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SOME AGRONOMIC CHARACTERISTICS AND ESSENTIAL OIL COMPOSITION OF HYSSOP (*Hyssopus officinalis* L.) UNDER CULTIVATION CONDITIONS

Süleyman Kizil[1], Vedat Güler[2], Saliha Kirici[3], Murat Turk[4]

[1] Dicle University, Diyarbakır, Turkey,

[2] Ministry of Agriculture and Rural Affairs, Diyarbakir, Turkey,

[3] Department of Field Crops, Cukurova University, Adana, Turkey,

[4] Ceyhan Vocational School of Higher Education, Cukurova University, Adana, Turkey

Abstract. Hyssop (*Hyssopus officinalis* L.) is an aromatic and medicinal perennial herb native to Southern Europe and temperate regions of Asia. The study aimed to determine the effect of different plant parts harvested at different flowering stages: pre-flowering, full-flowering and post-flowering, in some agronomical characteristics, essential oil yield and components of hyssop plant in semi-arid climatic conditions of South Eastern Anatolia, Diyarbakir, Turkey. The results showed that both flowering stages and plant parts had important effects on fresh, dry herbage, dry leaf yield of hyssop, as well as essential oil content. It was concluded that under semi-arid climatic conditions, hyssop plant could be grown successfully in conformity to the limits of ISO standards and harvest from top parts of the plants at full flowering stage could be preferred for maximum dry leaf yield and essential oil contents.

Key words: Lamiaceae, ontogenetic variation, leaf yield, volatile oil, *iso*-pinocamphone

INTRODUCTION

Hyssop (*Hyssopus officinalis* L.), belonging to the family Lamiaceae, is an aromatic perennial herb native to Southern Europe including France, Hungary and Holland [Leung and Foster 2003] and some temperate regions of Asia. The plant is known

Corresponding author: Suleyman Kizil, Dicle University, Faculty of Agriculture, Department of Field Crops, 21280 Diyarbakir, Turkey; e-mail: suleymankizil@gmail.com

'Zulfa otu' in Turkey and is widely used to flavour soups [Ozer et al. 2005]. Whereas, flowering tops and leaves of hyssop are sometimes also used to flavour teas, tonics, non-alcoholic beverages, vegetable dishes, soups, salads, sauces, pickles, meats and candied products [Baytop 1984, Leung and Foster 2003, Ozer at al 2005]. Kreis et al. [1990] and Fraternale et al. [2004] has also reported that crude extract of dried leaves have an anti-HIV activity.

Although toxic effects such as clonic and clonic-tonic convulsions related to the presence of pinocamphone and *iso*-pinocamphone in the oil have been reported, the essential oil of hyssop may be used as expectorant and antiseptic [Mazzanti et al. 1998]. Moreover, hyssop herb has low content of heavy metals, i.e.: 5.73 mg kg^{-1} of Fe, 0.34 mg kg^{-1} of Cu, 0.58 mg kg^{-1} of Zn, and 0.64 mg kg^{-1} of Mn which enable its use in medical treatments [Blazewicz et al. 2007].

Analysis of hyssop essential oil showed that the terpenes are the main constituents: essentially pinocamphone, *iso*-pinocamphone and pinene [Ghfir et al. 1997]. The main volatile constituents of *H. officinalis* essential oil from a variety of locations are β-pinene, limonene, β-phellandrene, 1,8-cineole, pinocamphone, *iso*-pinocamphone, pinocarvone, germacrene-D and methyl eugenol. The oils extracted from different subspecies, plant populations of varied origin or morphology differ in percentage composition of the major volatile constituents [Garg et al. 1999]. Although, the presence of the *Iso*-pinocamphone, β-pinene, pinocamphone and *cis/trans* sabinene hydrate remains peculiar to these products; the chemical composition of these essential oils vary considerably even from oils of the same type of hyssop under different type of biotic or abiotic stress conditions. In fact, there are oils with a higher level of pinocamphone (up to 80%) or *iso*-pinocamphone (up to 50%), although the ISO 9841 Standard [1991] recommends 5.5 to 17.5% for pinocamphone and 34.5 to 50% for *iso*-pinocamphone [Mazzanti et al. 1998]. Nemeth [2005] reported that during shoot development hyssop pinocamphone increased and *iso*-pinocamphone decreased; whereas, other major constituents of the oil (limonene, β-pinene) did not change significantly, content of *iso*-pinocamphone varied 71–74% until flowering, 64–69% during flowering and 61–67% in the seed ripening and second flowering stages. Baser and Kirimer [2006] reports that composition of essential oil from herbal parts of Turkish hyssop contained 27% pinocarvone, 19% β-pinene, 14% *iso*-pinocamphone and 14% pinocamphone.

Because secondary metabolites are particularly prone to qualitative and quantitative variations depending on factors such as genetic drift, physiological conditions, season and harvesting time, the phonological stage of the plant is another source of variance that considerably influences concentrations of secondary metabolites [Chen et al. 2012]. Besides, agricultural practices such as spacing and harvesting have a critical effect on the quantitative and qualitative characteristics of many aromatic plants, which finally result in plant growth and yield increment. Ontogeny is a depiction of a plant's timeline for growth and development. It has been long considered as one of the most important factors that influence oil accumulation in plants as it also largely determines the proper time for harvesting raw material [Lee and Ding 2016]. An understanding of distribution of essential oil in different plants organs called morphogeny or morphogenetic variation determines the proper parts of plant for harvesting raw material. Agronomical studies on *H. officinalis* are also very limited.

