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HERB YIELD AND BIOACTIVE COMPOUNDS OF TARRAGON (*Artemisia dracunculus* L.) AS INFLUENCED BY PLANT DENSITY

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Abstract. Growth and yield of herbal plants are closely connected with meteorological conditions and agrotechnical procedures. We undertook studies on determining the effect of tarragon planting density upon fresh and dry herb yield, as well as the contents of nutritious and physiologically active substances: L-ascorbic acids, chlorophylls, carotenoids, tannins and flavonoids, as well as the contents, yield and chemical composition of essential oil. Significant effect of estragon planting density was demonstrated upon fresh and air-dry herb yield, grated herb yield and essential oil yield. Tarragon plants growing in higher density (40 × 40 cm) had higher yield of fresh, dry and de-stalked herb, as well as higher oil yield than the plants growing less densely (50 × 50 cm). The biological value of analyzed plant material was high and depended upon planting density to a really insignificant extent. Plants growing more densely had significantly higher concentration of carotenoids and significantly lower contents of essential oil compared to the remaining ones. The essential oil of *Artemisia dracunculus* L. was characterized by the presence of 46 compounds of the share >0.05% and 23 compounds occurring in trace amounts. The predominant compound in the examined tarragon oil was methyl eugenol, which occurred in larger quantity in the year 2012 (37.56%), than in 2011 (34.33%). Other main compounds were: elemicin, determined in the amount of 21.95 and 26.22%; sabinene, in the amount of 14.16 and 16.37%, as well as *E*-asarone, in the concentrations of 8.68 and 3.49% (respectively: in 2011 and 2012). *A. dracunculus* presented in the foregoing studies can be defined as a methyl eugenol-elemicin/sabinene chemotype (respectively: 36, 24 and 15%).

Key words: plant spacing, bioactive compounds, essential oil, methyl eugenol, elemicin, sabinene

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INTRODUCTION

Within genus *Artemisia* there are 500 known plant species, commonly occurring mainly in Asia, Europe and North America, distinguished by the characteristic aroma and biological activity. *Artemisia* species are frequently used in treating such diseases as: malaria, liver disease, neoplasms, inflammatory diseases and infections caused by microorganisms. *Artemisia dracunculus* L. (tarragon) is a popular seasoning plant used both in fresh state (leaves) and after it has been dried (herb). Tarragon herb is also applied for curative purposes: it contains essential oil in the amount of 0.05–0.95% [Hagi et al. 2010, Suleimenov et al. 2010, Zawiślak and Dzida 2012, Tak et al. 2014], flavonoids, phenolic compounds [Karabegović et al. 2011, Weinoehrl et al. 2012], cumarin compounds [Obolskiy et al. 2011], carotenoids [Daly et al. 2010], tannins, bitter tastes [Obolskiy et al. 2011], and mineral compounds [Zawiślak and Dzida 2012]. What is also worth noting, is the presence of artemisine, a compound from the group of sesquiterpene lactones with antibacterial properties and vast therapeutic application possibilities [Mannan et al. 2011]. Tarragon raw material acts as a cholagogue, spasmolytic, anti-hypoglycemic and antibacterial agent, and it increases the secretion of digestion juices [Obolskiy et al. 2011]. It also is an antioxidant [Souri et al. 2004] and antidiabetic [Weinoehrl et al. 2012]. It can be taken into consideration in treating cardiovascular diseases [Yazdanparasat and Shahriyary 2008]. Tarragon essential oil reveals anti-convulsive and sedative activity [Sayyah et al. 2004]. The chemical composition of tarragon raw material, just like the composition of its essential oil, are differentiated and depend upon genetic, ontogenetic and environmental factors [Chauhan et al. 2010, Hagi et al. 2010, Verma et al. 2010, Tak et al. 2014]. On the basis of tarragon oil predominant factors six main groups were distinguished: (1) methyl chavicol, (2) methyl eugenol, (3) α-terpinolene, (4) capillene, (5) 5-phenylo-1,3-pentadine, (6) (*E*)-β-ocimene/(*Z*)-β--ocimene, representing a different chemical profile of the oil [Eisenman et al. 2013]. Besides, as the main reported components of tarragon oil there are: *trans*-anethol (Iran and Turkey), limonene (Iran) and *trans*-ocimene (Iran) [Abad et al. 2012]. The most controversial compounds are methyl chavicol and methyl eugenol, substances with positive physiological activity determining the pharmacological effect, but also with possible carcinogenic effect [Obolskiy et al. 2011].

