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Quantity and Quality of Hyssop (*Hyssopus officinalis* L.) Affected by Precision Harvesting

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ABSTRACT

The amount of active compounds of medicinal plants (e.g. essential oil) varies in different plant parts. Thus, it is important to harvest those parts containing the highest levels of active compounds. In the present study the effect of harvest height on quantity and quality of *Hyssopus officinalis* was investigated based on a randomized complete block design with three replications. Treatments included four harvest heights including 15, 25, 35, and 45 cm (in basipetal order) and the residual stalks. After measuring the essential oil, the component values analyzed by GC and GC/MS. Regarding essential oil content (% v/w), yield and dry matter of different heights, the 15 cm height had the highest amount of essential oil (1.02±0.01%) and the lowest yield (2.17±0.13 g/m^2) and dry matter (213.72±15.32 g/m^2). On the contrary, the 45 cm height had the lowest amount of essential oil (0.75±0.01%) and the highest yield (5.7±0.57 g/m^2) and dry matter (757.52±63.5 g/m^2). For all harvest heights, *cis*-pinocamphone (53.93-44.6%), β-pinene (15.33-12.5%) and *trans*-pinocamphone (12.2-8.17%) had the highest levels among the compounds of the essential oil. The findings obtained from present study revealed that, if the quality is the matter, the essential oil extracted from the top 15 cm had the highest quality and purity although it was less in quantity.

Introduction

Hyssop (*Hyssopus officinalis*) is a native plant to central and southern Europe, western Asia, and northern Africa. This perennial plant has leaves with the opposite arrangement and purple-blue flowers usually used in traditional medicines (Lawless, 2002). Its essential oil uses as a flavouring agent in most food products and has applications in perfume, soap, and cosmetics industries (Wesołowska et al., 2010). Hyssop is a treatment for reducing gastrointestinal disorders, treating laryngitis, or sedating wounds in medicine. In traditional medicines in most parts of the world, it uses for treating vertigo, inflammation, and spasms, and can be used as an antiseptic agent (Kizil et al., 2008; Jangi et al., 2019). Some parts of Hyssop can inhibit the activity of human immunodeficiency viruses (HIVs) (Gollapudi et al., 1995; Kreis et al., 1990) and additionally because of their cis-

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pinocamphone and β-pinene components have antibacterial, antifungal, and antioxidant properties (Kreis et al., 1990). The essential oil yield depends on several factors including parameters related to the harvesting. The harvest time and method affect the quality and quantity of essential oil (Zutic et al., 2004; Ebadi et al., 2017; Etehadpour and Tavassolian, 2019). In the traditional harvest, the plant cut from the ground level and then processed for extracting their active compounds. However, precise harvesting refers to a process where only the target segments of the plant harvest. This process can include harvesting special organs or cut from a specific height (not from the ground level). Since all aerial parts are not cut, this type of harvest for perennial medicinal plants allows for re-growth, reduces time that is needed for the next harvest, and thus increases harvest frequencies (Mighri et al., 2009; Fallah and Hosseini, 2020). At the same time, in precise harvesting, the plants may grow irregularly before the first harvest due to non-uniform seeds and genetic diversity. However, at the second harvest, where all plants have already cut from a similar height, this non-uniformity resolve, and it would be possible to apply different harvest treatments. For example, in Russia, by cutting bay laurel twice at a 40 cm height, 5 tons of dried leaves produced totally, which was twice as much as cutting the whole plant (at 8-10 cm) once a year (De Guzman et al, 1999). Since many of perennial medicinal plants, have several harvest times during the growing season due to the lack of harvest of total shoots, it is possible to increase the number of harvests by harvesting at higher heights (experimentally), as well as obtaining more valuable active compounds. Some researchers have shown the quality of essential oils in aromatic plants varies at different harvest heights (Hefendehl, 1970; Adzet et al., 1992; Hose et al., 1997). In perennial aromatic plants, harvest height is very important. In a study on Lemon balm (*Melissa officinalis*), by defining three harvesting heights, the upper two thirds and the whole plant showed that although the harvest of the upper third of the plant was only a quarter of the total weight, it had 53.8% more essential oil than the whole plant (Mrlianová et al., 2002). Studies showed the essential oil content in the stems is low compared to the leaves, and this is for the importance of harvesting from the right height (Schultze et al., 1993). Also, in a study by Saebi et al. (2020) the effect of harvest height on heavy metals accumulated at different heights of hyssop evaluated. Their results showed that by this method, heavy metals concentration at heights close to the upper parts of the plant will be significantly reduced (Saebi et al., 2020). The precision harvesting is a subset of precision agriculture that allows utilizing different plant areas based on the physical and chemical information. Studying the harvest height of medicinal plants can help to find the best cutting point as the first link in the process (Oztekin and Martinov, 2014) producing premium raw materials that can meet the requirements of the food and pharmaceutical industries for high-quality products. Accordingly, it will be possible to improve the quality and value of industrial products and allow for better use of active compounds available in medicinal plants. Studies on other medicinal plants suggested that the distributions of active compounds are not similar in different parts of the plants. Research on medicinal plants has shown the significance of upper branches as they hold more essential oils (Wolski et al., 2006). Considering the special significance of the quality of essential oils extracted from these plants, this study was planned to investigate the qualitative and quantitative parameters of essential oil at different heights of Hyssop.