Therefore, the aim of this study was to determine the effect of different plant parts harvested at different flowering stages on some agronomic characteristics, essential oil yield and oil components of hyssop under semi-arid climatic conditions of South Eastern Anatolia, Turkey.

MATERIALS AND METHODS

Field Experiment. The experiment was conducted at the research station of the Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir (Turkey) ecological conditions (latitude 37°53'N and longitude 40°16'E, 680 m above sea level), during years, 2005, 2006 and 2007.

The soil was analysed for total soil organic matter, total salts, soil saturation percentage, lime, phosphorus (P_2O_5), potassium (K_2O), soil pH, and electrical conductivity as per methods described by Klute [1986, tab. 1]. The soil had pH of 7.45, EC of 3.8 mmhos cm^{-1}, 1.16% organic matter, 0.61 kg ha^{-1} phosphorus and 0.16% total salts. Climatic conditions varied slightly between the years in which the experiment was carried out mean temperatures for the period January to June 2005, 2006, and 2007 were 12.2, 12.7 and 10.8°C, respectively. Long-term, 2005, 2006 and 2007 mean relative humidity for January to June period was 59.3%, 50.3, 59.1 and 74.7%, respectively. January to June mean long-term precipitation was 54.5 mm, with mean precipitation for 2005, 2006, and 2007 of 43.4, 64.2 and 55.5 mm, respectively (tab. 2).

Table 1. Soil analysis report of the experimental site

Total salts (%)	0.16
Potassium (K_2O) (kg ha^{-1})	0.0
Electrical conductivity (mmhos cm^{-1})	3.8
Organic matter (%)	1.16
Saturation percentage with water (%)	66
Phosphorus (P_2O_5) (kg ha^{-1})	0.61
pH	7.45
Sampling depth (cm)	0–40

Fresh 10 cm long cuttings of uniform length were taken in the morning from mother plants of hyssop (*Hyssopus officinalis* L.) grown in Collection Garden of Medicinal and Aromatic Plants, (Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey) to avoid wilting. Care was taken by avoiding unhealthy, damaged, over vigorous or abnormal growth, with shorter internodes using clean tools. All of the selected material was free from visible damage by insect pests and diseases. These cuttings were planted on sand bed in the greenhouse in the first week of February, 2005 keeping it moist avoiding excess of moisture and development of any type of fungus during rooting. These cuttings were regularly checked, by removing any dead and dis-

eased cuttings and fallen leaves on the growing cuttings. The rooted cuttings of hyssop with height of 12–15 cm were transplanted after hardening on April 24, 2005 into experimental plots with dimension of 60 × 25 cm with 6 cm long rows making the surface area of one plot equal to 10.8 m^2. Each plot contained 78 healthy rooted plants produced from cuttings. The field trial was arranged in the complete randomized block design with 3 replications. The plots were applied 40 kg N ha^{-1} N and 40 kg P_2O_5 ha^{-1} before planting. The soil of each experimental plot was watered soon after planting of cuttings to avoid wilting of the transplanted material. These were watered by sprinkler irrigation, hand weeded and when needed during three years of experimentation. The first year (2005) plants were harvested on the November 15, 2005 leaving their roots and bottom parts of the plant uncut 5–10 cm above the soil manually using sickle for ratooning. The experimental data was not taken for reason of maintenance and prevent winter damages to the developing plants. Moreover, this practice (ratooning) helped in earlier maturing of the experimental plants in the subsequent years (2006 and 2007).

Table 2. Meteorological data of experimental site for long years, 2005, 2006 and 2007

Months		Jan.	Feb.	Mar.	Apr.	May	June	Mean	July	Aug.	Sep.	Oct.	Nov.	Dec.
Long term	temp.	1.7	3.5	8.2	13.8	19.2	26.0	12.1	31.0	30.3	24.8	17.1	9.6	4.1
	hum.	76	71	65.0	63	55	26	59.3	26	31	31.7	48	66	75
	precip.	73.1	68.5	65.9	70.2	42.0	7.8	54.5	0.7	0.5	2.6	30.9	54.3	70.2
2005	temp.	2.3	3.0	8.4	14.1	19.6	25.8	12.2	32.4	31.8	25.0	16.2	7.5	5.3
	hum.	66.4	61.7	53.3	19.6	43.5	25.1	50.3	11.0	20.0	31.0	40.0	60.4	73.0
	precip.	58.7	46.8	58.4	36.8	26.5	33.1	43.4	0.0	0.0	0.7	14.9	38	94.3
2006	temp.	0.4	4.3	9.2	14.5	19.4	28.5	12.7	31.4	32.6	25.0	17.6	7.8	0.7
	hum.	77.1	70.7	62.1	68.9	53.3	23.3	59.1	25.0	16.4	35.9	70.9	72.7	69.0
	precip.	121.3	121.0	26.6	77.9	38.4	0.0	64.2	6.1	0.0	3.5	104.5	67.3	25.9
2007	temp.	-5.4	3.0	8.8	10.3	20.6	27.2	10.8	31.8	31.5	25.1	18.2	8.6	2.4
	hum.	88.5	78.6	73.4	79.3	75.5	52.0	74.7	44	24	25	36	49	61
	precip.	44.5	79.8	55.5	88.2	45.5	19.5	55.5	0.0	0.2	0.0	4.7	15.7	43.5

