In Vitro Propagation Affects the Composition of Narrow-Leaved Lavender Essential Oils

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Received: 28 April 2017; accepted: 04 July 2017

The aim of this study was to identify and determine by means of gas chromatography–flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS) method the volatile compounds of essential oils obtained from three varieties of narrow-leaved lavender grown in the field and in *in vitro* cultures. The essential oils were isolated by hydrodistillation in Deryng apparatus. It was found that the analyzed essential oils varied in terms of chemical composition depending on the variety and conditions of growth. Sixty-four to 87 different compounds were identified in the oils. Essential oils of all 3 varieties obtained in *in vitro* cultures contained large amounts of borneol (13–32%). This compound was also dominant in plants obtained from in vivo conditions in varieties Ellagance Purple (11%) and Blue River (13%), and in the Munstead variety, the dominant compound was linalool (13%). High concentration of *epi*-α-cadinol (10–20%) was found in essential oils obtained from *in vitro* cultured plants. Globulol was found in high concentration (10%) in the Munstead variety grown in *in vitro* conditions. However, significant quantitative and qualitative differences were found with respect to composition of essential oils obtained from plants grown in the field and *in vitro* conditions. There was a lack of (*E*)-β-ocimene, 3-octyn-2-one, 1-octen-3-yl acetate, sabina ketone, pinocarvone, *trans*-carveol, nerol, *epi*-longipinanol, or humulene epoxide II. In comparison to oils obtained from field-grown plants, the oils isolated from plants grown in *in vitro* conditions contained the less volatile compounds identified in the final stage of GC–FID and GC–MS analysis, i.e., thymol, carvacrol, γ-gurjunene, *trans*-calamene, α-calacorene, khusinol, and 8-cedren-13-ol.

Keywords: *Lamiaceae*, *Lavandula* spp., secondary metabolites, GC/MS

Introduction

The lavender genus (*Lavandula*) belongs to the mint family (*Lamiaceae* Lindl.). It comprises 39 species, numerous hybrids, and approximately 400 registered varieties [1]. *Lavandula* is native to the Mediterranean region and is commercially grown, among others, in France, Spain, the United Kingdom, Bulgaria, Australia, China, and in the United States [2]. The most commonly grown and best-known species of the *Lavandua* genus are *Lavandula stoechas*, *Lavandula dentata*, and most of all *Lavandula angustifolia.*

Narrow-leaved lavender (*L. angustifolia* Mill. syn. *Lavandula officinalis* Chaix) is used in many industries mainly due to its essential oils characterized by a specific aroma. The oils are used in perfume [3, 4] and cosmetics industry [5, 6]. Apart from the aroma, the oils show a number of medicinal properties including anti-bacterial properties and are, therefore, used in medicine and pharmaceutical industry [3, 7–10]. Essential oils are mixtures of mainly monoterpene and sesquiterpene compounds; however, their composition depends on various factors connected with both biological material used for isolating the oil as well as with the physical factors of this technological process [11–13].

Essential oils are contained in the secretory tissue covering the entire above ground portion of the plant; therefore, essential oil can be isolated from flowers [14–16], stem [17], or leaves [18]. However, the type of material used for oil isolation affects the concentration of particular compounds of essential oils [19]. The method of oil isolation also has a significant effect on its composition. Reverchon and Della Porta [20] isolated essential oils using two methods: hydrodistillation (steam distillation — the most widely used commercial method), and supercritical fluid extraction. The analysis of the composition of the obtained oils showed significant differences in the amount of, among others, linalyl acetate — using supercritical extraction, its concentration was 34.7%, and using hydrodistillation, only 12.1%.

Numerous studies point to significant variations in terms of composition of essential oils isolated from species of the *Lavandula* genus. Linalool, camphor, and 1,8-cineole were the main components of essential oil isolated from *Lavandula latifolia* Med. [21] and *Lavandula intermedia* Emeric ex Loiseleur [22]. The main components of oil obtained from *Lavandula pedunculata* (Miller) Cav. were camphor and 1,8-cineole [15] and in the case of *Lavandula pinnata* L., α- and β-phellandrene [23]. In essential oil of *Lavandula viridis* L'Hér, 1,8-cineole and camphor were the main components [24], and in oil obtained from *L. stoechas* L., fenchon, camphor, myrtenyl acetate, and 1,8-cineole [25]. Main compounds dominating the aroma of essential oil isolated from *L. angustifolia* are linalool, which amounts to 25–38%, and linalool acetate, 25–45% [26–28]. Nevertheless, the studies were limited to field-grown plants or growing in natural conditions.

The method of plant tissue culture allows for quick proliferation of tissue in controlled sterile conditions. So far, it has been used in production of large number of plant cuttings genetically identical to the mother plant [29, 30]. Nowadays, as the technique of *in vitro* culture develops and its costs decrease, the aim is to apply this technique for proliferation of plant tissue in order to obtain secondary metabolite, including essential oils. However, for this to happen, it is necessary to determine the influence of the conditions of *in vitro* plant cultures on the variations in the amount and composition of essential oils — as was found in

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DOI: 10.1556/1326.2017.00317

First published online: 10 October 2017

Thymus caespititius [31], *Achillea millefolium* [32], *Agastache rugosa* [33], and *Lantana camara* [34].

At present, there is a little information on the composition of essential oils isolated from tissues of plants of *Lavandula* genus, proliferated in *in vitro* cultures. The composition of isolated essential oils was identified as for, among others, *L. pedunculata* [35, 15], *L. viridis* [24], *L. vera*, and *L. viridis* [36]. The study by Prasad et al. [37] identified the effects of proliferation of shoots of *L. officinalis* syn., *L. angustifolia* var. *Sher-e-Kashmir* in *in vitro* cultures by comparing the composition of essential oils obtained from the mother plant with that obtained from clones which were previously proliferated in *in vitro* cultures and later grown in the field. However, there is a shortage of publications on the quantitative and qualitative composition of essential oils obtained from tissues of *L. angustifolia* during proliferation in *in vitro* cultures. The present study aims to provide the qualitative and quantitative analysis of the composition of essential oils obtained from three varieties of narrow-leaved lavender Ellagance Purple, Blue River, and Munstead grown in natural conditions as mother plants as well as those proliferated in *in vitro* conditions.