Materials and Methods

Plant material

This research project conducted by planting hyssop in the Faculty of Agriculture, Tarbiat

Modares University, Tehran, Iran (35°70'E, 40°51'N and 1200m above sea level) (Govahi et al., 2015). The planting date was April 2018 and the harvest time was in the last days of June. For uniform growth, the seeds planted in the greenhouse (direct sowing) and irrigated evenly with a drip irrigation system (Figure 1). Each plot was 3 m wide and included six planting rows. Row spacing was 50 cm to allow proper distance between the blocks for water passage. The seeds purchased from the Pakan Bazr Co. (Isfahan, Iran) and precisely planted. Weed control was done by hand weeding. Soil characteristics of the field are given in Table 1.

Fig. 1. Examination plot for *Hyssopus officinalis*

Table1. Physico-chemical properties of soil collected from experiment field in 2018

Descriptive Statistics	pH	EC	Sand	Silt	Clay	Organic matter	N	P	K	Fe	Zn	Mn
Unit		(ds/m)			(%)					(mg/kg)		
Mean	7.4	1.6	63.5	16.5	20	0.9	0.5	44	120	6.7	0.8	2.6
SD	0.3	0.29	4.6	1.11	2.3	0.08	0.02	0.45	8.53	0.25	0.03	0.09

SD: Standard Deviation

The experiment conducted based on a randomized complete block design (RCBD) in three replications. Treatments included four harvest heights (15, 25, 35 and 45 cm in basipetal order) as well as three treatments related to residues (15, 25 and 35 cm remaining plant height). The plant harvested from the defined heights by hand. For simplicity, the heights in basipetal order were designed as T15, T25, T35 and T45 while the residues (lower heights) were marked as R15, R25 and R35 as shown in Figure 2.