Source: Diyarbakir meteorological bulletin, Turkey (2007)

Herbage harvest from the ratooned plants of year 2006 was conducted at pre-flowering stage on May 16, at the full-flowering stage on June 02 and at post-flowering stage on June 23, 2006. In the next year (2007), the ratooned plants were harvested at pre-flowering stage on June 06, at the full-flowering stage on June 18 and at post-flowering stage on July 04, 2007 manually as described earlier. Plant height in 2006 was recorded as 43.1 cm at pre-flowering stage, 47.5 cm at full-flowering stage and 48.6 cm at post-flowering stage. Plant height in 2007 was recorded as 42.8 cm at pre-flowering stage, 47.5 cm at full-flowering stage and 49.6 cm at post-flowering stage. Soon after each harvest, all plant materials were separated equally into equally distributed three parts; bottom (1/3), middle (1/3) and top (1/3) to determine variations in fresh herbage weights for each part. Later, all plants were dried under shade at an airy place

for one week. Different parts of plants were also weighed to determine dry herbage yield. Dry leaf yield was determined after separating the leaves and stems on the dried herb samples.

Essential oil extraction. Dried leaves of each part of plants (30 g) from different phenological stage (pre-flowering, full flowering and post flowering) were separately hydro distilled for 2 h using a Clevenger-type apparatus. The essential oils were stored in glass vials and kept at ±4°C until analysis.

Gas chromatography–mass spectrometry (GC–MS) analysis. Qualification and quantification were carried out by using a Finnigan-Trace GC–MS (Strada Rivoltana-Rodana (Milan) Italy) equipped with an auto sampler. One micro litre of sample volume was injected using split method with 50 split ratio. Chromatographic separations were accomplished with a Zebron ZB-5 capillary column (5% phenyl – 95% dimethylpolysiloxane, 0.25 mm i.d. × 60 m, film thickness 0.25 m) with injections in the split mode with 50 split ratio. Analysis was carried out using helium as the carrier gas, flow rate 1.0 mL min^{-1}. The column temperature was programmed from 50 to 240°C at 3°C min^{-1}. The sample size was 2 ml. The injection port temperature was 250°C. The ionization voltage applied was 70 eV, mass range *m*/*z* 41 – 400 a.m.u. The separated components were identified tentatively by matching with GC–MS results of National Institute of Standards and Technology (NIST) 05 mass spectral library data because their reference reagents were not available. The quantitative determination was carried out based on peak area integration.

Data pertaining to the mean fresh & dry herbage yield, dry leaf yield, essential oil yield and its main components were subjected to analysis to variance (ANNOVA) by using MSTAT-C (Michigan State University) statistical computer program using harvest period as main factor and plant parts as sub factors for each year. The significantly different means for each parameter were grouped using the least significant difference (LSD) test ($P < 0.05$).

RESULTS AND DISCUSSION

Field Experiment. Fresh and dry herbage yield of hyssop are very important because the plant has been used traditionally in treatment of many diseases and also used commercially for extraction of compounds of pharmaceutical importance. The significant effect of flowering stages × plant parts interactions on fresh herbage yields for 2006 and 2007 years and dry herbage yield in the 2006, demonstrated significant ($P < 0.05$) variation in dry herbage yield. Ecological factors including rains had a positive effect on fresh and dry herbage yield resulting in more fresh and dry herbage yield during 2006 compared to these parameters during 2007 (tab. 3). It was also observed that the developing stage was important in terms of fresh and dry herbage yield as the maximum fresh (15207 kg ha^{-1}) and dry herbage yield (5348 kg ha^{-1}) were obtained at the full-flowering stage but no significant differences were found for the total values of plants harvested at the pre-flowering and post flowering stages of the plantation of 2006. Additionally, in the interaction of flowering stages × plant parts, the highest fresh

herbage yield (5607 kg ha^{-1}) was obtained from bottom part of plants at full-flowering stage, but non significant difference were noted from pre-flowering stage values (5431 kg ha^{-1}) of the same year.

The total fresh herbage yield of hyssop plants harvested at pre-flowering stage was the maximum (13511 kg ha^{-1}) but non significant differences were found between the total values of plants harvested at the full-flowering and post flowering stages in 2007. The minimum fresh herbage yield was obtained from top parts of plant in 2006 and 2007 (tab. 3). In the general, the highest amount of fresh and dry herbage yields were achieved at the full and pre-flowering stages from bottom parts of plants. The fresh and dry herbage yields decreased from bottom part to top parts of plants during both years (2006 and 2007), as bottom parts of hyssop plant contained higher amount of fibre and woody texture.

