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THE INFLUENCE OF HARVESTING APPLIED AT DIFFERENT STAGES OF PLANT DEVELOPMENT ON *Hyssopus officinalis* L. PRODUCTION

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ABSTRACT

The yield and quality traits of hyssop (*Hyssopus officinalis* L.) depending on the plant developmental phases were investigated under Turkey's Eskisehir ecological conditions in 2019 and 2020. The experiment were conducted in randomized complete block design with 3 replications. The developmental stages examined were before-flowering, beginning of flowering, full flowering and after flowering stage. The effect of harvest times on examined all parameters was very significant ($p \leq 0.05$). The maximum yields of fresh herb and dry leaf + flower were obtained from after-flowering stage. However, no significant difference was observed between full flowering and after-flowering period for dry leaf + flower yield. On the other hand, the essential oil content (0.93%) and essential oil yield (24.01 L ha^{-1}) in the full flowering stage were found to be higher. The main components of hyssop essential oil were as follows: pinocamphone (38.41–41.85%), isopinocamphone (22.73–22.99%) and β-pinene (7.92–8.94%). Maximum pinocamphone content was observed in the before-flowering period. To harvesting in full flowering of hyssop plant can be a reasonable strategy in terms of high dry leaf + flower yield, essential oil content and yield.

Key words: hyssop, harvest times, isopinocamphone, ontogenetic variability, pinocamphone

INTRODUCTION

Species belonging to the genus *Hyssopus* (Lamiaceae) are perennial and semi-woody aromatic plants. So far 12 species of this genus have been identified in the world [Fatemeh et al. 2011, Kurkcuoglu et al. 2016]. *Hyssopus officinalis* species is the only species of this genus growing in Turkey. This species is known as "zufa otu, zulfa otu, corduk and curduk otu" in Turkey [Kurkcuoglu et al. 2016]. The dried flowers and leaves of hyssop have been used in folk medicine and herbal tea mixtures for colds, digestive disorders, treatment of throat inflammation, expectorant, wound healing, anti-inflammatory and antitussive. Essential oils obtained from flowers and leaves are used a wide range of products from the food to the cosmetics [Fatemeh et al. 2011]. Additionality, extracts obtained from the leaves and flowers of the plant have been used as antifungal, antibacterial, antimicrobial, antioxidant, antiseptic, antispasmolytic and antiviral [Fatemeh et al. 2011, Tavakoli and Aghajani 2016]. The essential oil content of the plant varies between 0.75 and 1.50% depending on the genotype of the plant, climate and soil conditions and the cultivation practices [Kotyuk 2015]. The chemical composition of hyssop oil is controlled by ISO 9841, being iso-pinocamphone

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(25–45%), pinocamphone (8–25%) and β-pinene (7–20%) are the main components recognized as standards [ISO 2007, Moro et al. 2011].

The quality, which is as important as high yield in medicinal and aromatic plants, is desired to comply with the criteria recognized in the pharmacopoeias. As a matter of fact, the medicinal and aromatic plants industry demands high-quality raw materials with a standard chemical composition. The biosynthesis of secondary metabolites in medicinal and aromatic plants is not only genetically controlled but also strongly influenced by environmental factors such as climate and soil conditions and cultural practies such as plant density, fertilisation, harvest time. Furthermore, the percentage composition and biologically active compounds of essential oil in aromatic plants vary depending on various plant growth conditions and developmental stages [Pfefferkorn et al. 2008, Kotyuk 2015, Can et al. 2021]. It were revealed that harvest periyods affect essential oil content and components of many aromatic plants [Pfefferkorn et al. 2008, Nurzyńska-Wierdak 2009, Karaca and Sonkaya 2020]. Therefore, it is of great importance to determine the growth periods in which the plants have the highest essential oil content and the most suitable essential oil composition and to harvest during these periods. It were conducted some studies on the effect of ontogenetic variability on yield, essential oil content and composition in aromatic plants such as *Lavandula stoechas*, *Origanum onites* and *Mentha* × *piperita* in Turkey [Kaya et al. 2012, Can et al. 2021, Yesil and Ozcan 2021]. Howewer, studies on the hyssop plant, which has a high agricultural potential in Turkey, are very few.

