro- carvone	1.61	0.07	~		0.09	2.01
53	14.82	Methyl thymyl ether	1.36	0.04	3.65	-	-	4.25
59	15.08	Thymoquinone	1.14	0.64	0.01	375	-	-
66	16.04	Thymol	4.43	0.26	8.92	-	13.26	-
68	16.33	Carvacrol	56.32	81.32	40.94	72.46	31.45	44.65
71	17.05	Elixene	0.95	0.09	0.78	-	0.65	-
73	17.45	Eugenol	0.17	0.10	1.39	6.84	2.42	5.47
76	17.70	Carvacryl acetate	0.06	0.10	2.31	==	1.08	-

Herbalism nr 1(9)/2023

1	2	3	4	5	6	7	8	9
82	18.97	Caryophyllene	8.25	0.92	1.56	0.11	7.24	3.75
83	19.14	β-Copaene	0.02	0.04	-	0.06	-	17
86	19.52	Humulene	0.54	0.18	0.06	0.04	1.68	0.51
88	19.83	Germacrene D	0.25	3.81	5.96	877	-	4.28
89	19.99	y-Elemene	0.08	0.13	0.99	140	2.18	
91	20.08	β-Bisabolene	0.75	0.92	-	-	0.41	2.14
94	20.74	4-epi-cubedol	0.07	0.03	-	7	0.15	0.12
95	20.80	Caryophylle- ne oxide	0.02	0.02	-	0.27	0.04	0.85
Total number of components		41	41	34	26	32	30	
rield of essential oil [mL/100 g dry mass]*		0.2	0.2	0.2	0.2	0.1	0.2	

<sup>\*</sup>Data quoted with ± 0.05% precision.

Source: own research.

Thus, carvacrol was chiefly responsible for flavour and aroma of this essential oil. However, it also introduced antimicrobial and some fungicidal properties [31]. Watering with LPGPA and LPGPC considerably increased the level of that phenol whereas watering with LPGPN, LPGPO and particularly LPGPM reduced its level.

Caryophyllene, a sesquiterpene, is known for its anticancer and analgesic properties [32]. Watering with LPGP-treated water decreased the original content of that component in the essential oil (8.25%) in the order LPGPM (7.24%) > LPGPO (3.75%) > LPGPN (1.56%) > LPGPA (0.92%) > LPGPC (0.11%). It is biosynthesized from the common terpene precursors dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). First, single units of DMAPP and IPP are reacted via an SN1-type reaction with the loss of pyrophosphate, catalyzed by the enzyme GPPS2, to form geranyl pyrophosphate (GPP). This further reacts with a second unit of IPP, also via an SN1-type reaction catalysed by the enzyme IspA, to form farnesyl pyrophosphate (FPP). Finally, FPP undergoes QHS1 enzyme-catalysed intramolecular cyclization to form caryophyllene [33].

 $\gamma$ -Terpinene was a subsequent component in the order arranged according to its decreasing content in the essential oil. That terpene exhibits antimicrobial properties against various human pathogens [34]. Watering with LPGP-treated water reduced the content of this terpene in the order: control (6.40%) > LPGPA (4.10%) > LPGPN (2.13%) > LPGPO (2.00%) > LPGPM (0.37%) > LPGPC (0.35%).

Apart from a pleasant aromatic scent, thymol, a monoterpenoid phenol, introduced to the essential oil strong antiseptic properties. Hence, it is used as medical disinfectant and non-persisting pesticide [35, 36]. Watering the plant with LPGPO tremendously increased the content of this compound in the essential oil from 4.43% in control to 13.26%. Also watering with LPGPN doubled the initial concentration therein. LPGPA strongly decreased the content of thymol to 0.26% and watering with either LPGPC or LPGPM completely inhibited formation of thymol.

p-Cymene, the aromatic hydrocarbon contributes to the aroma of the essential oil. It can be dangerous for human health [37, 38]. Watering with the LPGP-treated water considerably but not completely inhibited biosynthesis of that compound. LPGPN and LPGPO were the most efficient in this respect.