Materials and methods

Field-grown plant. The material used in the study was field-grown plants of narrow-leaved lavender (*L. angustifolia* L.) of three varieties: Ellagance Purple, Blue River, and Munstead and cultured in *in vitro* conditions. Field-growing plants were obtained from experimental cultivation by the Department of Horticulture of the West Pomeranian University of Technology in Szczecin conducted in the period 2013–2014. The seeds for initiation of the culture were obtained from voucher specimen number 195 from the Institute of Natural Fibres and Medicinal Plants in Poznan, Poland. The fragments of stems without inflorescence, harvested in mid-July from plants at full bloom, were used to initiate *in vitro* culture and air dried in order to obtain a sample for isolation of essential oil.

***In vitro* plants.** The fragments of shoots of the aforementioned field-growing plants were used as original explants for the initiation of *in vitro* cultures. The explants, 1 cm long fragments of shoots, were placed on Murashige and Skoog medium (MS) media of mineral composition according to Murashige and Skoog [38], supplemented with vitamins: nicotinic acid, 0.5 mg dm^{-3}; pyridoxine HCl, 0.5 mg dm^{-3}; thiamine HCl, 0.1 mg dm^{-3}; glycine, 2 mg dm^{-3}; agar, 8 g dm^{-3}; sucrose, 30 g dm^{-3}; and inositol, 100 mg dm^{-3}. pH was adjusted to 5.7 with solutions of 0.1 M NaOH and HCl prior to autoclaving. Media were sterilized by autoclaving at 121 °C for 20 min at 103 kPa. The sterile shoots induced to grow were proliferated on the media with a mineral composition according to Murashige and Skoog [38] supplemented with 2 mg dm^{-3} kinetin and 0.2 mg dm^{-3} indole-3-acetic acid. Proliferation cycle was repeated 4 times after 6 weeks each. The cultures were placed in phytotrons at 23 ± 1 °C with a 16 h light–8 h night photoperiod with a photosynthetic photon flux density (PPFD) of 30 μmol m^{-2} s^{-1} supplied by 21 W cool white fluorescent lamps. Then, the proliferated lavender shoots (together with leaves) were air dried and constituted a sample for isolation of essential oils.

Gas chromatography with mass spectroscopy (GC–MS) of essential oils. The analysis was conducted in Central Agroecology Laboratory of the University of Life Sciences in Lublin. Dried material in weight of 20 g of field-grown and *in vitro* plant tissue were used for the purpose of oil isolation. Isolation of essential oils was repeated in three replicates. The percentage content of essential oil was determined using hydrodistillation method of steam distillation in Deryng apparatus, according to European Pharmocopoeia [39].

The chemical constituents of the essential oil were analyzed by capillary gas chromatography–flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS). The oil was stored at 4 °C until the GC–FID and GC–MS analysis. The qualitative and quantitative composition of essential oil was determined by GC–MS method using gas chromatograph Varian Chrompack CP-3800 equipped with a with a mass detector (4000 GC-MS/MS) and a flame ionization detector (FID). A VF-5ms column (equivalent of DB-5) was used. Parameters of chromatographic column were as follows: length, 30 m; internal diameter, 0.25 mm; stationary phase film thickness, 1 μm. The carrier gas was Helium (He) with constant flow rate 0.5 mL/min. The temperature of the dispenser was 250 °C (split 1:100). The dosing was 1 μL of the solution (10 μL of sample in 1000 μL of hexane). Temperature gradient was applied (50 °C for 1 min, then an increase to 250 °C at a rate of 4 °C/min and 250 °C for 10 min). The range recorded was 40–1000 *m*/*z*, and the scan rate was 0.8 s/scan. Retention index was determined on grounds of a series of alkanes C10–C40.

Software and statistical analysis. The obtained results of the hydrodistillation assays were statistically analyzed using analysis of variance. For two-way cross-classification, evaluating the significance of differences with Tukey's confidence intervals and performing least significant difference (LSD) calculations at the level of significance $\alpha = 0.05$.

The HP Chemstation software was used for the collecting and processing the data. The qualitative analysis was based on identification of compounds in samples by comparing MS spectra with standard spectra of NIST Mass Spectral Library [40] and with data available in the literature [41]. The compounds which showed conformity of mass spectra with the standard library spectra of more than 95% were taken into account. Relative percentage content of the analyzed compounds was based on the peak area of the total ionic current of all the compounds present in a given sample. The quantitative composition of essential oil was determined assuming that the sum of individual compounds amounts to 100%. The analysis was repeated in three replicates for each experiment. The obtained results of the assays were statistically analyzed using analysis of variance for one-way cross-classification, separately for each compound and variety, evaluating the significance of differences between lavender grown in the field and in *in vitro* cultures with Student *t* test calculated at a confidence level of $P \leq 0.05$.

Results and discussion

From dry plant material of field-grown narrow-leaved lavender, 0.53% of essential oil was obtained from Ellagance Purple, 0.52% from Blue River, and 0.90% from the Munstead variety. Propagation of plants in *in vitro* cultures resulted in a decrease in the content of essential oil in shoots and leaves — distillation efficacy was 0.51% for Ellagance Purple, 0.20% Blue River, and 0.84% for the Munstead variety (Table 1). Statistically significant

Table 1. Hydrodistillation efficacy of *Lavandula angustifolia* varieties field-grown and propagated *in vitro* (%). The values represent the means of three replicates ± SE

Cultivar (A)	Plant type (B)		Mean
	Field-grown	*In vitro*	
Ellagance Purple	0.53 ± 0.03	0.51 ± 0.04	0.52
Blue River	0.52 ± 0.03	0.20 ± 0.01	0.36
Munstead	0.90 ± 0.04	0.84 ± 0.04	0.87
Mean	0.65	0.52	
LSD 0.05 (Tukey's test) for:			
Cultivar (A)	0.180		
Plant type (B)	0.120		
Interaction (A × B)	0.254		
Interaction (B × A)	0.216		

difference of hydrodistillation efficacy between means of Blue River field-grown and *in vitro* essential oils was observed. However, between essential oils isolated from field-grown and *in vitro* plants of Ellagance Purple and Munstead, cultivar was not observed.