Growth and yield of herbal plants are closely connected with meteorological conditions and agrotechnical procedures [Azizi and Kahrizi 2008, Aćimović 2013]. The herb yield obtained in southern Finland was 10–40% higher than in its northern part, and shortening the vegetation period affected biomass production stronger than it influenced the essential oil contents and chemical composition [Galambosi et al. 2002]. A significant co-relationship of the plant age and planting density with plant height, leaf surface index and dry leaf yield of *Artemisia annua* L., as well as artemisine yield was demonstrated [Damtew et al. 2011]. It was different in the case of essential oil, the content of which did not depend upon the age of harvested plant, but upon planting density. The maximum oil concentration was found at the lowest planting density [Damtew et al. 2011]. Besides, the variability of yield, essential oil and artemisine contents in *Artemisia annua* L. remained dependent upon the kind of soil and harvest term [Omer et al. 2013]. Studies conducted in northern India [Singh et al. 2008] proved that increased

spacing between rows in cultivation of South American marigold caused increased oil concentration and decreased dry biomass and oil yield, as well as change in the essential oil composition. Atmospheric conditions during tarragon cultivation, as well as sowing term, significantly affected the height of plants, fresh herb yield, as well as essential oil contents [Jadczak and Grzeszczuk 2008, Zawiślak and Dzida 2012]. Morphological variability was also found among wild plants [Chauhan et al. 2010]. *Artemisia dracunculus* L. characterized as a species with vast morphological and phytochemical variability [Obolskiy et al. 2011], has not been described in greater detail in literature with respect to variability of other biologically active substances than essential oil. However, as biologically active components of tarragon, except essential oil, among others, also flavonoids, carotenoids, phenylopropanoids, cumarines and alcamides are mentioned [Daly et al. 2010, Obolskiy et al. 2011, Weinoehrl et al. 2012]. Bearing in mind the above relationships and available information, we undertook studies on determining the effect of tarragon planting density upon fresh and dry herb yield, as well as the contents of nutritious and physiologically active substances: L-ascorbic acids, chlorophylls, carotenoids, tannins and flavonoids. Also the contents, yield and chemical composition of essential oil in the studied tarragon plants were assessed.

MATERIAL AND METHODS

The study was conducted in the years 2011–2012 at the experimental field of the University of Life Sciences in Lublin (51°14'N latitude, 22°38'W longitude). Seed of tarragon (*Artemisia dracunculus* L.) came from the company PNOS Ożarów Mazowiecki (Poland). Tarragon seedlings were produced in greenhouses. The seeds, sown in boxes filled with peat substrate, were germinated after ten days. The seedlings were pricking out to multipots. Seedlings were planted in the field on 10 May, at a spacing of 40 × 40 cm and 50 × 50 cm. Experimental fields were established in randomized blocks with four replications. Plot size amounted to 3.2 cm^2 and 4.0 cm^2, respectively. Plants were grown on podzolic soil developed from dusty medium loam containing 1.8% pf organic matter and with pH of 6.7. The soil under cultivation was prepared in accordance with the recommendations of agricultural technology to the test species. Mineral fertilization was used in the following dosages and forms: 70 kg N · h^{-1} in the form of calcium nitrate, 26.4 kg P · h^{-1} as a granular triple superphosphate, 83 kg K · h^{-1} in the form of potassium sulphate. Phosphorus and potassium fertilizers were applied at the full rate during the preparation of the field before planting. Nitrogen fertilizer was applied in two doses: half of the rate was incorporated during field preparation, and the other half – after the seedlings became established. Manual weeding and loosening of the soil were done. In the experiment was not used any chemical treatments during the growing season of plants. Climatic conditions during the study period contributed to the development of tarragon plants. Air temperature and precipitation for the period from May to July were optimal and generally exceeded the values from the years 1951–2005 (respectively: 14.6, 18.0, 19.9°C compared with 13.0, 16.2, 17.8°C and 49.2, 65.3, 120.6 mm with respect to 57.7, 65.7, 83.5 mm). Air temperature and uniform distribution of rainfall conducive to the growth and development of studied plants.

Tarragon herb was harvested by cutting shoots 8 cm above the soil surface, at the beginning of the flowering period (on 20 July).