3-Octanol is used chiefly as a fragrant compound. Although slightly irritating at higher concentration it is safe in use [39]. Its concentration in control sample (2.35%) was significantly reduced when the plant was watered with LPGPM and LPGPA to 1.05% and 0.68%, respectively, and totally inhibited by watering with LPGPN, LPGPC and LPGPO.

cis- $\beta$ -Terpineol, the terpene alcohol is used as a fragrant component. It is absorbed by skin, therefore, it is irritating at higher concentration. At low concentration it is safe in use [40]. Watering the plant with LPGPN increased the yield of that terpene by about 120% whereas the watering with other kinds of the LPGP-treated water considerably but not completely inhibited its biosynthesis and, hence, reduced its content in the essential oil.

Sabinene is one of the chemical compounds that contribute to the spiciness of black pepper [41]. In Greek oregano its biosynthesis was strongly promoted by watering its plantation with LPGPN and LPGPO lifting its content from 1.18% in control to 8.25 and 7.09%, respectively. Watering with LPGPA and LPGPC reduced the sabinene content to slightly over 0.10% and watering with LPGPM completely eliminated sabinene from the essential oil.

trans-Dihydrocarvone, terpene of a medium intensity strength of aroma should not be used for fragrance and flavouring [42]. Its biosynthesis is stimulated when the plant was watered with LPGPM and strongly inhibited by watering with other LPGP-treated kinds of water.

Methyl thymyl ether is appreciated for its burnt, smoky, and woody tasting. It causes skin, eye and respiratory tracts irritation [43]. Watering the plant with LPGPN and, particularly with LPGPM strongly promoted its biosynthesis whereas LPGPA, LPGPC and LPGPO inhibited it.

Thymoquinone, a component considered as useful in treating cancer [44, 45] resided in the essential oil extracted after watering the plant with non-treated water (control) in the amount of 1.14%. Watering with either LPGPA or LPGPN reduced the amount of that component to 0.64 and 0.01%, respectively, whereas watering with remained kinds of water under study completely inhibited formation of that component.

The last in the order 3-carene is reported as useful in relieving inflammation related to arthritis or fibromyalgia and boosting bone health, subsequently benefitting people with osteoporosis, osteoarthritis, or other bone diseases. It is also suggested as memory stimulating terpene [46, 47]. The plant watering with LPGPM, LPGPN and LPGPC stimulated biosynthesis of that terpene whereas LPGPA and LPGPO inhibited its formation.

Insight in Table 2 shows that watering plantations with various kinds of LPGPtreated water did not provide any novel component of the essential oil which would be absent in the oil from the control plantation.

Separate studies are required to explain mechanisms of biosynthesis of particular components of the essential oil. Some speculations on that subject were presented in our earlier papers [12, 13]. Recently Krause et al. [48] presented the course of biosynthesis of thymol and carvacrol. It involved the cyclization of geranyl diphosphate to  $\gamma$ -terpinene, followed by a series of oxidations via p-cymene. That finding could rationalize an increment of the carvacrol content and simultaneously decrease of some other components in the essential oil isolated from the plant watered with LPGPA. This water contained singlet oxygen as guests of aqueous clathrates. On decomposition of LPGPA clathrates, singlet oxygen turned in the triplet oxygen molecules providing the medium of oxidative properties and, hence, supporting biosynthesis of carvacrol . An increase in the yield of biomass from the plants watered with LPGPN and LPGPC could be rationalized in terms of the content of the molecules of nitrogen and carbon dioxide, respectively, in their excited states. These molecules were incorporated by the plant for biosynthesis of proteins as presented in our earlier papers [12, 13].