Table 2 shows the detailed composition and amounts of particular compounds identified in essential oils of Ellagance Purple, Blue River, and Munstead varieties of narrow-leaved lavender grown in in vivo and *in vitro* conditions. The GC–MS analysis allowed for identification of 92.44–97.71% of compounds in the analyzed essential oils. Most of the compounds belong to monoterpenoids group and monoterpenoid esters. The chemical composition of essential oils isolated from shoots of the three varieties of narrow-leaved lavender field and *in vitro* grown varied greatly (Table 2). In the oil, isolated from field-grown plant, 83 compounds were identified in Ellagance Purple,

Table 2. Essential oil composition (%) of varieties *Lavandula angustifolia* isolated from field-grown parent plants and the respective *in vitro* shoot cultures

Compound	RT (min)	RI	Ellagance Purple				Blue River				Munstead			
			Field-grown		*In vitro*		Field-grown		*In vitro*		Field-grown		*In vitro*	
Tricycylene	6.838	926	tr.	–	tr.	–	0.10^{ns}	–	0.14^{ns}	±0.15	0.11^{a}	–	0.05^{b}	–
α-Thujene	6.921	931	0.11^{b}	–	0.23^{a}	±0.02	0.21^{ns}	–	0.73^{ns}	±0.84	0.17^{b}	–	0.36^{a}	±0.01
α-Pinene	7.157	939	1.67^{b}	±0.00	2.69^{a}	±0.17	1.17^{ns}	±0.00	1.33^{ns}	±0.45	0.88^{b}	±0.03	3.25^{a}	±0.02
α-Fenchene	7.620	951	0.07	–	–	–	0.07	–	–	–	–	–	–	–
Camphene	7.673	953	0.52^{b}	–	1.21^{a}	±0.07	1.37^{ns}	±0.06	0.98^{ns}	±1.19	1.67^{a}	±0.09	1.19^{b}	±0.00
Thuja-2.4-(10)-diene	7.797	957	tr.	–	tr.	–	tr.	–	tr.	–	tr.	–	tr.	–
Benzaldehyde	8.090	963	tr.	–	–	–	tr.	–	–	–	tr.	–	–	–
Verbenene	8.341	967	0.60^{a}	±0.07	0.28^{b}	±0.07	0.64^{a}	±0.04	0.36^{b}	±0.11	0.33^{b}	±0.02	0.58^{a}	±0.01
Sabinene	8.384	976	0.26^{b}	±0.04	0.98^{a}	±0.02	0.27^{a}	±0.02	0.09^{b}	±0.03	0.12^{b}	–	0.87^{a}	±0.01
β-Pinene	8.560	981	5.17^{b}	±0.06	6.50^{a}	±0.04	2.11^{a}	–	0.29^{b}	±0.09	0.83^{b}	–	6.26^{a}	±0.01
3-Octanone	8.777	986	0.19	±0.26	–	–	0.27	±0.01	–	–	0.34	±0.02	–	–
Myrcene	8.901	991	0.07^{b}	–	0.34^{a}	±0.01	0.16^{b}	±0.02	0.55^{a}	±0.17	0.58^{ns}	–	0.37^{ns}	±0.16
Dehydro-1.8-cineole	8.949	992	0.20^{a}	–	0.10^{b}	±0.01	0.13	±0.01	–	–	–	–	–	–
3-Octanol	9.161	993	–	–	–	–	tr.	–	–	–	tr.	–	–	–
trans-Isolimonene	9.250	995	–	–	–	–	–	–	0.08	±0.02	–	–	0.15	–
α-Phellandrene	9.498	1004	–	–	tr.	–	–	–	–	–	–	±0.03	–	–
δ-2-Carene	9.573	1011	0.78^{b}	–	2.74^{a}	±0.19	1.29^{b}	±0.02	4.49^{a}	±1.52	0.66^{b}	±0.03	4.47^{a}	±0.01
α-Terpinene	9.850	1018	0.06^{b}	–	0.07^{a}	–	tr.	–	–	–	0.06^{b}	–	0.11^{a}	±0.00
p-Cymene	9.945	1027	1.29^{a}	±0.01	0.82^{b}	±0.04	1.47^{a}	±0.02	0.91^{b}	±0.27	0.71^{b}	–	1.14^{a}	±0.01
o-Cymene	10.118	1032	2.40^{a}	±0.04	1.31^{b}	±0.08	3.11^{a}	±0.05	1.21^{b}	±0.36	1.62^{b}	±0.07	1.80^{b}	±0.03
Sylvestrene	10.278	1033	1.65^{b}	±0.03	2.17^{a}	±0.10	3.79^{ns}	±0.03	2.64^{ns}	±0.82	2.41^{a}	±0.10	2.05^{b}	±0.03
1.8-Cineole	10.389	1049	4.04^{b}	±0.05	4.84^{a}	±0.27	5.48^{a}	–	0.86^{b}	±0.09	2.70^{a}	±0.07	2.00^{b}	–
(*Z*)-β-Ocimene	10.485	1052	0.19	±0.01	tr.	–	0.10^{a}	–	0.05^{b}	±0.01	1.08	±0.04	tr.	–
(*E*)-β-Ocimene	10.852	1055	0.12	–	–	–	0.11	–	–	–	0.73	±0.03	–	–
o-Cresol	11.064	1062	tr.	–	–	–	tr.	–	–	–	tr.	–	–	–
γ-Terpinene	11.289	1067	0.14^{ns}	–	0.14^{ns}	–	0.14^{ns}	±0.01	0.09^{ns}	±0.03	0.18^{ns}	–	0.20^{ns}	–