Therefore, in this study carried out for two years, it was aimed to determine the effect on yield, essential oil content and composition of harvest at different developmental stages of the hyssop (*Hyssopus officinalis* L.) plant.

MATERIAL AND METHODS

Research site and microclimatic conditions. The field experiments were conducted at the experimental field of Forest Directorate (39°44'26.19"N and 30°26'14.14"E) located in Turkey's Eskisehir Province. Trial plots were established in 2016. However, this study contains the data of the fourth and fifth years of the plantation (2019 and 2020). The climate of experiment area was characterized as continental climate by warm and drought during summer, cold and snowy in winter months. The mean temperature was 12.8°C and 13.0°C, and the total precipitation was 426.8 mm and 301.6 mm in 2019 and 2020, respectively. The soil of experimental field was clay-loam texture, slightly alkaline (pH 7.43), EC (0.23 dS m^{-1}) medium lime (8.0%), low organic matter (1.62%), low available P_2O_5 (34 kg ha^{-1}) and high available K_2O (1924 kg ha^{-1}) content.

Plant material and experimental design. The seeds of *Hyssopus officinalis* L. were obtained from in Zeytinburnu Medicinal Plants Garden, Istanbul, Turkey (it was deposited in hebarium under vouch no 2008). The seeds were sown into a mixture of sand (1/3) and peat (2/3) on 21 March 2016 in greenhouse conditions. The seedlings were transplanted to experimental plots at plant density (50x40 cm intervals) on 26 April 2016 when the plants were at 4–6 leaves stage. The experiments were conducted in randomized complete block design with three replications and the size of each experimental plot was 9.6 m^2. Triple super phosphate and ammonium sulphate were used as fertilizer sources. All of the phosphorus (80 kg ha^{-1} P_2O_5) and half of the nitrogen (50 kg ha^{-1} N) were applied to the soil when the plants started to grow in the spring of 2019 and 2020. The others half of nitrogen (50 kg ha^{-1} N) was applied as top dressing three weeks after the first application. Plants were irrigated with a drip irrigation system and irrigation was applied 4 times a year, with 100 mm of water per plot every 2 weeks. Weed control was done by hand and no plant protection products were used. In the study, the first and last rows of each plot and one plant from both ends of the rows were discarded as side effect. The center two rows of each four-row plot were cut at 10 cm above ground. Cut was done manualy and all above ground parts of plant was collected. The developmental stages of hyssop under agroecological conditions similar to the study area in Turkey are generally as following: vegetation period (June), beginning of flowering (first half of July), full flowering (second half of July) and seed formation (August). The harvests were made at four different times in parallel with these developmental phase of plant: before flowering (03.07.2019 and 01.07.2020), beginning of flowering (12.07.2019 and 08.07.2020),

full flowering (22.07.2019 and 18.07.2020), after flowering (31.07.2019 and 28.07.2020).

Essential oil extraction and analysis. The harvested plants were dried in the oven at 35°C for 48 h. 1.0 liter of water was added to 100 g of dry leaf + flower sample from each application and extracted by water-distillation for 3 h using the clevenger apparatus. The obtained samples of essential oil were stored in a refrigerator at 4°C until the composition analysis. The analysis of the essential oil samples were performed by gas chromatography (Agilent 7890B) coupled to mass spectrometry (Agilent 5977A) using capillary column (HP Innowax Agilent 19091N-116: 60 m × 0.320 mm and 0.25 µm). Helium was used as the carrier gas at 1.3 mL min^{-1} flow rate. Essential oils were dissolved with hexane to analyze its composition (20 µL essential oil was diluted in 1 mL n-hexane). GC-MS analysis was carried out at split mode (40:1). The injection volume was adjusted as 1 µl and injection temperature as 250°C. The samples analyzed with the column held initially at 70°C after injecting with 5 min hold time. Afterward, the temperature increased to 160°C with 3°C min^{-1} heating ramp and 5 min hold time. Eventually, the temperature reached to 250°C with 6°C min^{-1} heating ramp and 5 min hold time. The detector and ion source temperatures were 270°C and 230°C, respectively. The retention indices were determined by injecting C7-C30 n-alkanes (Sigma-Aldrich) to (GC/FID) system (Agilent Technologies, 7890B) under the same conditions as GC-MS analysis. The identification of the components were performed by comparing the spectra taken from the MS detector with the spectra of the Wiley, NIST libraries and with data available in the Adams literature. The components percentage was obtained based on GC-FID analyses.