In the fresh plant material it was determined the content of L-ascorbic acid, chlorophyll, carotenoids and essential oil. L-ascorbic acid content was determined by spectrophotometry according to J.H. Roe with a modification by Ewelin [Korenman 1973], absorbance was measured at 520 nm. Chlorophyll and carotenoids by means of spectrophotometric method [Lichtenthaler and Wellburn 1983]; measuring the absorbance at a wavelength $\lambda = 662$ nm (chlorophyll a) and $\lambda = 645$ nm (chlorophyll b) and $\lambda = 470$ nm for carotenoids. The tarragon essential oil was determined by hydro distillation [Farmakopea Polska VII 2006]. Distillation time was 3 hours, the sample size of the raw material was 20 g of fresh herb, and distilled water – 400 ml.

The remainder of the herb was dried for five days at 30°C. The whole dried herb was ground through 5 mm mesh sieves, thereby obtaining the ground herb (without steams). The essential oil, tannins and flavonoids content were determined in dried ground herb. The content of essential oil in dry herb tarragon was determined by hydro distillation, as in the fresh material. The obtained oil was stored in a dark glass container at a temperature of -10°C until the time of chromatographic separation.

Qualitative and quantitative analysis of the composition of the essential oil was performed by gas chromatography (GC-MS) (Varian 4000 gas chromatograph GC/MS) in conjunction with mass detector, retention time on the column VF-5ms (equivalent DB-5); film thickness 0.25 μm; carrier gas, helium at a rate of 0.5 ml min^{-1}; injector and detector temperature, 220ºC and 200ºC, respectively; split ratio, 1:100; injector volume, 1 μl. A temperature gradient was applied (50ºC for 1 min, then incremented by 4ºC min^{-1} to 250ºC and held at this temperature for 10 min); ionization energy, 70 eV; mass range, 40–1000 Da; scan time, 0.8 s. Kováts retention indices were determined on the basis of series of alkanes C_6-C_{40} [Van den Dool and Kratz 1963]. The qualitative analysis was carried on the basis of MS spectra, which were compared with the spectra of the Mass Spectral Library [2008] and with data available in the literature [Adams 2004]. The identity of the compounds was confirmed by their retention indices, taken from the literature [Adams 2004] and our own data.

Tannins were determined spectrophotometrically, after their extraction from the dried raw material. Assay was performed without direct exposure to light, used water free of CO_2. Weighed 1 g of finely powdered (0.16 mm sieve) of the raw material to a volumetric flask with a capacity of 250 ml, 150 ml of water was added and kept for 30 min in a boiling water bath. The mixture was cooled stream of water, quantitatively transferred to a volumetric flask with a capacity of 250 ml, filled up with water and allowed to sediment total raw material. The supernatant was filtered through filter paper, the first 50 ml was discarded, the remaining filtrate was used for the determination. In order to determine the total polyphenol content of the filtrate was supplemented with 5.0 ml of water to 25.0 mL of this solution was added 1.0 ml of tungsten molybdenum phosphoric reagent, then 10.0 ml of water, to make sodium carbonate solution (290 g · l^{-1}) to 25.0 ml. After 30 min the absorbance was measured at 760 nm, using water as a reference (A_1). In order to determine polyphenols are not associated with leather powder, to the filtrate was added 10.0 ml of 0.10 g of leather powder, shaken vigorously for 1 hour and filtered. 5.0 ml of the filtrate was

supplemented with water to 25.0 ml, and then 2.0 ml of this solution was added 1.0 ml of tungsten molybdenum phosphoric reagent, then 10.0 ml of water, to make sodium carbonate solution (290 g · l^{-1}) to 25.0 ml. After a further 30 min, the absorbance was measured at 760 nm using water as a reference (A_2). Comparative A solution was prepared: just before the assay 50.0 mg of pyrogallol was dissolved in water and made up to 100.0 ml with water. 5 ml of the resulting solution was supplemented with water to 100.0 ml to 2.0 ml of this solution was added 1.0 ml of tungsten molybdenum phosphoric reagent, 10.0 ml of water, to make sodium carbonate solution (290 g · l^{-1}) to 25.0 ml. After the next 30 min the absorbance was measured at 760 nm, using water as a reference (A_3). Tannin content (%) was calculated based on the pyrogallol:

$$\frac{62.5 \cdot (A_1 - A_2) \cdot m_2}{A_3 \cdot m_1}$$

A_1 – absorbance of polyphenols in the test solution,

A_2 – absorbance of polyphenols are not associated with powder leather in the test solution,

A_3 – absorbance of the solution comparative pyrogallol,

m_1 – starting weight of raw material,

m_2 – the sample with pyrogallol in g.