trans-Linalool oxide	11.716	1088	0.96	±0.01	–	–	0.59	±0.01	–	–	0.47	–	–	–
cis-Sabinene hydrate	11.724	1089	–	–	0.21	±0.01	tr.	–	0.09	±0.03	–	–	0.13	±0.01
m-Cymenene	12.111	1090	0.10	±0.02	–	–	0.11	±0.01	–	–	0.06	–	–	–
Terpinolene	12.130	1093	–	–	0.14	±0.01	–	–	0.27	±0.07	–	–	0.24	±0.00
p-Mentha-2.4(8)-diene	12.269	1094	–	–	0.24	±0.01	–	–	0.20	±0.06	–	–	0.22	–
cis-Linalool oxide	12.280	1095	0.67	±0.04	–	–	0.44	±0.03	–	–	0.43	±0.02	–	–
p-Cymenene	12.463	1097	0.27^{a}	±0.06	0.12^{b}	±0.01	0.21^{a}	±0.04	0.11^{b}	±0.03	0.13^{b}	–	0.15^{a}	–
Linalool	12.755	1098	5.94^{a}	±0.26	2.32^{b}	±0.05	3.71^{ns}	–	3.31^{ns}	±0.88	12.67^{a}	–	0.36^{b}	±0.00
trans-Sabinene hydrate	12.817	1099	–	–	tr.	–	–	–	–	–	–	–	tr.	–
3-Octyn-2-one	12.881	1117	0.25	–	–	–	0.10	±0.01	–	–	0.09	–	–	–
1-Octen-3-yl acetate	13.001	1121	0.09	–	–	–	0.42	±0.01	–	–	0.50	–	–	–
Endo-fenchol	13.485	1122	–	–	0.05	–	–	–	–	–	–	–	–	–
trans-Mentha-2.8-dien-1-ol	13.557	1123	0.06	–	–	–	0.23	±0.01	–	–	0.11	±0.00	–	–
trans-*p*-Mentha-2.8-dien-1-ol	13.559	1124	–	–	0.13	–	–	–	0.25	±0.08	–	–	0.13	–
cis-Menth-2-en-1-ol	13.645	1128	0.12	–	–	–	0.42	±0.02	–	–	0.21	±0.00	–	–
α-Campholenal	13.742	1129	0.29^{a}	±0.01	0.06^{b}	–	0.22	±0.02	–	–	0.12^{ns}	±0.02	0.12^{ns}	–
cis-Limonene oxide	13.951	1132	0.21^{a}	–	0.11^{b}	–	0.28^{a}	±0.09	0.09^{b}	±0.03	0.09^{b}	–	0.15^{a}	±0.00
cis-*p*-Mentha-2.8-dien-1-ol	14.086	1136	0.11^{b}	±0.01	0.15^{a}	–	0.26^{ns}	±0.02	0.28^{ns}	±0.07	0.12^{b}	–	0.15^{a}	–
Nopinone	14.193	1138	0.46	–	–	–	0.19	–	–	–	–	–	–	–
trans-Pincarveol	14.253	1139	2.01^{a}	±0.04	1.59^{b}	±0.03	1.04^{a}	±0.20	0.10^{b}	±0.03	0.45^{b}	–	1.58^{a}	±0.00
trans-Verbenol	14.420	1141	0.68	±0.02	–	–	0.40	–	–	–	0.25	±0.00	–	–
cis-Verbenol	14.428	1142	–	–	0.66	–	–	–	0.35	±0.09	–	–	0.72	±0.03
Camphor	14.487	1144	1.30^{b}	±0.01	1.79^{a}	±0.04	1.89^{a}	±0.16	1.04^{b}	±0.21	1.33^{a}	±0.14	0.62^{b}	±0.00
Sabina ketone	14.854	1156	0.11	–	–	–	0.09	±0.01	–	–	tr.	–	–	–
Pinocarvone	15.030	1162	2.64	±0.02	–	–	1.63	±0.01	–	–	0.72	–	–	–
Borneol	15.393	1165	11.32^{b}	±0.02	32.17^{a}	±0.89	13.36^{b}	±0.06	25.75^{a}	±5.41	9.32^{b}	±0.28	13.38^{a}	±0.07
p-Cymen-8-ol	15.666	1183	2.05^{a}	±0.04	1.50^{b}	±0.01	2.01^{a}	±0.08	1.10^{b}	±0.22	4.22^{a}	±0.17	2.02^{b}	±0.00
Cryptone	15.900	1188	2.17^{a}	±0.04	0.81^{b}	±0.03	5.12^{a}	±0.11	1.01^{b}	±0.18	2.84^{a}	±0.08	0.88^{b}	±0.13
α-Terpineol	16.217	1189	3.20^{a}	±0.00	2.87^{b}	±0.07	1.91^{a}	–	0.56^{b}	±0.09	2.61^{a}	±0.10	2.38^{b}	±0.00
Myrtenal	16.622	1193	0.25^{b}	±0.01	0.57^{a}	±0.01	0.21^{b}	±0.12	0.68^{a}	±0.10	0.12^{b}	±0.03	1.22^{a}	–
Verbenone	16.724	1204	1.29^{a}	±0.03	0.30^{b}	±0.01	1.50^{a}	±0.02	0.21^{b}	±0.06	0.61^{a}	±0.04	0.43^{b}	±0.01
trans-Carveol	17.009	1217	0.47	–	–	–	0.76	±0.02	–	–	0.37	±0.02	–	–
4-Methylene-isophorone	17.069	1221	0.15	–	–	–	0.26	±0.02	–	–	0.13	–	–	–
Nerol	17.165	1228	tr.	–	–	–	0.23	–	–	–	0.40	–	–	–
Isobornylformate	17.342	1233	0.60	–	–	–	0.68	–	–	–	0.45	±0.02	–	–
cis-Sabinene hydrate acetate	17.357	1235	–	–	0.30	±0.01	–	–	0.15	±0.04	–	–	0.14	±0.01
cis-Carveol	17.477	1237	–	–	0.08	–	–	–	0.07	±0.02	–	–	–	–
Cumin aldehyde	17.837	1239	0.81	±0.03	–	–	3.71	±0.02	–	–	1.96	±0.05	–	–