Table 3. Fresh and dry herbage yield of different plant parts of *H. officinalis* L. at different flowering stages

	Harvest Periods	2006				2007			
		bottom	middle	top	total	bottom	middle	top	total
Fresh herbage (kg ha^{-1})	pre-flowering	5431 a	4831 bc	3878 d	14333	6222 a	4600 b	2689 ef	13511
	full-flowering	5607 a	5065 b	3994 d	15207	4644 b	3689 cd	2933 de	11266
	post-flowering	4694 c	4804 bc	4573 c	14921	4100 bc	3466 cde	2116 f	9682
	mean	5244	4900	4148		4989	3919	2579	
	LSD (0.05)	flowering stages × plant parts: 295				flowering stages × plant parts: 800			
Dry herbage (kg ha^{-1})	pre-flowering	1756 b	1297 c	1110 c	4163	2274	1557	907	4738
	full-flowering	2274 a	1753 b	1321 c	5348	2086	1503	1098	4687
	post-flowering	1762 b	1749 b	1763 b	5274	2270	1745	1103	5118
	mean	1931	1599	1398	–	2209 a	1602 b	1036 c	–
	LSD (0.05)	flowering stages × plant parts: 404				plant parts: 217			

Flowering stages and parts of plant significantly affected the dry leaf yield that varied and changed from 2006 to 2007. According to flowering stages, the highest total dry leaf yield (2735 kg ha^{-1}) was determined at post flowering stage in 2006. Among different parts of the plant, the maximum dry leaf yield was obtained from top part of plant (1016 kg ha^{-1}) in 2006. In 2007, maximum leaf yield (899 kg ha^{-1}) was also obtained from bottom parts of plants at pre-flowering, middle parts at full flowering (868 kg ha^{-1}) and top parts at post flowering stages 862 kg ha^{-1}). This situation clearly shows that harvest performed at full flowering stage and top part of the plant produced higher yield compared to other stages and parts. Less dry leaf yield was recorded from intermediate and bottom parts of hyssop plant due to higher amount of fibre and woody texture, which was not the situation when the samples were taken from top parts with less to no fibre or woody texture (tab. 4). Total leaf yield obtained in this study is compatible with Jankovsky and Landa [2002] reported 0.67 to 3.26 tones dry leaf yield for hyssop.

The essential oil content of two experimental years (2006 and 2007) for hyssop obtained from different parts of plants in the ontogenetic stages of pre-flowering, full-flowering and post-flowering stages are given in Table 4. The content of essential oil of hyssop plant was significantly increased at full flowering stage (1.41%) and thereafter, it started to decrease in 2006. In the same year, regard to morphogenetic variation of essential oil, the highest value (2.04%) was obtained from top parts of plant. The significant effect of interactions of flowering stages × plant parts on content of essential oil was demonstrated for 2007 (tab. 4). The content of essential oil was the maximum (3.62%) in top parts of plants at full flowering stage in 2007. The least values were obtained from bottom parts of plant at all phenological stages in 2007. The mean of essential oil content increased from bottom part to top part during both years (2006, 2007), especially essential oil content of 2007 was higher than that of 2006. Thus, the essential oil content of hyssop increased at all stages with the advancement of crop age.

Table 4. Dry leaf yield and essential oil content of different plant parts of *H. officinalis* L. at different flowering stages

	Harvest Periods	2006				2007			
		bottom	middle	top	total	bottom	middle	top	total
Dry leaf yield (kg ha^{-1})	pre-flowering	555	664	789	2009 b	899 a	848 ab	666 c	2413
	full-flowering	627	826	955	2408 ab	738 bc	762 abc	779 abc	2279
	post-flowering	540	891	1304	2735 a	648 c	868 ab	862 ab	2378
	mean	575 b	793 ab	1016 a	–	761	826	769	–
	LSD (0.05)	flowering stages: 522; plant parts: 349				flowering stages × plant parts: 148			
Essential oil content (%)	pre-flowering	0.19	0.91	1.74	0.95 c	1.81 e	2.82 c	3.15 b	2.59
	full-flowering	0.39	1.25	2.59	1.41 a	0.83 f	2.49 d	3.62 a	2.31
	post-flowering	0.35	0.97	1.81	1.04 b	0.83 f	1.97 e	2.81 c	1.87
	mean	0.31 c	1.04 b	2.04 a	–	1.15	2.42	3.19	–
	LSD (0.05)	flowering stages: 0.14; plant parts: 0.46				flowering stages × plant parts: 0.23			

A sharp decline in mean oil content was recorded from bottom-part of hyssop plants during two years (2006 and 2007). This may be the result of essential oil transportation from below to above. Nemeth [2005] has recently found that hyssop reach a high essential oil content of the drug above 1.0%. The oil yields of top part of hyssop at all development stages in this study were higher than 1.0%.

Moreover, low oil content at all stages of growth in 2006 could be related to low temperature and precipitation during 2006. This results in considerable increase of secondary metabolites like essential oils at full blooming stage. Ontogeny is a depiction of a plant's timeline for growth and development. It has been long considered as one of the most important factors that influence oil accumulation in plants as it also largely determines the proper time for harvesting raw material [Lee and Ding 2016]. For the maximum yield values of dry leaves and essential oil content, hyssop plant should be harvested at pre and full flowering stages.