#### Conclusions

Watering plantations of Greek oregano influences the yield and quality of the crops as well as composition of essential oil isolated from the dried plant. The effect of watering depends on the method of the treatment of water with glow plasma. The selection of particular kind of the plasma treated water for watering provides essential oil of diverse suitability as the spice, flavouring agent and also a potential mean applicable from the point of view of herbal medicine and aromatherapy. Regarding the kind of water taken for watering always carvacrol was a dominating component of the essential oil. Never Application of particular kind of treated water always changed the yield of particular components of the oil up to a total inhibition of its formation but never resulted in formation of novel components. Separate studies are required to explain mechanisms of biosynthesis of particular components of the essential oil.

## Literature

- Oszczeda Z., Elkin I., Strek W., Equipment for treatment of water with plasma, Polish Patent PL, 216025 B1, 28 February, 2014.
- [2] Reszke E., Yelkin L., Oszczęda Z., Plasming lamp with power supply, Polish Patent PL, 2017, 227530 B1.
- [3] Białopiotrowicz T., Ciesielski W., Domański J., Doskocz M., Fiedorowicz M., Grąż K., Kołoczek H., Kozak A., Oszczęda Z., Tomasik P., Structure and physicochemical properties of water treated with low-temperature low-frequency plasma, Current Physical Chemistry, 2016, 6, s. 312–320.
- [4] Chwastowski J., Ciesielska K., Ciesielski W., Khachatryan K., Kołoczek H., Kulawik D., Oszczęda Z., Tomasik P., Witczak M., Structure and physicochemical properties of water treated under nitrogen with low-temperature glow plasma, Water, 2020, 12, 1314.
- [5] Ciesielska A., Ciesielski W., Kołoczek H., Kulawik D., Kończyk J., Oszczęda Z. Tomasik P., Structure and some physicochemical and functional properties of water treated under ammonia with low-temperature low-pressure glow plasma of low frequency, Open Chemistry, 2020, 18, s. 1–12.
- [6] Ciesielska A., Ciesielski W., Khachatryan K., Kołoczek H., Kulawik D., Oszczęda Z., Soroka J.A., Tomasik P., Structure and physicochemical properties of water treated under carbon dioxide with low-temperature glow plasma of low frequency, Water, 2020, 12, 1920.
- [7] Ciesielska A., Ciesielski W., Khachatryan K., Kołoczek H., Kulawik D., Oszczęda Z., Soroka J.A., Tomasik P., Structure and physicochemical properties of water treated under methane with low-temperature glow plasma of low frequency, Water, 2020,12, 1638.
- [8] Chwastowski J., Ciesielski W., Khachatryan K., Kołoczek H., Kulawik D., Oszczęda Z., Soroka J.A., Tomasik P., Witczak M., Water of increased content of molecular oxygen, Water, 2020, 12, 02488.
- [9] Wolski K., Talar-Krasa M., Leshschenko A., Dradrach A., Adamczewska-Sowińska K., Oszczęda Z., Application of nanowater and biopreparations in agriculture (in Polish), Studia i Monografie Politechniki Opolskiej, 2014, 404, s. 265–372.
- [10] Pisulewska E., Ciesielski W., Jackowska M., Gastoł M., Oszczęda Z., Tomasik P., Effect of water treated with low-pressure, low-temperature glow plasma of low frequency on planted peppermint (*Mentha piperita*), EJPAU, Seria Biotechnologia, 2018, 21(3), #01.
- [11] Ciesielska K., Ciesielski W., Kulawik D., Oszczęda Z., Tomasik P., Cultivation of cress involving water treated under different atmospheres with low temperature, lowpressure glow plasma of low frequency, Water, 2020, 12, 2152.
- [12] Ciesielska K., Ciesielski W., Girek T., Kołoczek H., Oszczęda Z., Tomasik P., Reaction of Lavandula angustifolia Mill. to water treated with low-temperature, low-pressure glow plasma of low frequency, Water, 2020, 12, 3168.
- [13] Ciesielski W., Gastoł M., Girek T., Kulawik D., Oszczęda Z., Pisulewska E., Tomasik P., Specific controlling essential oil composition of basil (*Ocimum basilicum L.*), Water, 2020, 12, 3332.
- [14] Tomasik-Krótki J., Strojny J., Scaling of sensory impressions, Journal of Sensory Studies, 2008, 23, s. 251–266.
- [15] Szott K., Krzywda M., Strojny J., Tomasik P., Sensual taste cloro associations and their link to temperament, Study. Social Science (Canada), 2014, 5(3), s. 1–8.