(*Continued*)

Table 2. *(contd.)*

Compound	RT (min)	RI	Ellagance Purple				Blue River				Munstead			
			Field-grown		*In vitro*		Field-grown		*In vitro*		Field-grown		*In vitro*	
Carvone	17.885	1242	0.49^{ns}	±0.16	0.31^{ns}	±0.01	–	–	0.35	±0.10	–	–	0.30	±0.17
Geraniol	18.067	1245	1.04^{a}	±0.04	0.18^{b}	±0.02	0.64^{a}	±0.03	0.27^{b}	–	12.28	±0.44	–	–
Piperitone	18.235	1147	–	–	–	–	0.21	±0.01	–	–	0.09	–	–	–
Thymoquinone	18.689	1198	0.33	–	–	–	0.36	±0.01	–	–	0.10	±0.13	–	–
Linalyl acetate	19.177	1238	0.97^{a}	±0.08	0.12^{b}	±0.01	1.68^{a}	±0.02	0.43^{b}	±0.04	0.85^{a}	±0.04	0.49^{b}	±0.10
Neo-isopulegyl acetate	19.247	1270	0.06	±0.07	–	–	0.73	±0.01	–	–	1.37	±0.02	–	–
Iso-3-thujyl acetate	19.251	1278	–	–	0.05	–	–	–	0.39	±0.04	–	–	0.09	–
α-Terpinen-7-al	19.345	1280	tr.	–	–	–	0.14	–	–	–	0.07	±0.00	–	–
p-Cymen-7-ol	19.512	1287	0.56		–	–	1.61	±0.01	–	–	0.80	±0.03	–	–
Thymol	19.521	1290	–	–	0.12	–	–	–	0.28	±0.02	–	–	0.27	±0.01
Carvacrol	19.756	1292	–	–	0.13	–	–	–	0.10	±0.03	–	–	0.21	±0.00
Perilla alcohol	19.778	1298	0.25	–	–	–	0.21	±0.07	–	–	0.10	±0.01	–	–
Myrtenyl acetate	20.761	1305	0.08	–	–	–	0.07	–	–	–	–	–	–	–
3- Oxo-*p*-menth-1-en-7-al	21.062	1350	0.44	–	–	–	0.73	±0.01	–	–	0.36	±0.02	–	–
Neryl acetate	21.684	1365	0.24^{b}	–	3.77^{a}	±0.21	0.31^{b}	–	1.10^{a}	±0.02	0.70^{b}	±0.04	1.56^{a}	±0.07
Linalylisobutanoate	22.346	1370	9.77	±0.18	–	–	6.07	±0.09	–	–	5.12	±0.20	–	–
Longicyclene	22.466	1373	–	–	–	–	0.07	–	–	–	–	–	–	–
α-Funebrene	23.135	1375	–	–	–	–	–	–	0.07	±0.02	–	–	–	–
α-*cis*-Bergamotene	23.480	1398	–	–	tr.	–	–	–	0.22	–	–	–	0.07	–
E-caryophyllene	23.665	1399	1.62^{a}	±0.01	1.31^{b}	±0.05	2.05^{b}	±0.02	4.39^{a}	±0.08	0.94^{b}	±0.05	3.54^{a}	±0.17
β-Cedrene	23.870	1418	–	–	0.07	–	–	–	0.09	±0.07	–	–	0.22	±0.01
α-*trans*-Bergamotene	24.104	1427	0.17	–	–	–	0.25	–	–	–	0.10	±0.00	–	–
Coumarin	24.259	1429	0.09	–	–	–	0.08	±0.03	–	–	0.12	±0.00	–	–
Aromadendrene	24.458	1439	0.09	–	–	–	0.13	–	–	–	0.06	–	–	–
β-Duprezianene	24.460	1441	–	–	tr.	–	–	–	0.24	±0.02	–	–	–	–
epi-β-Santalene	24.552	1447	0.08	–	–	–	0.10	–	–	–	tr.	–	–	–
β-Copaene	24.558	1448	–	–	tr.	–	–	–	0.27	±0.02	–	–	–	–
(*Z*)-β-farnesene	24.739	1450	–	–	tr.	–	–	–	0.39	±0.03	0.38	–	–	–
α-Himachalene	24.898	1463	–	–	0.10	±0.03	–	–	0.36	±0.01	–	–	–	–
trans-Muurola-3.5-diene	25.048	1464	–	–	0.09	–	–	–	0.43	±0.02	–	–	0.12	±0.01
Dehydro-aromadendrene	25.182	1466	–	–	0.06	±0.01	–	–	0.11	±0.03	–	–	0.07	–
γ-Amorphene	25.628	1467	–	–	–	–	0.10	±0.04	–	–	0.11	±0.07	–	–
9-*epi*-(*E*)-caryophyllene	25.720	1469	–	–	tr.	–	–	–	0.21	±0.02	–	–	–	–
γ-Gurjunene	26.341	1473	–	–	0.12	±0.01	–	–	0.53	±0.09	–	–	0.18	±0.01
β-Bisabolene	26.432	1509	–	–	–	–	–	–	–	–	0.13	±0.00	–	–
α-Amorphene	26.447	1510	–	–	3.60	±0.23	–	–	0.14	±0.04	–	–	0.15	±0.01
γ-Cadinene	26.621	1513	1.90	±0.02	–	–	1.60^{b}	±0.01	8.38^{a}	±1.01	1.40^{b}	±0.04	4.68^{a}	±0.36
trans-Calamenene	26.840	1525	–	–	0.24	±0.02	–	–	0.62	±0.11	–	–	0.30	±0.03
epi-Longipinanol	27.149	1529	0.45	–	–	–	0.44	–	–	–	0.32	±0.02	–	–
α-Calacorene	27.473	1548	–	–	0.11	±0.01	–	–	0.26	±0.07	–	–	0.16	±0.01
Caryophyllene oxide	27.772	1564	0.67^{a}	±0.01	0.16^{b}	±0.08	0.42^{a}	±0.02	0.11^{b}	±0.03	0.42^{b}	±0.03	0.95^{a}	±0.06
Globulol	28.704	1583	6.85^{a}	±0.01	2.07^{b}	±0.23	4.40^{a}	±0.03	1.58^{b}	±0.54	4.62^{b}	±0.08	9.95^{a}	±0.88
Khusimone	29.418	1589	0.14^{ns}	±0.01	0.12^{ns}	±0.01	0.10^{ns}	±0.01	0.17^{ns}	±0.06	0.10^{ns}	–	0.15^{ns}	–
Humulene epoxide II	29.509	1614	0.15	±0.02	–	–	0.08	±0.01	–	–	0.09	–	–	–
Cubenol	29.656	1642	0.80^{ns}	±0.03	0.97^{ns}	±0.12	0.59^{b}	±0.03	1.85^{a}	±0.73	0.61^{b}	±0.04	1.44^{a}	±0.04
epi-α-Cadinol	30.449	1653	7.45^{ns}	±0.53	9.85^{ns}	±1.49	5.87^{ns}	±0.14	20.18^{ns}	±7.30	7.05^{b}	±0.01	15.81^{a}	±0.55
Epoxyallo-alloaromadendrene	30.610	1655	0.31	±0.18	–	–	0.18	±0.01	–	–	–	–	–	–
Himachalol	30.800	1657	0.42^{a}	±0.00	0.17^{b}	±0.06	0.30^{ns}	±0.02	0.41^{ns}	±0.23	0.30^{b}	±0.00	0.47^{a}	±0.08
14-Hydroxy-9-*epi*-(*E*)-caryophyllene	31.218	1658	0.52^{a}	±0.02	0.19^{b}	±0.04	0.24^{ns}	±0.03	0.37^{ns}	±0.24	1.68^{ns}	±2.79	0.37^{ns}	±0.02
cis-14-nor-Murol-5-en-4-one	31.634	1661	1.03	±0.04	–	–	0.76	±0.02	–	–	0.80	±0.00	–	–
14-Hydroxy-α-muurolene	32.368	1663	0.32	±0.02	–	–	0.29	±0.02	–	–	0.24	±0.01	–	–
Khusinol	32.384	1665	–	–	0.19	±0.03	–	–	0.42	±0.33	–	–	0.40	±0.01
8-Cedren-13-ol	32.810	1668	–	–	tr.	–	–	–	0.10	±0.09	–	–	0.09	–
Nootkatone	33.149	1776	0.63^{a}	±0.02	0.29^{b}	±0.05	0.42^{ns}	±0.01	0.52^{ns}	±0.40	0.44^{b}	±0.01	0.53^{a}	±0.03
Total identified compounds			83		72		87		69		82		64	
Total identified (%)			96.03		94.98		95.20		95.76		97.71		92.44	
Monoterpene hydrocarbons			14.87		19.70		15.79		14.16		12.00		22.88	
Oxygenated monoterpenes			57.56		55.57		61.02		39.18		65.92		29.91	
Sesquiterpene hydrocarbons			3.86		5.70		4.30		16.71		3.12		9.49	
Oxygenated sesquiterpenes			19.74		14.01		14.09		25.71		16.67		30.16	