Statistical analysis. All the data of this study were subjected to analysis of variance using SPSS program. The mean comparisons were performed by the Tukey test at a significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

For the parameters examined, the results obtained from analysis of variance were given in Table 1 and Table 2. The results showed that plant height of hyssop were significantly affected by years and plant developmental stages. However, year × harvest periods interaction had not a significant effect on this parameter (Tab. 1). There was a significant difference between the mean plant height values of the first (58.26 cm) and second (59.80 cm) years of the study. This difference could be due to the annual change of climatic

Table 1. Effect of different harvest times on the plant heihgt, fresh herb yield and fresh leaf + flower yield of hyssop (*Hyssopus officinalis* L.)

Treatments	Plant height (cm)			Fresh herb yield (t ha^{-1})			Fresh leaf + flower yield (t ha^{-1})		
	2019	2020	mean	2019	2020	mean	2019	2020	mean
BF	51.37	52.77	52.07 b	18.29	19.59	18.94 d	5.74	6.14	5.94 d
BG	58.77	60.10	59.43 ab	20.32	21.30	20.81 c	6.67	7.65	7.16 c
FF	59.97	61.73	60.85 ab	23.40	24.75	24.08 b	8.28	9.07	8.68 b
AF	62.93	64.60	63.77 a	24.92	26.39	25.65 a	10.18	10.25	10.21 a
Mean	58.26 B	59.80 A	59.03	21.73	23.01	22.37	7.72	8.28	8.00
Year		**			ns			ns	
Harvesting		*			**			**	
Interaction		ns			ns			ns	

BF – before-flowering, BG – beginning of flowering, FF – full flowering, AF –after-flowering
* significant at a level of $p \leq 0.05$, ** significant at a level of $p \leq 0.01$, ns – non-significant, Means followed by the same lowercase letter in the columns do not differ statistically from each other by the Tukey's test, at 5% probability level.

Table 2. Effect of different harvest times on the dry leaf + flower yield, essential oil content and essential oil yield of hyssop (*Hyssopus officinalis* L.)

Treatments	Dry leaf + flower yield (t ha^{-1})			Essential oil content (%)			Essential oil yield (L ha^{-1})		
	2019	2020	mean	2019	2020	mean	2019	2020	mean
BF	1.52	2.56	1.63 c	0.61	0.61	0.61 c	9.22	10.61	9.92 d
BG	1.74	2.62	1.95 b	0.67	0.70	0.68 b	12.38	14.16	13.27 c
FF	1.87	2.65	2.59 a	0.93	0.93	0.93 a	23.64	24.39	24.01 a
AF	2.03	2.89	2.77 a	0.71	0.69	0.70 b	18.73	20.04	19.38 b
Mean	1.79	2.68	2.23	0.73	0.74	0.73	16.00 B	17.30 A	16.65
Year		ns			ns			*	
Harvesting		**			**			**	
Interaction		ns			ns			ns	

Explanations as in Table 1.

factors such as temperature. The plant height varied between 52.07 and 63.77 cm depending on the developmental phase of hyssop plant. The highest plant height was obtained from plants harvested after-flowering. However, there was no statistically significant difference between plant heights in the harvest at the beginning of flowering, full flowering and after-flowering periods (Tab, 1). In the observations conducted by Aghaei et al. [2019] the plant height of hyssop was between 35.00 and 71.33 cm. On the other hand, Roslon et al. [2002] revealed that hyssop plants reach a height of 70 cm in full blooming. Similarly, Yesil and Ozcan [2021] also reported that the plant height of *M. piperita* L. during the budding period was significantly lower than the plant heights determined in other harvest periods (first flowering, 50% flowering and 100% flowering).

