Changes of Essential Oil, Photosynthetic Pigments, and Morphological Characteristics of Hyssop (*Hyssopus officinalis* L.) at Different Harvesting Time

Yousefzadeh S (Ph.D.)[1*], Naghdi Badi H (Ph.D.)[2]

1- Department of Agriculture, Payame Noor University, Tehran, Iran
2- Medicinal Plants Research Centre, Institute of Medicinal Plants, ACECR, Karaj, Iran
* Corresponding author: Department of Agriculture, Payame Noor University of Marand, East Azerbaijan, Iran
Tel: +98-41-42231557 (254)
Email: s_yousefzadeh@pnu.ac.ir

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Abstract

Background: Hyssop (*Hyssopus officinalis* L.) is herbaceous perennial plants of Lamiaceae family grown in Europe, the Middle East, Asia, and Northern Africa.

Objective: In this research, the effect of harvesting time was studied on essential oil, photosynthetic pigment, and some morphological characteristics of hyssop.

Methods: A Field experiment was carried out during 2015/2016 at the Research farm of Payam Noor University of Marand, Iran. The experimental design was laid out as randomized complete block design with three replicates. The three harvesting time (pre-flowering, beginning of flowering and full flowering stages) were arranged in experimental plots.

Results: Results indicated that the percentage of essential oil varied for 0.459 to 0.618 in different stage of plant growth. Hyssop essential oil yields increased with time and the highest value of measured traits included plant high, stem diameter, number of secondary and flowering branches, dry weight, chlorophyll (a, b and total), carotenoid, total anthocyanins and total flavonoids was obtained for collected plants in full flowering stage.

Conclusion: Overall, the harvesting in full flowering stage was the best time in respect of essential oils content and morphological characteristics in hyssop.

Keywords: ***Hyssopus officinalis*, Essential oil, Harvesting stage, Phytochemical traits**

Introduction

Hyssop (*Hyssopus officinalis* L.), which is known as ‘‘Zufa’’ in Persian, is herbaceous perennial plants belong to Lamiaceae family that widely grown as essential oil crop in Europe, the Middle East, Asia, and Northern Africa [1-2]. Hyssop has small blue flowers borne on the upper part of the branches with up to 60 cm tall [3]. The essential oil of hyssop is mainly used in perfumery, cosmetics, beverages, foods, and flavoring industries [2] and extraction from the aerial parts are used for cough relief, antispasmodic, simulative, carminative, expectorant, as well as to treat chronic bronchitis and certain skin diseases [4-5]. Extracts of the plant are also used for their antimicrobial, antioxidant properties and exhibit strong antiviral activity against HIV [6, 7, and 8]. According to Kohlmünzer (2007) in hyssop percentage of essential oil was 1% [9] while zawislak (2011), reported that essential oil of hyssop ranged from 0.67 to 1.65% in vegetative and full blooming stages, respectively [10]. Other researchers reported that the amount of essential oil in the vegetative body of *Hyssopus officinalis* L. is different and is between 0.3-1% and the most amount of essential oil in this plant is in the flowering branches [11]. Other study showed that hyssop oil content ranged from 0.13 to 0.26% [12].

The essential oil biosynthesis is mainly attributed to genetic factors, but other factors have important effect on production of essential oil such as climate and soil condition, harvesting time, development stage and plant age [13]. Harvesting time is one of the important agents to cause variation of essential oil content in medicinal and aromatic plants. In this regard, Nemeth et al. demonstrated that the essential oil contents in hyssop significantly changes during plant growing [14]. Ozguven and Tansi reported that the greatest essence content of Thymus vulgaris plants obtained at full flowering and post-flowering stages [15]. The similar results recorded Artemisia annua [16], and Thymus vulgaris [17]. Thus, selecting the best harvesting time in medicinal plant is necessary for obtain the maximum essential oil content and commercialization. Essential oil content is varied in different growth stages in medicinal plants and harvesting time had important function on essential oil content. Thus, selection of optimal harvest time is necessary for achieve maximum essential oil yield in medicinal plants. This study was done the first time in East Azerbaijan province about harvesting time on essential oil content and photosynthetic pigment. Also, due to different environment condition in East Azerbaijan province this study is attracted for many researchers. However, to the best of our knowledge, there is no sufficient information about harvesting time for hyssop in East Azerbaijan climate condition. Therefore, the objective of the study was to determine the harvesting time on essential oil, photosynthetic pigment and some morphological characteristics of hyssop.