Flavonoids were determined spectrophotometrically, after their extraction from the raw material. Weighed 0.5 g on average, a raw material powder (0.315 mm sieve) into a round bottom flask, was added 20 ml of acetone, 2 ml of HCl (281 g · l^{-1}), 1 ml methenamine (5 g · l^{-1}) and maintained for 30 min under reflux on a water bath under reflux. The hydrolyzate was filtered through cotton wool into a volumetric flask of 100 ml, together with the cotton pellet placed in a flask, 20 ml of acetone and then refluxed for 10 min. 20 ml solution was dispensed into a separatory funnel, 20 ml of water and extracted with ethyl acetate, 15 ml portions and three times with 10 ml. The combined organic layers were washed twice with 40 ml of water, filtered into a volumetric flask of 50 ml and supplemented with ethyl acetate. To determine the two samples were prepared: To 10 ml of the stock solution was added 2 ml of a solution of aluminum chloride (20 g · l^{-1}) and supplemented with a mixture (1:19) of acetic acid (1.02 kg · l^{-1}) of methanol and 25 ml. To prepare the comparative solution was supplemented with 10 ml of the mixture (1:19) of acetic acid (1.02 kg · l^{-1}) of methanol and 25 ml. After 45 min, the absorbance of the solutions at 425 nm using the reference solution which is a comparator. The total content of flavonoids (%) is expressed in terms of quercetin, according to the formula:

$$\frac{A \cdot k}{m}$$

A – the absorbance of the solution of the research,

k – convection factor for quercetin; $k = 0.875$,

m – the sample with the raw material in g.

All chemical analyzes were performed in quadruplicate. The yield of essential oil was determined according to the formula given by Farahani et al. [2009]: essential oil yield = essential oil percentage × herb yield. The obtained results were statistically analyzed by analysis of variance and significance of differences was determined using Tukey's test at 0.05 probability level. Essential oil composition was presented independent of the plant density in the two years of the study.

RESULTS

Herb and essential oil yield of tarragon. Significant effect of estragon planting density was demonstrated upon fresh and air-dry herb yield, grated herb yield and essential oil yield (tab. 1). More densely growing plants (40 × 40 cm) had better yielding parameters than those growing less densely (50 × 50 cm). The share of grated herb in dry tarragon herb did not differ significantly in plants grown in different densities. The biological value of analyzed plant material was high and depended upon planting density to a really insignificant extent (tabs 2, 3). 100 g of fresh tarragon herb contained on average: 10.69 mg of L-ascorbic acid, 125.49 mg of chlorophyll a + b, 30.43 mg of

Table 1. Tarragon herb and essential oil yield (2011–2012)

Plant spacing (cm)	Yield of fresh herb	Yield of dry herb	Yield of herb without stems	Share of herb without stems in dry herb (%)	Yield of essential oil (kg · 100 m^{-2})
	(kg · 100 m^{-2})				
40 × 40	227.86	73.96	32.24	43.59	0.55
50 × 50	196.04	60.37	22.05	36.52	0.37
Mean	211.95	67.16	27.14	40.05	0.46
LSD $_{0.05}$	21.964	6.993	4.02	-	0.069

Table 2. Concentration of chosen active substances in tarragon fresh herb (2011–2012)

Plant spacing (cm)	L-ascorbic acid	Chlorophyll			Carotenoids	Essential oil (ml · 100 g^{-1})
		a	b	a + b		
	(mg · 100 g^{-1} f.m.)					
40 × 40	10.89	75.37	48.25	123.62	39.62	0.71
50 × 50	10.50	85.37	42.00	127.37	21.25	0.84
Mean	10.69	80.37	45.12	125.49	30.43	0.77
LSD $_{0.05}$	n.s.	n.s.	n.s.	n.s.	7.113	0.077

n.s.– not significant

Table 3. Concentration of chosen active substances in tarragon dry herb without steams (2011–2012)

Plant spacing (cm)	Essential oil (ml·100 g^{-1})	Tannins (%)	Flavonoids (%)
40 × 40	1.73	0.33	0.71
50 × 50	1.70	0.37	0.71
Mean	1.71	0.35	0.71
$LSD_{0.05}$	n.s.	n.s.	n.s.

n.s.– not significant

carotenoids and 0.77 ml of essential oil. Plants growing more densely had significantly higher carotenoids concentrations (39.62 mg 100 · g^{-1} f. w.) and significantly lower contents of essential oil (0.71 ml 100 · g^{-1} f. w.), compared to the remaining ones (respectively: 21.25 mg 100 · g^{-1} f. w. and 0.84 ml 100 · g^{-1} f. w.). Dried and grated tarragon herb was characterized by high contents of essential oil (1.71 ml 100 · g^{-1} f. w.), comparable in plants growing in various densities (tab. 3). No significant differences were also found in the share of tannins and in the herb of examined plants.