Different harvest times had a significant effect on fresh herb yield. However, the effect of years and year × harvest time interaction on fresh herb yield were found to be not statistically significant (Tab. 1). The fresh herb yield was higher in the latest harvest period and the highest fresh herb yields were recorded from after flowering stage (25.65 t ha^{-1}), the lowest fresh herb yields were obtained from before flowering stage (18.94 t ha^{-1}) – Table 1. As the harvest time was delayed, the increase in vegetative cover of the plants resulted in an increase in the yield of fresh herb. Since the plants harvested in the earliest period contain less stems, less fresh herb yield was obtained in this period. Some studies report that the developmental stage of harvested plants significantly affects yield. For example, Ozyazici and Kevseroglu [2019] reported that ontogenetic variability has an effect on fresh herb yield of *Mentha spicata, Origanum onites* and *Melisa officinalis*. Also, Badi et al. [2004] reported that the highest fresh herb yield in *Thymus vulgaris* L. obtained from the harvest at the beginning of flowering period. On the other hand, Nurzyńska-Wierdak [2009] recorded the highest fresh herb yields of *Origanum vulgare* L. at full blooming phase of plants.

Statistically significant differences ($p \leq 0.01$) were observed in the fresh and dry leaf + flower yields of hyssop in different harvest period (Tabs 1 and 2). Fresh leaf + flower yields increased from before-flowering towards after-flowering stage. Whereas, dry leaf + flower yields significantly increased from before-flowering towards full-flowering stage. The highest fresh and dry leaf + flower yields were obtained from after-flowering stage (10.21 and 2.77 t ha^{-1}, respectively). However, there was no statistically significant difference between the dry leaf + flower yields obtained in full flowering and after-flowering periods. On the other hand, the lowest fresh and dry leaf + flower yields were recorded from before-flowering period (5.94 and 1.63 t ha^{-1}, respectively) – Tables 1 and 2. Katar et al. [2021] reported that they obtained 12.22 t ha^{-1} fresh leaf + flower yield from a 3-year-old

hyssop plant harvested during full flowering. The difference of these results may be due to the difference of the plant material used in the studies (different genotypes and ages) and the changing ecological conditions of study areas such as temperature, light intensity etc. In parallel with this study, Yesil and Ozcan [2021] reported that fresh leaf yield increased significantly from before-flowering period towards full flowering period in *M. piperita* L. and they obtained the highest fresh leaf yield during the full flowering period.

The essential oil content of hyssop was significantly affected by the changing harvest periods. The essential oil content significantly increased from before flowering towards full flowering phase. The maximum and minimum essential oil content was determined from full flowering stage (0.93%) and before-flowering stage (0.61%) – Table 2. In the hyssop plant, the essential oil content, which reached the maximum level at the full flowering period, decreased significantly with further delay of harvest. In parallel with these findings, it were mentioned that the essential oil content increased until full flowering stage and then began to decline [Németh 2005]. In addition, in the studies conducted by Jankovsky and Landa [2002], Rey et al. [2004] and Katar et al. [2021], the essential oil contents were found to be 0.10–1.80%, 0.80–1.30% and 0.17–0.96%, respectively.