Method and materials

Field experiment was carried out during 2015/2016 at the Research farm of Payam Noor University of Marand, Iran (38°22′ N, 45°46′ E and 1500 m above sea level). The

experimental design was laid out as randomized complete block design with three replicates. Three harvesting time (pre-flowering, beginning of flowering and full flowering stages) were arranged in experimental plots. The composite soil samples were collected one week before planting and some of its physicochemical properties were evaluated. Details of the properties of soil were shown in Table 1. Each plots had six rows with 3 m long that was spaced 0.50 m apart.

Seedlings of hyssop were planted by hand on 10 May 2015. Weeds were controlled by hand three times after the planting. The plants were harvested in pre-flowering, beginning flowering and full flowering stages, on 15 June, 27 July and 10 August 2015, respectively. At each stage data were recorded for plant and studied traits included: plant height (PH), stem diameter (SD), Number of secondary branches (NSB), number of flowering branches (NFB), essential oil content (EOC), dry weight (DW), essential oil yield (EOY), total anthocyanins (TA), chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), Carotenoid (C) and total flavonoids (TF). Photosynthetic pigments included chlorophyll (a, b and total) and carotenoid determined by Arnon [18]. 1 gm of fresh leaves was suspended in10 ml of 80% ethanol and homogenized thoroughly with the help of motor and pestle. The whole contents were transferred into a centrifuge tubes. The contents were allowed to stand over-night and centrifuged at 6000 RPM. The supernatant was collected and the absorbance was read at 480, 510, 645 and 663nm on the spectrophotometer. The chlorophyll and carotenoid content (mg/g fresh weight) were calculated by following equations:

Chlorophyll-a = 12.7 (A663) –2.60(A645) x V/1000 x w

Chlorophyll-b = 22.9 (A645) – 4.68(A663) x V/1000 x w

Carotenoid = 7.6 (A480) – 1.49 (A510) x V/1000 x w

Total chlorophyll = 20.2 (A645) + 8.02 (A663) x V/1000 x w

(Where, V is the volume of acetone and w is the weight of sample used). Total anthocyanins measure by Krizek et al [19]. Leaf samples were homogenized in a mortar and pestle with 3 ml 1% HCl-methanol solvent (1: 99, v: v). The homogenate was centrifuged at 18 000 g for 30 min at 4°C, and then the supernatant was filtered through Whatman #1 to remove particulate matter and was stored in darkness at 5°C for 24 h. The amount of anthocyanin was determined from the absorbance at 550 nm. Anthocyanin content was expressed as μmol/g FW and the concentration of anthocyanin was calculated using the extinction coefficient of anthocyanin

Table 1- Soil physical and chemical properties

Soil texture	Ec (dSm^{-1})	pH	Organic carbon (%)	N (%)	P (mg/kg)	K (mg/kg)
Sandy loam	1.09	7.66	1.2	0.06	47	605

ε = 33 000/mol2 cm. Flavonoids were estimated according to the method of Krizek et al. [19]. Leaf samples were homogenized in a mortar and pestle with 3 ml 1% acetic acid-ethanol solvent (1:99, v:v). The homogenate was centrifuged at 18 000 g for 30 min, and then the supernatant was incubated in a water bath for 10 min at 80°C and then allowed to cool to room temperature. The amount of flavonoids was determined from the absorbance at 270, 300 and 330 nm. Flavonoid content was expressed as μmol/g FW and the concentration of flavonoids was calculated using an extinction coefficient of flavonoids ε = 33 000/mol2 cm. Essential oils were extracted from dried aerial parts of the collected samples of each treatment by hydro distillation for 3h, using a Clevenger-type apparatus. Analysis of variance and mean comparisons were performed using the GLM procedure of SAS [20]. The least significant difference (LSD) method at a probability level of 0.05 was used to determine significant differences among means.

Results

According to analysis of variance (Table 2), harvesting time had significant effect on plant height, stem diameter, number of flowering branches, dry weight, essential oil yield ($P \leq 0.01$) and essential oil content ($P \leq 0.05$). The results of the ANOVA for the other traits (Table 3), showed that harvesting time had significant effect ($P \leq 0.05$) on chlorophyll (a, b and total), total anthocyanins, and total Flavonoids. Its effect was not significant on carotenoid amount (Table 3).

Morphological characteristics

Results showed that the highest and lowest plant height (55.88 cm) was observed in full flowering and pre-flowering stage (29.75 cm) (Table 4). In full flowering stage, plant height approximately was twice more than the pre-flowering stage. As shown in Table 4, the highest stem diameter, number of secondary branches, number of flowering branches and dry weight were observed in flowering stage (H3). In addition, the lowest amounts of mentioned traits were recorded at pre-flowering stage (H1). Concerning the effect of harvest time, the greatest value of morphological traits was gained in the full flowering stage with passage of time and it might be due to the increment of growth and development of plant.