RT (min), retention time on VF-5ms capillary column; RI, retention index was determined on grounds of a series of alkanes C10–C40; ^{a,b}, means followed by the same letter(s) within every varieties are not significantly different at $p = 0.05$ (Student t test); ^{ns}, not statistically significant; tr., trace <0.05% or 0.001 mg/mL; –, not detected.

87 in Blue River, and 82 in the Munstead variety. In comparison, the number of compounds identified in the essential oils isolated from *in vitro* plants was smaller — 72 in Ellagance Purple, 69 in Blue River, and 64 in the Munstead variety. A decrease in the number of constituent compounds in essential oils of *Caryopteris clandonensis* proliferated *in vitro* was also found by Łuczkiewicz et al. [42]. According to Avato et al. [43], the decrease in the number of compounds produced is connected with juvenility of plant tissue in *in vitro* conditions which is associated with a drop in production of more complex metabolites produced in the subsequent stages of metabolic pathways.

In essential oils isolated from all 3 varieties of field-grown plants, the dominant compounds were borneol (from 9% in Munstead variety to 13% in Blue River), linalool (from 3.71% in Blue River to 13% in Munstead), and globulol (from 4% in Blue River to 7% in Ellagance Purple variety). Daferera et al. [44] isolated the essential oil of a slightly different concentration from narrow-leaved lavender grown in natural conditions. The authors found high concentration of linalool (45%), linalyl acetate (33%), and 1.8-cineole (5%), which were identified to be the main compounds out of 8 identified and constituted 82% of the total composition of the oil. The analysis of the

composition was made with the use of GC–MS, and Lickens-Nickerson method was used for isolation of essential oil applying distillation with organic solvents lighter than water. The analysis of composition of essential oils obtained from seven varieties of narrow-leaved lavender: Jubileina, Hemus, Hebar, Raya, Sevtopolis, Drujba, and Karlovo using GC–MS method was done by Zagorcheva et al. [16]. The plants were harvested in summer, and isolation of oil was done from fresh flowers using steam distillation. In the course of the study, 32 compounds were identified, with linalool having the highest concentration (19–34%), followed by linalyl acetate (21–33%), lavandulyl acetate (3–7%), and caryophyllene (1–4%). However, in the presented study, those compounds were in lower concentration. The study on oils isolated from flowers of *L. angustifolia* Mill. by Wesołowska et al. [45] shows the highest concentration of linalool (29–31%), linalool acetate (12–18%), and α-terpineol (8–12%) among the identified compounds (depending on the variety from 43 to 47). Twenty-nine compounds present in lavender essential oils were found by Daferera et al. [46] with linalool (26%), linalyl acetate (18%), and α-terpineol (6%). However, according to Adaszyńska et al. [47], essential oil of narrow-leaved lavender of Munstead, Munstead Strain, Lavender Lady, Ellagance Purple, and Blue River varieties contained linalol (24–16%), linalyl anthranilate (12–2%), 1-terpinen-4-ol (10–6%), terpineol (*p*-menth-1-en-8-ol) (8–4%), and linalool oxide (5–1%). From 18 to 21 different compounds were identified with GC–MS analysis of the essential oils. According to Cong et al. [48], 17 different compounds comprise lavender essential oil isolated from *L. angustifolia*. The highest concentration was found for linalool (45%), geraniol (11%), lavandulol acetate (11%), 3.7-dimetylo-2.6-octadien-1-ol (10%), and izoterpineol (7%). The research conducted by the authors of the present study shows high concentration of geraniol (12%) in essential oils isolated from field-grown lavender of Munstead variety.

The dominant compound in terms of composition of essential oil isolated from lavender plants proliferated in *in vitro* conditions, similarly to the oil isolated from field-grown lavender plants, was borneol — 32% in Ellagance Purple, 26% in Blue River, and 13% in the Munstead variety. However, there were some significant quantitative and qualitative differences in % of constituents of oils isolated from field-grown plants and *in vitro*. Linalyl isobutanoate, one of the main compounds present in oils isolated from field-grown plants in the concentration from 5% to 10%, was not found in oils isolated from plants grown *in vitro* regardless of analyzed variety — similarly to other compounds, such as (*E*)-β-ocimene, *cis*-linalool oxide, 3-octyn-2-one, 1-octen-3-yl acetate, sabina ketone, *trans*-carveol, 4-methylene-isophorone, nerol, *epi*-longipinanol, or humulene epoxide II. Sudria et al. [49, 50] studied the effect of conditions of culturing on the production of essential oils by *L. dentata* and found that the variation in the amount of oil produced in *in vitro* cultures, as well as its concentration, is connected with the addition of plant growth regulators to proliferation media, which affects the endogenous regulation of metabolic pathways.