Essential oil composition. Table 4 presents the composition of tarragon essential oil in two study years. The essential oil of *Artemisia dracunculus* L. was characterized by the presence of 46 compounds of the share >0.05% and 23 compounds occurring in trace amounts. In the plant material collected in the year 2011 the presence of 99.44%, and in 2012 99.20% of all essential oil compounds were determined. The predominant compound in the examined tarragon oil was methyl eugenol, which occurred in larger quantity in the year 2012 (37.56%), than in 2011 (34.33%). Other main compounds were: elemicin, determined in the amount of 21.95% and 26.22%; sabinene, in the amount of 14.16% and 16.37%, as well as *E*-asarone, in the concentrations of 8.68 and 3.49% (determined respectively: in the years 2011 and 2012). In the group of compounds occurring in the amount of more than 1% we determined the shares of myrcene, (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, terpinene-4-ol, citronelyl acetate, neryl acetate, and *E*-methyl isoeugenol. The shares of the above-mentioned components were differentiated in particular study years. In the examined essential oil small methyl chavicol content was found (0.26%). A group of predominant components of the examined oils were phenylopropanoids (62.80%), before monoterpene hydrocarbons (22.33%) and oxygenate monoterpenes (10.69%). We found high variability of the share of particular compound groups in the examined oil. The oil obtained from the plant material collected in the year 2011 was distinguished by a smaller share of phenylopropanoids and oxidized sesquiterpenes, but a greater share of monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxidized monoterpenes than the oil from the raw material collected in the year 2012.

Table 4. Essential oil composition of air-dry tarragon herb without steams (%)

	Compounds	RI	2011	2012
1	α-thujene	979	0.15 ±0.01	0.21 ±0.00
2	α-pinene	981	0.30 ±0.01	0.24 ±0.00
3	sabinene	993	14.16 ±0.33	16.37 ±0.15
4	β-pinene	994	0.47 ±0.02	0.19 ±0.00
5	myrcene	997	1.13 ±0.04	0.61 ±0.00
6	α-phellandrene	1008	0.05 ±0.00	tr.
7	α-terpinene	1017	0.31 ±0.00	0.46 ±0.01
8	p-cymene	1025	0.11 ±0.00	0.08 ±0.00
9	sylvestrene	1029	0.26 ±0.01	0.14 ±0.00
10	(Z)- β-ocimene	1033	2.96 ±0.12	1.19 ±0.00
11	(E)-β-ocimene	1044	2.06 ±0.07	0.69 ±0.00
12	γ-terpinene	1057	0.54 ±0.02	0.77 ±0.01
13	cis-sabinene hydrate	1069	0.21 ±0.01	0.32 ±0.00
14	terpinolene	1083	0.82 ±0.03	0.38 ±0.00
15	linalool	1095	0.07 ±0.00	0.06 ±0.00
16	trans-sabinene hydrate	1099	0.16 ±0.00	0.24 ±0.00
17	cis-para menth-2-en-ol	1124	0.12 ±0.00	0.13 ±0.01
18	trans-para-menth-2-en-ol	1143	tr.	0.05 ±0.00
19	neo-isopulegol	1152	0.05 ±0.01	–
20	terpinen-4-ol	1184	1.0 ±0.01	1.30 ±0.01
21	meta-cymen-8-ol	1189	0.08 ±0.00	0.05 ±0.00
22	methyl chavicol	1201	0.25 ±0.00	0.27 ±0.00
23	n.i.	1209	0.09 ±0.00	tr.
24	citronellol	1228	0.35 ±0.00	0.09 ±0.01
25	geraniol	1254	0.08 ±0.00	tr.
26	bornyl acetate	1293	tr.	0.07 ±0.00
27	citronellyl acetate	1352	1.93 ±0.06	1.07 ±0.00
28	eugenol	1359	tr.	0.10 ±0.00
29	neryl acetate	1379	1.01 ±0.04	0.77 ±0.00
30	β-elemene	1394	0.05 ±0.00	tr.
31	methyl eugenol	1405	34.33 ±0.35	37.56 ±0.20
32	e-caryophyllene	1431	0.05 ±0.00	0.08 ±0.00
33	β-acoradiene	1491	0.06 ±0.00	0.07 ±0.00
34	n.i.	1494	0.07 ±0.00	0.08 ±0.00
35	germacrene D	1500	0.72 ±0.02	0.59 ±0.01
36	E-metyl isoeugenol	1509	2.76 ±0.04	2.15 ±0.01
37	bicyclogermacrene	1514	0.44 ±0.01	0.33 ±0.02
38	γ-cadinene	1529	0.06 ±0.00	0.07 ±0.00
39	δ-amorphene	1532	0.33 ±0.00	0.34 ±0.01
40	elemicin	1556	21.95 ±0.26	26.22 ±0.43
41	E-nerolidol	1567	0.17±0.00	0.56 ±0.02
42	spathulenol	1588	0.88 ±0.01	1.13 ±0.00
43	caryophyllene oxide	1594	0.06 ±0.00	0.14 ±0.00
44	epoxy allo-alloaromadendrene	1647	tr.	0.05 ±0.00
45	E-asarone	1654	8.68 ±0.15	3.49 ±0.03
46	α-bisabolol	1700	0.11 ±0.00	0.49 ±0.07
Total (%)			99.44	99.20
	groupe components:			
	phenylopropanoid		59.29	66.30
	monoterpene hydrocarbons		23.32	21.33
	sesquiterpene hydrocarbons		1.71	1.53
	oxygenated monoterpenes		13.74	7.64
	oxygenated sesquiterpenes		1.38	2.4