Table 5. Essential oil components in different plant parts of *H. officinalis* L. at different flowering stages during 2006 growing season (%)

Components	RT	Pre-flowering			Full-flowering			Post-flowering		
		bottom	middle	top	bottom	middle	top	bottom	middle	top
α-Thujene	11.77	0.6	0.7	1.0	0.5	1.0	0.9	1.4	1.2	1.3
α-Pinene	12.05	1.4	1.3	1.6	0.9	1.5	1.4	2.2	1.9	1.5
Camphene	12.61	0.2	0.1	0.2	0.1	0.2	0.2	0.3	0.3	0.3
Sabinene	13.41	1.1	2.9	3.9	2.2	4.1	3.8	6.0	4.7	4.4
β-Pinene	13.59	8.6	21.0	16.3	16.8	17.6	14.7	20.6	16.5	11.2
β-Myrcene	13.88	0.7	2.0	2.6	1.4	2.6	2.3	3.4	2.5	2.4
α-Terpinene	14.92	0.6	0.5	1.1	0.7	1.2	1.4	2.7	2.1	1.9
Cymol	15.22	0.8	2.0	1.8	2.1	2.0	1.5	1.8	2.1	0.9
dl-Limonene	15.37	0.6	1.6	2.2	1.2	2.2	–	3.2	2.5	2.7
1.8-Cineole	15.49	2.1	1.5	2.3	1.1	2.4	2.2	2.8	2.5	1.8
Cis-Ocimene	15.92	2.0	1.4	2.9	1.6	2.4	2.0	2.2	2.0	1.8
η-Terpinene	16.42	2.6	0.6	2.5	0.5	0.3	2.8	4.4	3.6	3.3
Cis-Sabinene hydrate	16.78	3.3	0.1	0.3	0.3	0.5	4.2	1.7	0.5	6.5
α-Terpinolene	17.50	0.6	0.3	0.6	1.1	–	–	1.0	0.8	0.8
L-Linalool	17.79	1.4	1.3	1.7	0.1	–	0.2	–	0.9	–
1-Terpineol	17.91	0.3	0.4	0.4	–	0.4	0.5	0.4	–	1.6
α-Thujone	18.18	0.2	0.1	0.2	0.1	–	–	0.2	0.2	0.4
β-Terpineol	18.74	0.4	0.3	0.4	0.3	–	–	0.3	0.2	0.6
Pinocamphone	19.96	4.3	3.5	3.9	3.1	4.9	3.9	4.0	3.7	4.5
Pinocarvone	20.26	3.3	0.4	–	1.9	0.8	–	2.6	2.3	–
Iso-Pinocamphone	20.71	48.0	50.4	43.1	53.6	47.3	46.3	30.1	26.9	41.7
α- Terpineol	21.18	0.6	0.4	0.6	0.3	0.5	–	0.3	0.1	0.8
Myrtenol	21.42	1.9	1.2	2.2	1.2	1.3	2.2	1.1	0.5	2.9
Carvacrol	24.93	6.8	0.3	–*	–	–	0.1	–	–	–
Myrtenyl acetate	25.52	1.1	0.2	0.4	0.3	0.6	0.5	0.7	0.1	0.8
Nerolidil acetate	26.51	0.1	0.1	0.1	0.1	0.1	–	0.1	–	0.1
β-Bourbonene	27.60	0.6	0.4	–	0.6	0.9	0.6	0.5	0.5	0.4
Caryophyllene	28.71	1.0	0.5	0.6	1.3	0.6	0.6	0.4	1.3	0.2
Aromadendrene	29.96	0.2	0.2	0.2	0.2	0.3	0.2	0.3	–	0.3
Germacrene-D	30.52	1.1	0.7	1.1	0.7	0.8	1.3	0.8	3.7	0.6
Bicyclogermacrene	30.96	0.4	0.5	0.1	0.2	0.6	0.4	0.6	0.5	0.6
Elemol	32.36	1.2	1.4	2.9	1.0	–	2.2	1.1	0.5	1.0
Spathulenol	33.34	0.3	0.2	0.2	0.3	–	0.2	0.1	1.7	0.2
Caryophyllene oxide	33.52	0.2	0.2	0.1	0.3	–	0.2	–	0.8	–
Junipene	34.76	–	0.1	0.3	0.1	0.1	0.2	0.1	–	–
β-Cadinene	34.95	0.2	0.2	0.3	0.3	0.2	0.3	0.2	0.3	–
Torregol	35.34	0.2	0.2	0.3	0.2	0.2	0.3	–	–	–
Total (%)		99.0	99.4	98.5	96.6	97.6	97.6	97.6	89.4	97.5

RT – retention time

Table 6. Essential oil components in different plant parts of *H. officinalis* L. at different flowering stages during 2007 growing season (%)