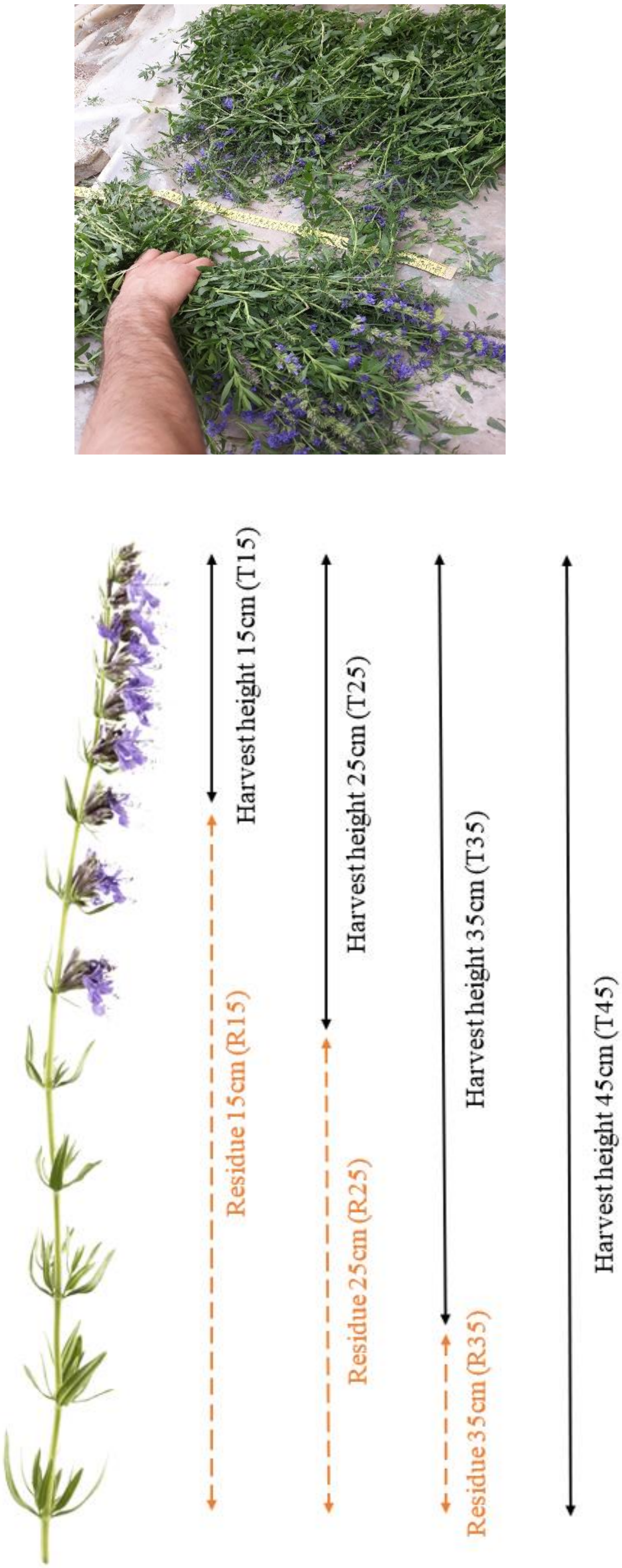

Fig. 2. Different heights of harvesting and residue in *Hyssopus officinalis*

Samples kept in shadow for a week at room temperature (20-25ºC) until they dried. The dried plants were transferred to the laboratory in paper bags and kept in a dry place at room temperature.

Essential oil extraction

For extraction of essential oil, 50 g of each sample weighed precisely mix with 600 mL of distilled water in a 1 L round-bottom flask. The essential oil of the samples extracted in a Clevenger apparatus for three hrs. The collected essential oil held in special glasses. Sodium sulphate (0.5 g) was added to each sample for dehydration (European Pharmacopoeia, 2004). To ensure better storage conditions, the samples were refrigerated at 4ºC.

Gas chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC/MS)

The essential oils were analyzed by a GC (Agilent 7890B) equipped with a flame ionization detector, and an HP-5 capillary Column (length 30 m, internal diameter 0.25 mm and 0.25 μm film thickness). Temperature program includes 2 min at 60 ˚C and increased to 250 ˚C with a ramp of 5 ˚C/min. GC–mass spectrometry (GC–MS) analysis performed by a Thermoquest–Finnigan gas chromatograph equipped with a fused silica capillary HP-5 column (60 m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with a trace mass spectrometer. Helium gas was used as the carrier at a flow rate of 1.1 mL/min in a split ratio of 1:100. Ionization voltage was 70 eV. Ion source and interface temperatures were 200 and 250 ˚C, respectively. Mass range adjusted from 45 to 456 amu. The same GC oven temperature program was used. Identification of essential oil components confirmed by comparison of each individual component's mass spectra with those of internal mass spectra library of the main library, Wiley 7.0 and Adams, while further identification based on comparison of peak retention indices by using a homologous series of n-alkanes (C8 to C24), verified under the same operating situations and data published in the literature (Adams, 2007). The quantification carried out based on the relative peak area percentage of individual components based on the GC-FID chromatograms (Adams, 2007; Wiley, 1998).