In comparison to the oils isolated from field-grown plants, the oils isolated from plants grown in *in vitro* conditions are characterized by the presence of the less volatile compounds, identified in the final stage of GC–MS analysis, i.e., thymol, carvacrol, epoxy allo-alloaromadendrene, khusinol, 8-cedren-13-ol, and *trans*-calamene. γ-Amorphene was found in trace quantity in essential oils of Munstead (0.11%) and Blue River (0.10%) variety obtained from in vivo plants, whereas in Ellagance Purple variety and in oils obtained from *in vitro* plants of the same varieties, the compound was not present. There was an increase in concentration of γ-cadinene to 5% for Munstead and 8% for Blue River variety. Additionally, α-amorphene was found in the Ellagance Purple variety of *in vitro* grown plant in the amount of 4%. *epi*-α-Cadinol, a compound which was found in all essential oils, was identified in substantial quantity in *in vitro* oils (10% in oil of Ellagance Purple, 20% in Blue River and 16% in the Munstead variety). The concentration of globulol is also noteworthy as it was identified in all isolated essential oils with the highest concentration (10%) in *in vitro* Munstead essential oil, however, in lower concentration in Ellagance Purple (2%) and in Blue River (2%) variety. Other compounds which were found only in plants propagated in *in vitro* cultures were, among others, terpinolene, *p*-mentha-2.4(8)-diene, *trans*-*p*-mentha-2.8-dien-1-ol, *cis*-verbenol, *cis*-sabinene hydrate acetate, and iso-3-thujyl acetate. Unexpectedly, in tested essential oils, limonene and 3-carene were not detected, which are components that occur in the lavender essential oil.

Zuzarte et al. [15] isolated essential oils from field-grown plant and from *in vitro* cultures of *L. pendunculata*, classified as belonging to two chemotypes: 1,8-cineole/camphor and fenchone. The GC–MS analysis showed that the main components of the oils were the same for field-grown as well as *in vitro* propagated plants; however, their concentration varied. Higher concentration of compounds in plants propagated in *in vitro* cultures classified as 1,8-cineole/camphor chemotype was found for, among others, α-pinene (14%) and bornyl acetate (10%); in terms of the fenchone chemotype, α-pinen (10%) and camphor (11.6%). The chemical uniformity of essential oils isolated from field-grown plants and *in vitro* shoot cultures propagated on MS media supplemented with 0.5 mg dm^{-3} BAP-6-benzyloaminopurine (BAP) and micropropagated plants of the same clone *L. viridis* was also observed by Nogueira and Romano [24]. In all analyzed oils, among 45 identified compounds, the same main compounds were determined. Monoterpene fraction identified in oils isolated from *in vitro* culture showed slight variation in terms of content of carbohydrate and oxidized components in comparison to oil obtained from mother plant.

Conclusion

In vitro method of propagating plant tissues allows for obtaining large bulk of plants in relatively short period of time, yet the method can affect the metabolism of plants and, consequently, the qualitative and quantitative composition of produced essential oils. In turn, this can affect the aroma and even modify antioxidative and antimicrobial action of the essential oils. Antimicrobial and antioxidant activities of lavender essential oils isolated from field-grown plants are confirmed. However there are not yet published results in respect to lavender essential oils isolated from *in vitro* plants. Presented results have shown that *in vitro* conditions lead to a change biochemical profile of the essential oils and increasing the concentration, e.g., borneol, γ-cadinene, *epi*-α-cadinol, or emergence of other chemical compounds. That might have an impact on differences in the antimicrobial and antioxidant activity of essential oils. Our previous research shows that essential oils isolated from *in vitro* propagated plants show higher antioxidant and antimicrobial activity especially in respect to bacteria presented on the human skin, in comparison with oils isolated from field-grown plants [51]. Essential oils with confirmed and more than average antioxidant and antimicrobial potential could be use in cosmetic industry as a natural preservative, which would extend cosmetics durability without the addition of synthetic preservatives.

Abbreviations

GC–MS gas chromatography–mass spectrometry
GC–FID gas chromatography–flame ionization detector
RI retention index