Components	RT	Pre-flowering			Full-flowering			Post-flowering		
		bottom	middle	top	bottom	middle	top	bottom	middle	top
α-Thujene	11.77	–	0.1	0.2	–	0.1	0.2	0.5	0.2	0.5
α-Pinene	12.05	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.3	0.6
Camphene	12.61	–	–	0.1	–	–	0.1	–	–	0.2
Sabinene	13.41	0.4	1.0	1.7	0.6	1.2	1.7	0.8	1.1	1.9
β-Pinene	13.59	12.3	11.3	15.3	12.9	11.8	11.8	10.2	9.6	8.9
β-Myrcene	13.88	–	0.3	0.5	–	0.3	0.4	0.1	0.2	0.6
α-Terpinene	14.92	–	0.4	0.5	0.2	0.7	0.7	0.6	0.5	0.6
Cymol	15.22	–	–	–	–	0.4	0.4	–	0.3	0.9
dl-Limonene	15.37	0.2	0.3	0.4	0.6	0.5	–	0.3	0.3	0.7
1.8-Cineole	15.49	0.6	0.9	1.0	0.9	1.2	1.1	0.9	1.0	1.2
Cis-Ocimene	15.92	1.4	–	–	1.6	–	–	–	–	0.3
η-Terpinene	16.42	0.4	0.6	0.7	0.2	–	0.9	0.4	0.6	1.1
Cis-Sabinene hydrate	16.78	2.6	4.5	4.1	2.3	6.0	5.2	3.3	5.5	5.8
α-Terpinolene	17.50	–	0.3	0.1	–	0.5	0.4	0.4	0.3	0.4
L-Linalool	17.79	–	0.2	0.3	–	0.2	0.1	–	0.2	0.8
1-Terpineol	17.91	–	0.1	–	–	0.2	0.2	–	0.1	0.3
α-Thujone	18.18	0.3	0.1	0.2	–	0.2	0.2	–	0.1	0.2
β-Terpineol	18.74	–	–	–	–	0.2	0.2	0.1	0.2	0.3
Pinocamphone	19.96	2.3	2.6	2.8	3.0	3.0	3.2	2.6	3.3	3.0
Pinocarvone	20.26	0.7	0.5	0.5	1.0	0.5	0.5	1.1	0.4	–
Iso-Pinocamphone	20.71	74.5	72.8	68.2	70.7	66.9	67.4	73.9	72.2	70.2
α-Terpineol	21.18	–	–	–	–	–	–	–	–	–
Myrtenol	21.42	0.4	0.3	0.4	0.4	–	0.4	0.3	0.5	0.4
Carvacrol	24.93	–	–	–	–	–	–	–	–	–
Myrtenyl acetate	25.52	–	0.3	0.4	–	0.2	0.5	–	0.2	0.2
Nerolidil acetate	26.51	–	–	–	–	–	–	–	–	–
β-Bourbonene	27.60	0.4	0.2	–	0.5	0.1	0.1	0.3	0.2	0.1
Caryophyllene	28.71	0.2	0.2	–	0.4	0.1	0.2	0.3	0.2	–
Aromadendrene	29.96	0.1	–	–	0.2	0.1	0.2	–	0.2	–
Germacrene-D	30.52	0.2	0.2	–	0.3	0.1	–	–	0.1	–
Bicyclogermacrene	30.96	–	0.1	–	0.2	0.1	–	–	–	–
Elemol	32.36	1.3	0.6	0.9	1.8	1.3	0.6	1.8	0.9	0.5
Spathulenol	33.34	0.5	0.4	0.3	0.6	0.5	0.4	0.5	0.4	0.2
Caryophyllene oxide	33.52	0.2	0.1	–	0.3	–	–	0.2	0.1	–
Junipene	34.76	–	–	–	–	–	–	–	–	–
β-Cadinene	34.95	0.2	0.2	–	0.2	0.2	0.1	0.3	0.2	–
Torregol	35.34	–	–	–	–	–	–	–	–	–
Total (%)		99.6	99.0	99.1	99.4	98.0	97.6	99.4	99.4	99.9

RT – retention time

Table 7. Main essential oil components in different plant parts of *H. officinalis* L. at different flowering stages during 2006 and 2007 years (%)

Components	Parts	2006				2007			
		pre flowering	full flowering	post flowering	mean	pre flowering	full flowering	post flowering	mean
iso-Pinocamphone	bottom	48.0 c	53.6 a	30.1 e	43.9 A	74.5	70.7	73.9	73.0 A
	middle	50.4 b	47.3 c	26.9 f	41.5 B	72.8	66.9	72.2	70.6 B
	top	43.1 d	46.3 c	41.7 d	43.7 A	68.2	67.4	70.2	68.6 B
	mean	47.2 A	49.1 A	32.9 B		71.8 A	68.3 B	72.1 A	
LSD (0.05)		F.S.: 2.59; plant parts: 1.10; int.: 1.91				F.S.: 0.83; plant parts: 2.13; int.: NS			
β-Pinene	bottom	8.6 e	16.8 b	20.6 a	15.3 B	12.3 bc	12.9 b	10.2 cde	11.8
	middle	21.0 a	17.6 b	16.5 bc	18.3 A	11.3 bcd	11.8 bcd	9.6 de	10.9
	top	16.3 bc	14.7 c	11.2 d	14.1 B	15.3 a	11.8 bcd	8.9 e	12.0
	mean	15.3	16.4	16.1		12.9 A	12.2 A	9.6 B	
LSD (0.05)		F.S.: NS; plant parts: 1.35; int.: 2.01				F.S.: 0.89; plant parts: NS; Int.: 2.32			
cis Sabinene hydrate	bottom	3.3 c	0.3 e	1.7 d	1.8 B	2.6 e	2.3 e	3.3 d	2.7 B
	middle	0.1 e	0.5 e	0.5 e	0.4 C	4.5 c	6.0 a	5.5 ab	5.3 A
	top	0.3 e	4.2 b	6.5 a	3.7 A	4.1 c	5.2 b	5.8 ab	5.0 A
	mean	1.3 C	1.7 B	2.9 A		3.7 B	4.5 A	4.9 A	
LSD (0.05)		F.S.: 0.33; plant parts: 0.49; int.: 0.85				F.S.: 0.58; plant parts: 0.40; Int.: 0.69			
Pinocamphone	bottom	4.3 abc	3.1 d	4.0 abcd	2.8	2.3	3.0	2.6	2.6 B
	middle	3.5 cd	4.9 a	3.7 bcd	4.0	2.6	3.0	3.3	2.9 A
	top	3.9 bcd	3.9 bcd	4.5 ab	4.1	2.8	3.2	3.0	3.0 A
	mean	3.9	3.9	4.1		2.6 B	3.1 A	2.9 A	
LSD (0.05)		F.S.: NS; plant parts: ns; int.: 0.95				F.S.: 0.26; plant parts: 0.19; Int.: NS			