## Herbalism nr 1(9)/2023

- [16] Kew World Checklist of Selected Plant Families, Royal Botanic Gardens, Oregano, Origanum vulgare L. Kew, Richmond, Surrey, UK. 2017.
- [17] Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Canada. Oregano and Marjoram (17 October 2012).
- [18] Figiel A., Szumny A., Gutiérrez-Ortíz A., Carbonell-Barrachina A.A., Composition of oregano essential oil (Origanum vulgare) as affected by drying method, Journal of Food Engineering, 2010, 98, s. 240–247.
- [19] Dragland S., Senoo H., Wake K., Holte K., Blomhoff R., Several culinary and medicinal herbs are important sources of dietary antioxidants, Journal of Nutrition, 2003, 133, s. 1286–1290.
- [20] Mockute D., Bernotiene G., Judzentiene A., The essential oil of Origanum vulgare L. Spp. Vulgare growing wild in Vilnius district (Lithuania), Phytochemistry, 2001, 57, s. 65–69.
- [21] Tair A., Weiss E-K., Palade L.M., Loupassaki S., Makris D.P., Ioannou E., Roussis V., Kefalas P., Origanum species native to the island of Crete: in vitro antioxidant characteristics and liquid chromatography—mass spectrometry identification of major polyphenolic components, Natural Products Research, 2014, 28, s. 1284–1287.
- [22] Teixeira B., Marques A., Ramos C., Serrano C., Matos O., Neng N.R, Noguera J.M.F., Saraiva J.A., Nunes M.L., Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oil, Journal of the Science of Food and Agriculture, 2013, 93, s. 2707–2714.
- [23] Cheikhyoussef A., Cheikhyoussef N., Ramadan M.F., Cold pressed oregano (Origanum vulgare) oil, [in:] Cold Pressed Oils, Ramadan, M.F. (ed.), Academic Press, Ch. 25, s. 289–293, 2020.
- [24] Issaoui M., Delgado A.M. Grading, labelling and standardization of edible oils, [in:] Ramadan M. (ed.), Fruit Oils: Chemistry and Functionality. Springer, Cham 2019.
- [25] Oregano, MedlinePlus, US National Library of Medicine, 2016 (Retrieved Nov. 8th 2020).
- [26] LaTonya M.M., Warning Letter: Young Living. Inspections, compliance, enforcement, and criminal investigations, US Food and Drug Administration (22 September, 2014). (Retrieved 7 Nov. 8th 2020).
- [27] McMillan, K.L., Warning Letter: Long Life Unlimited. Inspections, compliance, enforcement, and criminal investigations, US Food and Drug Administration (31 January 2018), (Retrieved Oct. 22nd 2020).
- [28] Zambrana, I.A., Warning Letter: Absonutrix. Inspections, Compliance, Enforcement, and Criminal Investigations. US Food and Drug Administration (25 July, 2017). (Retrieved Oct. 22nd 2020).
- [29] NIST 11 Library, 2021. https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:start
- [30] Duncan D.B., Multiple range and multiple F tests, Biometrics, 1955, 11, s. 1-42.
- [31] Andersen A., Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol, International Journal of Toxicology, 2006, 25, s. 29–127.
- [32] Fidyt K., Fiedorowicz A., Strządała L., Szumny A., β-caryophyllene and β- caryophyllene oxide-natural compounds of anticancer and analgesic properties, Cancer Medicine, 2016, 5, s. 3007–3017.
- [33] Yang J., Li Z., Guo L., Du J., Bae H.-J., Biosynthesis of β-caryophyllene, a novel terpenebased high-density biofuel precursor, using engineered Escherichia coli, Renewable Energy, 2016, 99, s. 216–223.