Essential oil content and yield

Our findings showed that the percentage of essential oil ranged for 0.459 to 0.618 % (Table 4). The greatest and the lowest percentage of essential oil were obtained in full flowering and pre-flowering stages. According Table 4, the highest and lowest essential oil yield was obtained in full flowering stage and pre-flowering stage, respectively. Essential oil yield obtained from full flowering stage was five times higher than pre-flowering stage.

Chlorophyll and carotenoid

As shown in Table 5, the highest chlorophyll and carotenoid content was observed in full flowering stage (H3). Further, the lowest contents of chlorophyll and total carotenoid were obtained in pre-flowering

Table 2- Analysis of variance for the measured morphological traits, essential oil content and essential oil yield of hyssop (*Hyssopus ofisinalis* L.)

S.O.V	Df	PH	SD	NSB	NFB	DW	EOC	EOY
Block	**2**	18.89*	8.8	2.02	0.594	54631.59	0.0004	1.252
Harvesting time	**2**	540.82**	40.26**	3.36	45.32 **	5990631.07**	0.019*	289.93**
Error	**4**	2.28	1.74	1.22	0.861	77896.96	0.001	3.86
CV		3.67	12.1	10.54	25.99	11.58	7.22	14.5

SOV, Source of variation; DF, Degrees of freedom; CV, Coefficient of variation.

**, * and ns are significant at 1 and 5% probability level and non- significant, respectively. The traits are: plant height (PH), stem diameter (SD), Number of secondary branches (NSB), number of flowering branches (NFB), essential oil content (EOC), dry weight (DW), essential oil yield (EOY).

Table 3- Analysis of variance for the photosynthesis pigments of hyssop (*Hyssopus officinal's* L.)

S.O.V	Df	CA	CB	TC	C	TA	TF
Block	2	0.014	0.018	0.046	0.001	0.186	971.55
Harvesting time	2	0.2002*	0.066*	0.493*	0.001ns	1.416*	5958.08*
Error	4	0.0371	0.004	0.048	0.0005	0.216	665.92
CV		23.59	11.77	16.01	13.55	7.51	7.06

SOV, Source of variation; DF, Degrees of freedom; CV, Coefficient of variation.

**, * and ns are significant at 1 and 5% probability level and non- significant, respectively.The traits are: chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), Carotenoid (C), total anthocyanins (TA) and total flavonoids (TF).

Table 4-Mean comparison of the measured morphological traits and essential oil content of hyssop (*Hyssopus offisinalis* L.) for three harvesting stage

Harvesting stage	PH (cm)	SD (mm)	NSB	NFB	DW (Kg/ha)	EOC (%)	EOY (Kg/ha)
H1	**29.75c**	**7.69c**	**9.31b**	**0.00c**	**1249.2c**	**0.459c**	**5.784 c**
H2	**37.49b**	**10.14b**	**10.74a**	**3.0b**	**1994.3b**	**0.513 b**	**10.29 b**
H3	**55.88a**	**14.9a**	**11.38a**	**7.7a**	**3982.7a**	**0.618 a**	**24.61 a**

The traits are: plant height (PH), stem diameter (SD), number of secondary branches (NSB), number of flowering branches (NFB), dry weight (DW) and essential oil content (EOC). Harvest time: H1:pre-flowering, H2: beginning of flowering and H3: full flowering.

Table 5-Mean comparison of the photosynthesis pigments of hyssop (Hyssopus officinal's L.) for three levels of harvesting time

Harvesting	CA mg g^{-1} FW	CB mg g^{-1} FW	TC mg g^{-1} FW	C mg g^{-1} FW	TA mg g^{-1} FW	TF mmolg^{-1}FW
H1	0.581b	0.405c	0.98c	0.152b	5.61c	316.98c
H2	0.776b	0.560b	1.33b	0.185a	6.01b	373.46b
H3	1.093a	0.702a	1.79a	0.197a	6.95a	404.93a

Traits are: chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), Carotenoid (C), total anthocyanins (TA) and total flavonoids (TF). Harvest time: H1:pre-flowering, H2: beginning of flowering and H3: full flowering.

stage (H1). The chlorophyll and carotenoid content significantly increased in full flowering stage compare with other stages. In addition, chlorophyll content in full flowering stage was approximately two times higher than pre-flowering stage.