F.S. – flowering stages, N.S. – no significant

It is reported that there is a close co-ordination between ontogeny in aromatic plants and temperature, daylight [Sangwan et al. 2001]. Halva et al. [1992] reported that in dill, growth and essential oil accumulation improve with increase in the light level and was at the highest under full sunlight.

The secondary metabolite production is believed to be stimulated by stressful environment. Weather parameters such as temperature and precipitation have been reported to influence oil content and composition in several aromatic plants. Temperature and humidity are the most important factors influencing essential oil contents of plants belonging to Lamiaceae. Colder nights and warmer days adversely affected oil contents of many plants [Sangwan et al. 2001]. Hyssop produces maximum leaf growth with high oil content under warm sunny conditions. Cloudy days or low temperatures adversely affect both parameters.

Ceylan [1983] reported that variability in morphogenetic, ontogenetic, diurnal and ecological factors affected secondary metabolites of plants, especially essential oils, and

its oil components. Moreover the highest essential oil content is obtained during warm period of growth at full flowering periods.

Essential oil components. Volatile oil compositions vary depending on variety, growth stage, date of collection, climatic conditions, admixture of foreign plants and extraction technology. Thirty-seven components were identified in 2006, and thirty-two components were identified (except α-terpineol, nerolidil acetate, carvacrol, junipen and torregol) in 2007 (tabs 5 and 6). *Iso*-pinocamphone, β-pinene, pinocamphone and *cis/trans* sabinene hydrate were main components of each part of the plants and all developing stages during both years. Flowering stages, plant parts and flowering stages × plant parts interaction significantly affected contents of *iso*-pinocamphone during 2006. *Iso*-pinocamphone content in 2006 changed between 32.9–47.2% at different development stages and 41.5–43.9% for plant parts. The highest value (53.6%) was obtained from bottom part of the plant during full flowering stage and it varied between 26.9–53.6% in 2006. Among flowering stages, the highest value obtained from at full flowering stage as 49.1%. The composition of *iso*-pinocamphone increased in bottom part of plant, then decreased in middle part and again increased at top part of the plants in 2006. Flowering stages and plant parts affected significantly *iso*-pinocamphone contents, however, no significant interaction was found between them in 2007. The highest *iso*-pinocamphone (73.0%) was recorded from bottom parts of plants and its value varied 68.6 to 73.0% in 2007. Similarly, bottom part of plant had higher *iso*-pinocamphone and it reduced to top parts of plants at all development stages in 2007 (tab. 7). *Iso*-pinocamphone content changed with respect to flowering stages and the highest value of 72.1% was obtained from post-flowering stage. In generally, *iso*-pinocamphone content of year 2007was higher compared that of year 2006.

Plant parts and flowering stages × plant parts interaction affected significantly content of β-pinene in 2006. The highest β-pinene content (21.0%) was obtained from middle parts of plants during pre-flowering stage, the minimum β-pinene of 11.2% was also noted from top parts of plants during post flowering stage. In 2006, while the highest value of 18.3% was obtained from middle part of plant and the minimum value was noted from top part of plants as 14.1%. In 2007, β-pinene content was affected from flowering stages and flowering stages × plant parts interaction. The highest content of β-pinene (15.3%) was obtained from top of plants during pre-flowering stage and the lowest value of 8.9% was also noted from top of plants during post-flowering stage. β-Pinene content was found higher at pre and full flowering stages than at post flowering stage. The concentration of β-pinene fluctuated according to parts of plants in the experimental years (2006, 2007), but it was slightly reduced in 2007 (tab. 7).

It was observed that flowering stages, plant parts and flowering stages × plant parts interaction significantly affected *cis*-sabinene hydrate during both experimental years (2006–2007). Its content varied between 0.1–6.5% and 2.6–6.0% in 2006 and 2007, respectively. The highest contents of 6.5% were noted from top part of plants during post flowering stage for 2006 and 6.0% for 2007. In terms of development (flowering) stages, the highest values were obtained from post flowering stage of both years. Also pinocamphone content varied according to place of plant and developing (flowering) stage. The maximum value of 4.9 and 3.3% was obtained from middle part of

plants at full and post flowering stages in 2006 and 2007, respectively. There were large differences between results obtained during 2006 and 2007 in terms of essential oil components. In plants of 2006, monoterpene hydrocarbons (such as α-thujene, α-pinene, sabinene, β-myrcene, α-terpinene, cymol, dl-limonene, 1.8-cineole, *cis*-ocimene and η-terpinene) ranged 1–3%. Contrarily, *iso*-pinocamphone content remained 50%. In plants of 2007, monoterpenes were usually in poor amount; whereas, *iso*-pinocamphone content was over 70% (tab. 7). Moreover, 6.8% carvacrol was determined from bottom and middle parts of plant at pre-flowering stage and top part in the full-flowering stage in 2006 but was absent at other periods and in 2007 (tab. 6).