- [34] Tabasum F., Beenish B.N., Gousia G., Tahiya Q.T.A.B., Antioxidant potential and health benefits of cumin Antioxidant potential and health benefits of cumin, Journal of Medicinal Plants Studies, 2018, 6, s. 232–236.
- [35] Hu D., Coats J., Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory, Pest Management Science, 2008, 64, s. 775–779.
- [36] Nieto G., Biological activities of three essential oils of the Lamiaceae family, Medicines 2017, 4(3), 63.
- [37] Stelmakh V.A., Toxicological properties and the nature of the combined effect of aorthomenthane hydrocarbon mixtures, Gigiena truda i professionalnye zabolevanyia 1988, 9, s. 34–37.
- [38] Stelmakh, V.A., Talapin, V.I. & Pertsovskii, A.L. Experimntal data to establish MPEL of 1-methyl-2-isopropyl-benzene oin the air of work area, Gigiena truda i professionalnye zabolevanyia 1985, 3, s. 49–50.
- [39] TGSC Information System, 2020, http://www.thegoodscentscompany.com/data/rw1027901. html (Retrieved Nov. 8th 2021).
- [40] Barbieri G., Vallone S., Orsini F., Paradiso R., DePascale S., Negre-Zakharov F., Maggio A., Stomatal density and metabolic determinants mediate salt stress adaptation and water use efficiency in basil (*Ocimum basilicum* L.), Journal of Plant Physiology, 2012, 169, s. 1737–1746.
- [41] Shulgin A.T., Sargent T., Naranjo C., The chemistry and psychopharmacology of nutmeg and of several phenylisopropylamines, Psychopharmacological Bulletin, 1967, 4(3), s. 13.
- [42] trans-Dihydrocarvone, http://www.perflavory.com/docs/doc1511171.html#tosafiu (Retrieved, Nov. 8th, 2021).
- [43] PubChem 2020, 2-isopropyl-5-methylanisole (Compound) https://pubchem.ncbi.nlm. nih.gov/compound/Thymol-methyl-ether#section=Related- Substances (Retrieved on Nov. 8th 2021).
- [44] Abu Khader M.M., Thymoquinone in the clinical treatment of cancer: Fact or fiction? Pharmacognosy Review, 2013, 7(14), s. 117–120.
- [45] Woo C.C., Kumar P., Seth G., Tan K.H.B., Thymoquinone: Potential cure for inflammatory disorders and cancer, Biochemistry and Pharmacology, 2012, 83, s. 443–451.
- [46] Jeong J-G., Kim Y.S., Min Y.K., Kim S.H., Low concentration of 3-carene stimulates the differentiation of mouse osteoblastic MCC3T3E1 subclone 4 cells, Phytotherapy Research, 2008, 22, s. 18–22.
- [47] Lodi M., Terpenes 411:Delta 3-carene, EMBER Journal of Cannabis Culture, 2019, 3.
- [48] Krause S.T., Liao P., Crocoll C., Boachon B., Foerster C., Leidecker F., The biosynthesis of thymol, carvacrol, and thymohydroquinone in Lamiaceae proceeds via cytochrome P450s and a short-chain dehydrogenase, PNAS, 2021, 118(52), e2110092118.

#### Do cytowania:

Ciesielski W., Gastoł M., Girek T., Kulawik D., Oszczęda Z., Pisulewska E., Tomasik P., Specific modulation of flavor and aroma of Greek oregano (*Origanum vulgare var. hirtum*) and its essential oil, Herbalism, 2023, 1(9), s. 36–49.