Total Anthocyanins and total Flavonoids

Results showed that the greatest amount of total anthocyanin (6.95 mg g^{-1} FW) was gained in full flowering stage (H3) and the lowest anthocyanin (5.61 mg g^{-1} FW) was produced in pre-flowering stage. The harvesting time had significant on flavonoids content and the highest and lowest amount of flavonoids was obtained in the full flowering stage (H3) and pre-flowering stage (H1).

Discussion

In full flowering phase, plant height increased compare with other stages. Zawislak (2011) reported that the maximum plant height of hyssop was gained at full flowering (48.8 cm) and full blooming phases (47.9 cm) compare to vegetative and beginning of flowering stages [10]. Also, Roslon et al. (2002) reported that plants height of *Hyssopus officinalis* L. was achieved about 70 cm at the full blooming stage [21]. In fact, the height of plant increased with passage of time due to the increase of plant growth. Result showed that the maximum morphological traits were observed in full flowering stage. Accordind Zawislak (2011) morphological traits in hyssop significantly was affected by harvesting time, and the largest plant diameter and number of main shoots was recorded in full blooming and after flowering phases, respectively [10]. As well as, they reported that fresh and dry weight of plant considerably were in flowering phase more than vegetative phase. These results are in agreement with results of Naghdi-Badi et al. (2004) on thyme [17].

The highest essential oil content and essential oil yield were obtained in full flowering stage. According to Valtcho et al. (2012) findings, essential oil content of hyssop was from 0.13 to 0.26% [22], while Khazaie et al. (2008) found that percentage of essential oil changed from 0.83 to 1.2% [23]. The variation of oil content may be attributed to genetic differences, geographic origin, ecological factors, and climatic conditions [24-25]. The essential oil content was significantly improved in full flowering stage (H3) compare to pre-flowering (H1) and beginning of flowering stages (H2). Our result showed that essential oil content changed during plant growing and reached to highest amount in full flowering stage. Zawislak (2011) demonstrated that maximum and minimum oil content of hyssop was found in full blooming (1.65%) and vegetative phases (0.67%), respectively [10]. According to Hamrouni Sellami et al. (2009) findings, essential oil percentage of *Origanum majorana* L. was from 0.04% in late vegetative stage and reached to 0.09% in full flowering stage [26]. These results are in agreement with most of previous studies that the highest essential oil yield were observed in full-flowering stage, such as Ozguven and Tansi on *Thymus vulgaris* [15], Rohloff et al. on peppermint [27], Sefidkon et al. on *Satureja rechingeri* [28] and Verdian-Rizi on *Laurus nobilis* [29].

These finding showed that production of essence is low during vegetative phase. Similar finding reported by Hamrouni Sellami et al. on *Origanum majorana* [26].The essential oil yield has improved over time due to enhancement of dry biomass accumulation and essential oil percent. Similar to our finding, earlier researchers such as Badi et al. [17] on *Thymus vulgaris* and Oliveira et al. [30] on *Hyptis suaveolens* reported that the greatest essential oil yield was gained during the flowering stage. Therefore, suitable harvesting time is very important to obtain maximum essential oil yield.

Chlorophyll and carotenoid content improved with passage of time. According to Zawislak (2011) findings, harvesting stages had considerable effect on chlorophyll and carotenoid content in hyssop and the maximum and minimum amount of these pigments was recorded in beginning and full flowering stages, respectively [10]. It seems that the carotenoid content increased over time, which has important role in photooxidative protection [31] and plant adaptation. Also, the anthocyanin and flavonoid content significantly increased with passage of time. It has been reported that anthocyanin can help to reduce damage caused by free radical activity like platelet aggregation and endothelium-dependent vasodilation of arteries [32-33]. It seems that same as other studies, pigment amount of anthocyanin was increased during the time. These results are in agreement with results of Bakowska et al. [34] and Kucharska et al. [35] who, studied influence of UV irradiation time on anthocyanin-copigment stability.

Flavonoids are a huge group of phenolic secondary plant metabolites, which display antioxidant and free radical scavenging activity [36-37]. These results are in agreement with Papageorgiou et al. (2008) finding who reported that flavonoids were predominant during the flowering stage of *Origanum majorana* L. [38]. Alizadeh (2011) repoted that the most amount of phenolic content and antioxidant activity in hyssop herbal was obtained in flowering stage [39]. Although, Plants of Lamiaceae family showed high antioxidant activity [40], but no consistent results were obtained in the evaluation of antioxidant activity [41].

Conclusion

In summary, our finding showed that the full flowering was the best stage for harvesting hyssop and the most magnitudes of the measured traits were obtained this stage particularly essential oil yield as the most important trait. This management method could be result in good economic benefits for producers of hyssop (*Hyssopus afficinalis* L.).

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