It is necessary to assume variations in the composition during ontogenesis. In fact, *iso*-pinocamphone and β-pinene are generally the compounds characterizing the oils of *H. officinalis*. Moreover, hyssop oils from different phenotypes or from different areas show a great variability in chemical composition. The chemical composition can be related to many factors, such as the geography, climate and technological influence, origin, stages of flowering, parts used and harvesting time, as well as variations due to the presence of chemotypes [Fraternale et al. 2004, Jankovsky and Landa 2002].

According to the International ISO 9841 standards (1991E) the amount of % β-pinene, pinocamphone and *iso*-pinocamphone in hyssop oil should be 13.6–23.0%, 5.5–17.5% and 34.5–50.0%, respectively [Pandey et al. 2014]. The amounts of pinocamphone recorded in hyssop oil of all parts of plants and development stages were slightly less than standard values. The contents of *iso*-pinocamphone in all parts of plants and development stages were found to be within the desired ranges; furthermore, they were higher than the values recorded in 2007. However, β-pinene's values in 2006 were appropriate and compatible to ISO standards.

Jankovsky and Landa [2002] reported that the maximum quantities of 38.1% *iso*-pinocamphone, 20.3% pinocarvone, 12.2% 1,8-cineole and 10.2% β-pinene were found in essential oil samples of *H. officinalis.* Garcia-Vallejo et al. [1995] found that major constituents of *H. officinalis* are *iso*-pinocamphone (40.2%), β–pinene (14.2%), pinocamphone (10.3%) and β-phellandrene (9.5%) and did not found 1,8-cineole in commercial samples. Similarly, Kizil et al. [2008] found that *iso*-pinocamphone was the dominating component (47.9–51.4%) in the all analysed oil samples. Pandey et al. [2014] reported that major constituents of the hyssop's oils were *cis*-pinocamphone (49.7–57.7%), pinocarvone (5.5–24.9%) and β-pinene (5.7–9.3%). Furthermore leaf and stem oil compositions were similar, but flower oil compositions were different in terms of *cis*-pinocamphone and pinocarvone content. The composition of oils reported in this study is in agreement with the literature mentioned above.

Iso-pinocamphone, β-pinene, pinocamphone and *cis*-sabinene hydrate together constituted 67.6–90.0% of total hyssop oil at pre-flowering, 71.0–88.1% at full-flowering and 56.0–89.5% at post-flowering stage, in the experiment years (tab. 5, 6, 7). The results showed 71.8 and 72.1% *iso*-pinocamphone at pre-flowering and post-flowering stage during the second year. Similarly, Garg et al. [1999] and Ozer et al. [2005] described pinocamphone as the major component and Nemeth [2005] found (61–74%) volatile oils in their experiments. The results of this study show 34–50% *iso*-pinocamphone and 13–23%, β-pinene, in conformity to the limits of ISO standards.

CONCLUSION

H. officinalis and its essential oil components are very important from commercial point of view. The results of experiment describe appropriate stage, part of the plant and time of harvest for *H. officinalis.* The results confirmed that a relationship among herbage yields, leaves yields and ontogenetic variations and different plant parts effect essential oil and composition significantly under semi-arid climatic conditions. It was confirmed that pre, full and post-flowering ontogenetic stages of hyssop contain at least over 1.0% essential oil and high contents of *iso*-pinocamphone and β-pinene in the different above ground part of the plants. It was further confirmed that hyssop could be cultured profitably for commercial production of *iso*-pinocamphone and β-pinene etc. under semi-arid conditions.

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NIEKTÓRE CECHY AGRONOMICZNE ORAZ SKŁAD OLEJKÓW ETERYCZNYCH HYZOPU (*Hyssopus officinalis* L.) W WARUNKACH UPRAWY

Streszczenie. Hyzop (*Hyssopus officinalis* L.) jest aromatyczną i leczniczą wieloletnia rośliną zielną rodzimą dla Europy Południowej i umiarkowanych regionów Azji. Celem badania było określenie wpływu różnych części roślin zbieranych w różnych fazach kwitnienia (przed kwitnieniem, w pełni kwitnienia oraz po kwitnieniu) na pewne cechy agronomiczne, plon olejku eterycznego i składniki hyzopu w półpustynnych warunkach klimatycznych południowo-wschodniej Anatolii w Diyarbakir w Turcji. Wykazano, że zarówno fazy kwitnienia jak i części roślin mają istotny wpływ na świeżą i suchą masę ziela, plon suchych liści oraz zawartość olejku eterycznego. Stwierdzono, że w półpustynnych warunkach klimatycznych hyzop można uprawiać w zgodzie ze standardami ISO,

a plon części szczytowych na etapie pełnego kwitnienia jest najlepszy ze względu na maksymalny plon suchych liści oraz zawartość olejku eterycznego.

Słowa kluczowe: Lamiaceae, zróżnicowanie ontogenetyczne, plon liści, olejek lotny, iso-pinokamfon

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