n.i. – not identified; the following compounds were found in trace amounts (<0.05): camphene, benzaldehyde, dehydro-1,8-cineole, δ-2-carene, cis-thujone, Z-myroxide, trans-pinocarveol, camphor, β-pinene oxide, sabine ketone, myrtenol, γ-terpineol, cis-piperitol, metyl citronellate, carvacrol, α-copaene, α-humulene, globulol, khusimone, 1.10-epi-cubenol, khusinol, 8-cedren-13-ol, Z,E-farnesol

DISCUSSION

Herb and essential oil yield of tarragon. Herbal plant condensation on a surface unit most often causes the increase of fresh and dry weight herb yield. We found a tendency to biomass increase of the overground parts of basil as an effect of increased plant density in one of the sowing terms [Sadeghi et al. 2009]. Greater plant density allowed for obtaining higher fresh marjoram herb yield [Al-Kiyyam et al. 2008], as well as other medicinal plants [Kleitz et al. 2003]. Similarly, as the distance between rows in growing peppermint increases, the yield of fresh and dry leaf weight and the yield of fresh weight of overground parts decreased [Kassahun et al. 2011]. Positive effect of increased plant density with reference to the yield of fresh and dry tarragon herb, as well as the grated herb yield, was also demonstrated in the foregoing studies. Reverse dependencies were demonstrated, however, in the previous studies with marjoram [Nurzyńska-Wierdak and Dzida 2008]. It seems that these differences can be explained by deterioration of light conditions, to which some plant species with smaller leaves can be sensitive. Besides, high density of plants in a cornfield causes increased competition between vegetative and generative organs of a plant, which can be revealed by differentiated share of leaves and flowers in the herb.

The dependencies obtained in the foregoing studies, concerning essential oil contents and yield confirm the results achieved by Dantew et al. [2011] and Kassahun et al. [2011]: the essential oil contents was the highest at the largest distance between rows, whereas the oil yield was the highest in plants growing in the highest density. Other dependencies were revealed in fennel, where the increased distance between plants positively affected the essential oil contents [Khorshidi et al. 2009]. Wider spacing of South American marigold enhanced essential oil accumulation, but not growth of dry matter yield and the yield of oil [Singh et al. 2008]. For the plants of cumin, mean plant density was determined, as well as moderate climatic conditions as optimal for the best yield and essential oil contents [Azizi and Kahrizi 2008]. Marjoram plants, in turn, did not differ significantly between themselves as to the essential oil contents in the herb [Nurzyńska-Wierdak and Dzida 2008]. Essential oil biosynthesis in the conditions of differentiated plant densities may take different courses, which seems to depend upon the place of oil synthesis. More distinct tendencies and dependences in this respect can be demonstrated in plants forming exogamic oil cells, characteristic of leaves and flowers than endogenic, occurring in fruit, seeds and roots. Other variability factors should be considered as well: environmental, soil or ontogenetic. Variability of yield, essential oil and arthemisine concentration in *Artemisia annua* L. plants remained related to soil type and seasonal variability [Omer et al. 2013]. Studies conducted in Finland [Galambosi et al. 2002] proved that climatic conditions modified the contents and composition of essential oil in various herbal plant species.