PPFD photosynthetic photon flux density
LSD least significant difference

References

1. Upson, T.; Andrews, S. *The genus Lavandula* (1st ed.), Timber Press, Inc., USA, 2004.
2. Cavanagh, H. M. A.; Wilkinson, J. M. *Aust. Infect. Control.* **2005**, *10*, 35–38.
3. Cavanagh, H. M. A.; Wilkinson, J. M. *Phytother. Res.* **2002**, *16*, 301–308.
4. Lahlou, M. *Flavour Fragr. J.* **2004**, *19*, 159–165.
5. Aburjai, T.; Natsheh, F. M. *Phytother. Res.* **2003**, *17*, 987–1000.
6. Baumann, L. S. *Dermatol. Ther.* **2007**, *20*, 330–342.
7. Adam, K.; Sivropoulou, A.; Kokkini, S.; Lanaras, T.; Arsenakis, M. *J. Agric. Food Chem.* **1998**, *45*, 1739–1745.
8. Hui, L.; He, L.; Huan, L.; Xiao Lan, L.; Ai Guo, Z. *Afr. J. Microbiol. Res.* **2010**, *4*, 309–3130.
9. Lis-Balchin, M.; Deans, S. G.; Eaglesham, E. *Flavour Fragr. J.* **1998**, *13*, 98–103.
10. Lis-Balchin, M.; Hart, S. *Phytother. Res.* **1999**, *13*, 540–542.
11. Landmann, C.; Fink, B.; Festner, M.; Dregus, M.; Engel, K. H.; SchwabArch, W. *Biochem. Biophys.* **2007**, *465*, 417–429.
12. Van de Braak, S. A. A. J.; Leijten, G. C. J. J. in *Essential Oils and Oleoresins: A Survey in the Netherlands and Other Major Markets in the European Union. CBI*, Centre for the Promotion of Imports from Developing Countries, Rotterdam, 1999, 116.
13. Zheljazkov, V. D.; Cantrell, C. L.; Astatkie, T.; Jeliazkova, E. *J. Oleo Sci.* **2013**, *62*, 195–199.
14. Lane, A.; Boecklemann, A.; Woronuk, G. N.; Sarker, L.; Mahmoud, S. S. *Planta.* **2010**, *231*, 835–845.
15. Zuzarte, M.; Dinis, A. M.; Cavaleiro, C.; Salgueiro, L. R.; Canhoto, J. M. *Ind. Crop Prod.* **2010**, *32*, 580–587.
16. Zagorcheva, T.; Stanev, S.; Rusanov, K.; Atanassov, I. *J. Agri. Sci. Technol.* **2013**, *5*, 459–462.
17. Adaszyńska-Skwirzyńska, M.; Swarcewicz, M.; Dobrowolska, A. *Med. Chem.* **2014**, *4*, 734–737.
18. Lakušić, B.; Lakušić, D.; Ristić, M.; Marčetić, M.; Slavkovska, V. *Nat. Prod. Commun.* **2014**, *9*, 859–862.
19. Nadalin, V.; Lepojević, Ž.; Ristić, M.; Vladić, J.; Nikolovski, B.; Adamović, D. *Chem. Ind. Chem. Eng. Q.* **2014**, *20*, 71–86.
20. Reverchon, E.; Della Porta, G. *J. Agric. Food Chem.* **1995**, *43*, 1654–1658.
21. Munõz-Bertomeu, J.; Arrillaga, I.; Segura, J. *Biochem. Syst. Ecol.* **2007**, *35*, 479–488.
22. Desautels, A.; Biswas, K.; Lane, A.; Boeckelmann, A.; Mahmoud, S. S. *Nat. Prod. Commun.* **2009**, *4*, 1533–1536.
23. Figueiredo, A. C.; Barroso, J. G.; Pedro, L. G.; Sevinate-Pinto, I.; Antunes, T.; Fontinha, S. S.; Looman, A.; Scheffer, J. J. C. *Flavour Fragr. J.* **1995**, *10*, 93–96.
24. Nogueira, J. M.; Romano, A. *Phytochem. Anal.* **2002**, *13*, 4–7.
25. Giray, E. S.; Kirici, S.; Kaya, D. A.; Türk, M.; Sönmez, O.; İnan, M. *Talanta.* **2008**, *74*, 930–935.
26. Demissie, Z. A.; Sarker, L. S.; Mahmoud, S. S. *Planta.* **2011**, *233*, 685–696.
27. Da Porto, C.; Decorti, D.; Kikic, I. *Food Chem.* **2009**, *112*, 1072–1078.
28. D'Auria, F. D.; Tecca, M.; Strippoli, V.; Salvatore, G.; Battinelli, L.; Mazzanti, G. *Med. Mycol.* **2005**, *43*, 391–396.
29. Al-Bakhit, A. A. M.; Sawwan, J. S.; Al-Mahmoud, M. S. *J. J. Agr. Sci.* **2007**, *3*, 16–25.
30. Falk, L.; Biswas, K.; Boeckelmann, A.; Lane, A.; Mahmoud, S. S. *J. Essent. Oil Res.* **2009**, *21*, 225–228.
31. Mendes, M. D.; Figueiredo, A. C.; Oliveira, M. M.; Trindade, H. *Plant Cell Tiss. Org.* **2013**, *113*, 341–351.
32. Alvarenga, I. C. A.; Silva, S. T.; Bertolucci, S. K. V.; Pinto, J. E. B. P.; Pacheco, F. V. *Australian Journal of Crop Science.* **2015**, *9*, 948–953.
33. Zielińska, S.; Piątczak, E.; Kalemba, D.; Matkowski, A. *O. Plant Cell Tiss. Org.* **2011**, *107*, 161–167.
34. Affonso, V. R.; Bizzo, H. R.; De Lima, S. S.; Esquibela, M. A.; Sato, A. *J. Braz. Chem. Soc.* **2007**, *18*, 1504–1508.
35. Zuzarte, M.; Gonçalves, M. J.; Cavaleiro, C.; Dinis, A. M.; Canhoto, J.; Salgueiro, L. *Cav. Chem. Biodivers.* **2009**, *6*, 1283–1292.
36. Gonçalves, S.; Romano, A. *Biotechnol. Adv.* **2013**, *31*, 166–174.
37. Prasad, A.; Shukla, S. P.; Mathur, A.; Chanotiya, C. S.; Mathur, A. K. *Plant Cell Tiss. Organ. Cult.* **2014**, *120*, 803–811.
38. Murashige, T.; Skoog, F. *Physiol. Plant.* **1962**, *15*, 473–497.
39. *European Pharmacopoeia* 4th ed. Version 4.08., Strasbourg: EDQM. 3158–3159, **2004**.
40. NIST Mass Spectral Library *NIST/EPA/NIH* Gaithersburg, MD, USA, 2002.
41. Adams, R. P. *Identification of Essential Oil Compounds by Gas Chromatography/Quadrupole Mass Spectroscopy* Allured, Carol Stream, IL, USA, 2001.
42. Łuczkiewicz, M.; Jesionek, A.; Kokotkiewicz, A.; Migas, P.; Mardarowicz, M.; Szreniawa-Sztajnert, A.; Zabiegała, B.; Buciński, A. *Acta Physiol. Plant.* **2015**, *37*, 58.
43. Avato, P.; Fortunato, I. M.; Ruta, C.; D'Elia, R. *Plant Sci.* **2005**, *169*, 29–36.
44. Daferera, D. J.; Ziogas, B. N.; Polissiou, M. G. *J. Agric. Food Chem.* **2000**, *48*, 2576–2581.
45. Wesołowska, A.; Jadczak, D.; Grzeszczuk, M. *Herba Pol.* **2010**, *56*, 24–36.
46. Daferera, D. J.; Tarantilis, P. A.; Polissiou, M. G. *J. Agric. Food Chem.* **2002**, *50*, 5503–5507.
47. Adaszyńska, M.; Swarcewicz, M.; Dzięcioł, M.; Dobrowolska, A. *Nat. Prod. Res.* **2013**, *27*, 1497–1501.
48. Cong, Y.; Abulizi, P.; Zhi, L. *Chem. Nat. Comp.*, **2008**, *44*, 810.
49. Sudria, C.; Piñol, M. T.; Palazon, J.; Cusido, R. M.; Vila, R.; Morales, C.; Bonfill, M.; Cañigueral, S. *Plant Cell Tiss Organ. Cult.* **1999**, *58*, 177–184.
50. Sudria, C.; Palazón, J.; Cusidó, R.; Bonfill, M.; Piñol, M. T.; Morales, C. *Biol. Plant.* **2001**, *44*, 1–6.
51. Andrys, D.; Kulpa, D.; Grzeszczuk, M.; Bihun, M.; Dobrowolska, A. *Folia Hort.* (in press), **2017**.