When hyssop plant was harvested at different times, hyssop essential oil yield increased significantly until the full flowering period and decreased again after this stage. In addition, there was a significant difference between the experimental years in terms of essential oil yield. It was found that the mean essential oil yield (17.30 L ha^{-1}) was higher in 2020 compared to 2019 (16.00 L ha^{-1}). Considering that the essential oil yield depends on the essential oil content and the dry leaf yield, the higher of essential oil yield in 2020 can be explained by obtaining the higher of dry leaf yield and essential oil content in this year. The highest essential oil yield (24.01 L ha^{-1}) was determined at the full flowering phase which the highest essential oil content was observed. The minimum value of essential oil yield was found in the before-flowering stage (9.92 L ha^{-1}), similar to the other examined parameters (Tab. 2). These findings are in agreement with those obtained by Karaca Oner and Sonkaya [2020] and Yesil and Ozcan [2021], who founded that essential oil yield was the highest in full flowering stage of *O. onites* L. and *M. piperita* L. On the contrary, Ozyazici and Kevseroglu [2019] reported that the highest essential oil yield was determined at the 50% flowering period in *M. spicata* and *Lavandula angustifolia.* It was reported that the content and yield of essential oil in medicinal and aromatic plants vary depending on origin of plants, plants age, location of cultivation, harvest times, fertilization, drying, storage and distillation procedure [Sourestani et al. 2014, Kotyuk 2015, Can et al. 2021].

There were positive correlation between fresh herb yield and other parameters examined and fresh herb yield showed the strongest positive corelation with dry leaf + flower yield. Moreover, the essential oil content was only positively correlated with dry leaf + flower and essential oil yield at $p \leq 0.01$ level. On the other hand, no significant correlation was found between essential oil content and plant height, essential oil content and fresh leaf + flower yield (Tab. 3).

Thirty nine compounds were identified in the essential oil of hyssop depending on the different developmental phases in this study. The pinocamphone,

Table 3. Coefficient of correlation between the examined parameters of *Hyssopus officinalis* L. in relation to different harvest times

Specification	Plant height	Fresh herb yield	Fresh leaf + flower yield	Dry leaf + flower yield	Essential oil content
Fresh herb yield	0.516**	–	–	–	–
Fresh leaf + flower yield	0.574**	0.955**	–	–	–
Dry leaf + flower yield	0.566**	0.979**	0.954**	–	–
Essential oil content	0.367ns	0.472*	0.368ns	0.517**	–
Essential oil yield	0.522**	0.841**	0.767**	0.881**	0.857**

* significant correlation at a level of $p \leq 0.05$, ** significant correlation at a level of $p \leq 0.01$, ns – not significant.

Table 4. The effect of different harvest times on chemical composition of hyssop (*Hyssopus officinalis* L.) essential oil (the mean of 2019 and 2020)

R. time	R. index	Compounds (%)	BF	BG	FF	AF
8.77	1030	α-pinene	0.81	0.81	0.81	0.81
9.65	1070	camphene	0.14	0.14	0.14	0.14
10.71	1113	β-pinene	7.92	7.92	8.94	8.72
11.02	1123	sabinene	1.54	1.54	1.53	1.54
12.14	1159	β-myrcene	0.88	0.88	0.87	0.88
13.47	1201	limonene	0.73	0.73	0.72	0.73
13.86	1212	β-phellandrene	0.82	0.83	0.81	0.82
14.59	1231	*cis*-ocimene	0.58	0.58	0.57	0.58
15.25	1248	*trans*-β-ocimene	1.50	1.51	1.48	1.50
20.48	1380	myrtenyl methyl ether	0.60	0.58	0.59	0.59
23.08	1444	α-thujone	0.44	0.44	0.43	0.44
26.23	1521	pinocamphone	41.85	40.97	38.41	40.88
26.58	1530	α-gurjunene	0.19	0.18	0.20	0.19
26.99	1541	linalool	0.37	0.37	0.37	0.37
27.35	1550	isopinocamphone	22.91	22.99	22.73	22.92
28.16	1570	pinocarvone	0.82	0.82	0.81	0.82
28.60	1581	nopinone	0.19	0.19	0.19	0.19
29.25	1598	caryophyllene	0.96	0.93	1.03	0.96
30.47	1629	myrtenal	0.66	0.66	0.66	0.66
31.08	1646	(+)-aromadendrene	0.79	0.76	0.85	0.78
31.32	1652	pinocarveol	0.41	0.42	0.42	0.41
31.89	1667	estragol	0.24	0.24	0.25	0.24s
32.01	1670	α-humulene	0.10	0.07	0.15	0.09
32.60	1686	myrtenyl acetate	2.60	2.61	2.55	2.60
32.83	1692	α-terpineol	0.54	0.61	0.39	0.55
33.47	1709	germacrene D	1.41	2.36	3.72	1.90
34.35	1732	bicyclogermacrene	1.01	0.97	1.06	1.00
35.25	1756	α-amorphene	0.14	0.10	0.20	0.13
36.34	1785	myrtenol	2.56	2.56	2.56	2.56
44.32	1984	caryophyllene oxide	0.63	0.61	0.65	0.62
44.99	2003	methyl eugenol	0.35	0.35	0.34	0.35
45.68	2027	ledol	0.29	0.28	0.30	0.29
47.03	2075	elemol	1.47	1.38	1.52	1.46
47.35	2086	methyl p-anisate	0.18	0.17	0.19	0.18
48.19	2119	spathulenol	1.22	1.17	1.25	1.21
49.30	2166	τ-cadinol	0.72	0.69	0.77	0.72
50.47	2216	α-eudesmol	0.10	0.10	0.10	0.10
50.51	2220	isospathulenol	0.35	0.33	0.37	0.34
50.66	2227	β-eudesmol	0.31	0.28	0.33	0.30
–	–	total	99.31	99.11	99.27	99.57