Bioactive compounds of tarragon. The seasoning and curative value of tarragon is connected with the complex of active compounds with a wide spectrum of action. The most studies are conducted on the composition of *Artemisia dracunculus* L. essential oil, dynamics and variability of its particular components. The most important groups of tarragon bioactive compounds are, however, besides the essential oil, cumarines, flavonoids and phenolic acids [Obolskiy et al. 2011]. Curative properties of tarragon herb

are also connected with the presence of carotenoids [Daly et al. 2010]. The plant material analyzed in this paper was characterized by high biological value, determined concentrations of L-ascorbic acids, chlorophylls, carotenoids, tannins and flavonoids, as well as essential oils. The share of particular biosubstances in the examined plant material was comparable to the results of other studies [Jadczak and Grzeszczuk 2008, Daly et al. 2010, Weinoehrl et al. 2012]. The contents of above listed active components, excluding carotenoids and essential oil was not modified by density of plants, which can indicate stronger effect of genetic and ontogenetic variability than environmental variability. This hypothesis is confirmed by results obtained by other authors. Concentrations of flavonoids in extracts of French and Russian tarragon were differentiated and ranged from 0.35 to 1.75% [Weinoehrl et al. 2012]. Besides, the contents of essential oil and L-ascorbic acid in tarragon herb was comparable in two study years, however the ability of neutralizing free radicals was differentiated [Jadczak and Grzeszczuk 2008] – which indicates the share of other active components in forming the anti-oxidation complex of a plant, modified by climatic conditions. Studies on the dynamics of oil accumulation during ontogenesis revealed two peaks of oil contents: at the beginning of the formation of flower buds and at the beginning of blooming [Obolskiy et al. 2011]. The foregoing studies show that carotenoids and tarragon essential oil content, the antioxidant substances, depended upon agri-climatic factors. Additionally, the essential oil components of *A. dracunculus* L. *in vitro* demonstrate a differentiated free radical neutralizing activity [Obolskiy et al. 2011], and the essential oil composition remains under a strong effect of environmental conditions [Eisenman et al. 2013].

Essential oil composition. The chemical composition of tarragon oil is significantly differentiated, which is caused by different kinds of variability [Hagi et al. 2010, Verma et al. 2010, Eisenman et al. 2013]. The results presented in the foregoing paper indicate climatic variability modifying the share of particular components in tarragon oil. The essential oil obtained from raw material collected in the years 2011 and 2012 had a differentiated share of components, and the greatest differences concerned mono- and sesquiterpene compounds, as well as phenylopropanoids. Similarly, Medina-Holguin et al. [2007] demonstrated the differentiated share of 1,8-cineole, methyl eugenol and elemicin in the essential oil of *Anemopsis californica* during two years of studies, connected with climatic and environmental variability. The authors proved the maximum accumulation of methyl eugenol in plants growing on the stand below 1700 m, during persistent temperature between 12°C and 15°C and precipitation level below 25 per year. The share of predominant components in the examined tarragon oil indicates methyl eugenol chemotype [Eisenman et al. 2013], with a significant share of elemicin. The above-mentioned phenylopropanoid compounds are characterized by anti-microbial activity [Obolskiy et al. 2011, Eisenman et al. 2013].

The chemical profile of the examined tarragon oil was similar to that determined by Kowalski et al. [2007] and different from the oils of chemotypes from India [Chauhan et al. 2010, Verma et al. 2010, Tak et al. 2014], Iran [Sayyah et al. 2004, Hagi et al. 2010], Afghanistan [Jeppesen et al. 2012] and Kazakhstan [Suleimenov et al. 2010]. What is more, the composition of the examined oil differed as to concentrations of methyl eugenol and elemicin from these determined by Zawiślak and Dzida [2012], in plants growing in comparable environmental conditions. Comparing the results of our own

studies and those obtained by Zawiślak and Dzida [2012] qualitative and not quantitative differentiation of the examined tarragon oil can be noticed. That indicates the additional effect of climatic variability, modifying the chemical profile and the related activity of tarragon essential oil. The tarragon raw material and its essential oil undergo assessment not only as to biological activity and also the safety of application, the determinant of which is first of all the concentration of methyl chavicol and methyl eugenol. On the other hand, however, methyl chavicol is regarded as the main smell and taste component of tarragon oil [Eisenman et al. 2013], and methyl eugenol is probably one of a few monoterpenoids mediating in anti-convulsive activity of tarragon essential oil [Sayyah et al. 2004]. However, methyl chavicol and methyl eugenol alone do not have direct carcinogenic activity, but unstable molecules and active radicals that are created in result of their metabolic activation, they cause genotoxic effect [Obolskiy et al. 2011]. The examined tarragon essential oil, containing a substantial amount of methyl eugenol (36%), was characterized by a small share of methyl chavicol (0.26%). It should be added that the tarragon raw material is applied in small amounts as a spice or a drug in the form of non-toxic water extracts, with very small contents of methyl chavicol and methyl eugenol [Weinoehrl et al. 2012]. From among the components determined in the oil the presence of (*E*)-asarone should be noticed, which is a characteristic component of calamus oil [Satyal et al. 2013], in our opinion first determined for *A. dracunculus*. Also quite significant are big differences between contents of this compound in the samples of oil distillated from raw material collected in the years 2011 (8.68%) and 2012 (3.49%), indicating the modifying effect of climatic factors.