BF – before-flowering, BG – beginning of flowering, FF – full flowering, AF – after-flowering

isopinocamphone and β-pinene are the main components found in essential oil (Tab. 4). These three main components accounted for 72.68% of the essential oil at before-flowering, 71.88% at beginning of flowering, 70.08% at full flowering and 72.52% at after-flowering stage. The pinocamphone content was between 38.41–41.85%, isopinocamphone content was between 22.73–22.99% and β-pinene content was between 7.92–8.94%. While the highest pinocamphone content (41.85%) was determined in plants harvested in before flowering period, the lowest value (38.41%) was found in full flowering stage.

The pinocamphone content of essential oil decreased with the delay of harvest from before-flowering stage to full flowering period and reverse trend was showed in later harvest. As a second component, the isopinocamphone content was almost the same in all harvest periods. The isopinocamphone content was highest at beginning of floering stage (22.99%). While, the lowest isopinocamphone content (22.73%) was determined in before-flowering period, similar to the pinocamphone content. The β-pinene reached its highest value (8.94%) at full flowering stage in which the lowest value of pinocamphone was obtained (Tab. 4).

In some studies, the main components of hyssop essential oil were determined as isopinocamphone (38.47–40.25%), pinocamphone (13.32–14.92%), and n-decane (8.63–8.67%) [Moghtader 2014], *cis*-Pinocamphone (25.67–45.32%), *trans*-Pinocamphone (15.31–33.08%), and β-pinene (6.09–11.44%) [Tavakoli and Aghajani 2016], isopinocamphone (38.8–43.8%), pinocamphone (18.3–22.3%), and β-pinene (6.3–12.0)% [Acimovic et al. 2021]. Dudai [2005] reported that environmental conditions such as light, temperature, day length, water status and fertilizers influence compositions of essential oils. In addition, it were reported that the composition of the essential oil in medicinal and aromatic plants varies depending on different harvest times [Pfefferkorn et al. 2008, Kotyuk 2015, Can et al. 2021].

CONCLUSION

Hyssopus officinalis L. is not a widely grown plant in Turkey. However, it is an important aromatic plant with high agricultural potential. We found that it has a good industrial performance even in field with continental climate condition in this experiment. Furthermore, the results of this study revealed that the yield of hyssop plant was significantly affected by different harvest periods. The pinocamphone, isopinocamphone and β-pinene, the main components of hyssop essential oil, varied according to developmental stages on the harvest date. The dry leaf + flower yield, essential oil content and essential oil yield obtained during the full flowering period were higher than the other harvest periods. While, pinocamphone content was highest at before-flowering stage. As a result, harvesting in the full flowering period was the best treatment in respect of dry leaf + flower yield, oil content and oil yield per unit area.

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