A. dracunculus presented in the foregoing studies can be defined as a methyl eugenol-elemicin/sabinene chemotype (respectively: 36, 24 and 15%), as to the composition of essential oil it is the closest to both Danish [Obolskiy et al. 2011] and Polish chemotypes [Chauhan et al. 2010].

CONCLUSIONS

1. Tarragon plants growing in higher density (40 × 40 cm) had higher yield of fresh, dry and de-stalked herb, as well as higher oil yield than the plants growing less densely (50 × 50 cm). The share of grated herb in dry tarragon herb did not significantly differ in plants growing in various densities.

2. The plant material analyzed in this paper had high biological value, determined concentration of L-ascorbic acid, chlorophylls, carotenoids, tannins, flavonoids and essential oil. The biological value of analyzed plant material depended on planting density to a small extent. Plants growing more densely had significantly higher concentration of carotenoids and significantly lower contents of essential oil compared to the remaining ones.

3. The essential oil of *Artemisia dracunculus* L. was characterized by the presence of 69 compounds, among which the predominant ones were: methyl eugenol, elemicin and sabinene. The group of predominant components of the examined oils was phenylopropanoids, before monoterpene hydrocarbons and oxygenated monoterpenes.

4. The essential oil obtained from the raw material collected in the years 2011 and 2012 was characterized by differentiated share of components and the largest differences concerned mono- and sesquiterpene compounds, as well as phenylopropanoids.

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PLON ZIELA ORAZ SKŁADNIKI BIOAKTYWNE BYLICY ESTRAGONU (*Artemisia dracunculus* L.) W ZALEŻNOŚCI OD ZAGĘSZCZENIA ROŚLIN

Streszczenie. Wzrost i plonowanie roślin zielarskich związane są ściśle z warunkami meteorologicznymi oraz zabiegami agrotechnicznymi. Podjęto badania nad określeniem wpływu gęstości sadzenia roślin estragonu na plon świeżego i wysuszonego ziela oraz zawartość substancji o charakterze odżywczym i fizjologicznie aktywnym: kwasu L-askorbinowego, chlorofili, karotenoidów, garbników i flawonoidów, jak również zawartość, plon i skład chemiczny olejku eterycznego. Wykazano istotny wpływ gęstości sadzenia roślin estragonu na plon świeżego i powietrznie suchego ziela, plon ziela otartego oraz plon olejku eterycznego. Estragon rosnący w większym zagęszczeniu (40 × 40 cm) odznaczał się większym plonem świeżego, suchego i pozbawionego łodyg ziela oraz większym plonem olejku niż rośliny rosnące w mniejszym zagęszczeniu (50 × 50 cm). Wartość biologiczna analizowanego materiału roślinnego była wysoka i w niewielkim stopniu zależna od gęstości sadzenia roślin. Rośliny rosnące w większym zagęszczeniu odznaczały się istotnie wyższą koncentracją karotenoidów oraz istotnie niższą zawartością olejku

eterycznego w porównaniu z pozostałymi. Olejek eteryczny *Artemisia dracunculus* L. charakteryzował się obecnością 46 związków o udziale >0,05% oraz 23 związków występujących w ilościach śladowych. Związkiem dominującym badanego olejku estragonowego był metyloeugenol występujący w większej ilości w roku 2012 (37,56%) niż w roku 2011 (34,33%). Kolejnymi głównymi związkami był: elemicin, oznaczony w ilości 21,95 i 26,22%; sabinen w ilości 14,16 i 16,37% oraz E-azaron w koncentracji 8,68 i 3,49% (odpowiednio w roku 2011 i 2012). *A. dracunculus* prezentowany w niniejszych badaniach można określić jako chemotyp metyloeugenolo-elemicynowo-sabinenowy (odpowiednio: 36, 24 i 15%)

Słowa kluczowe: rozstawa sadzenia, składniki bioaktywne, olejek eteryczny, metyloeugenol, elemicin, sabinen

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