Data analysis

All the data analyzed by using SPSS (version 22). The least significant difference (LSD) procedure at a probability of 5% used to find out statistically significant differences among treatment means. All figures and diagrams was drawn using Excel software (version 2016).

Results

Biomass

As expected, the biomass yield of Hyssop increased with harvest height. The highest amount belonged to the 45 cm height (T45) with 757.52±63.5 g/m^2 (Table 2).

Essential oil content and yield

The essential oil of Hyssop was highly correlated with harvest height (Table 2). The highest amount of essential oil was obtained in T15 (1.02±0.01%) treatment. On the other hand, the lowest amount of essential oil was observed at T45 (0.75±0.01%). These results also showed distribute active compounds at different heights, the significance of harvesting upper branches, and the importance of selecting the best harvest height. This signifies the importance of harvesting the upper branches in increasing the amount of essential oil against decreasing the amount of harvested biomass yield. Regarding the essential oil in unharvested parts, the parts with more leaves and closer to the upper branches had more essential oil. The highest amount of essential oil was belonged to the residues from the 15 cm height (R15) (0.62±0.02%), and its lowest amount was obtained in the R35 (0.42±0.02%) treatment.

On other words, it may, by knowing the potential of different parts of medicinal plants, the harvest height can be optimized to minimize wastes and obtain high-quality essential oils through precision harvesting. There was no residue for the harvests made at T45.

Table2. Effects of harvesting height on dry matter, essential oil content and yield of Hyssop

Harvest height (cm)	Dry matter (g/m^2)	Essential oil content (%)	Essential oil yield (g/m^2)
T15	213.72±15.32[c]	1.02±0.01[a]	2.17±0.13[b]
T25	465.48±145.87[bc]	0.93±0.02[b]	4.38±1.44[ab]
T35	607.53±61.74[ab]	0.85±0.02[c]	5.16±0.63[a]
T45	757.52±63.5[a]	0.75±0.01[d]	5.7±0.57[a]
LSD	260.66	0.04	2.43
R15	531.62±37.09[a]	0.62±0.02[a]	3.27±0.2[a]
R25	345.49±57.21[b]	0.53±0.01[b]	1.83±0.32[b]
R35	186.98±17.57[c]	0.42±0.02[c]	0.78±0.05[c]
LSD	142.76	0.08	0.93

The heights in basipetal order were designed as T15, T25, T35 and T45 while the residues were marked as R15, R25 and R35. LSD, least significant difference

The essential oil yield of Hyssop was significantly affected by the harvesting height (Table 2). The maximum and minimum yield of essential oil was obtained at T45 and T15 (5.7±0.58 g/m^2 and 2.17±0.13 g/m^2, respectively). Accordingly, for residues matching to the studied heights, it was shown that the yield of essential oil was decreased with plant mass, where the residues related to the R35 height was the lowest (0.78±0.05 g/m^2). As the plant mass increased, the residue associated with the R15 height reached to 3.27±0.2 g/m^2 as the highest amount. Increase in the harvest height and yield decreased the yield of residues from unharvested parts, which was predictable as the plant mass in the remaining residues was decreased.

Essential oil composition

The components of Hyssop essential oil are given in Table 3 and the related chromatograms are shown in Figure 3. Seventeen compounds were identified in the essential oils of Hyssop. Data from harvesting height treatments showed that cis-pinocamphone (53.93±1.19%) increased in basipetal order. Its highest amount was obtained in T45, indicating that these compounds are higher at lower heights and with higher plant masses. However, β-pinene (15.33±0.38%) was higher at higher heights of the plant (i.e. T15). This shows an inverse relationship between distance from the ground and the increase in β-pinene levels at upper branches. The other compounds (trans-pinocamphone) were also higher at upper branches away from the ground level. The highest amount of this component was observed at T15 (12.2±0.26%).

For all the compounds shown in Table 3, similar relations by governing different harvest heights were also true for the residues from unharvested parts at the same height. In other words, this uniformity among the different components of the essential oil can be used as a criterion for determining the proper harvest height.

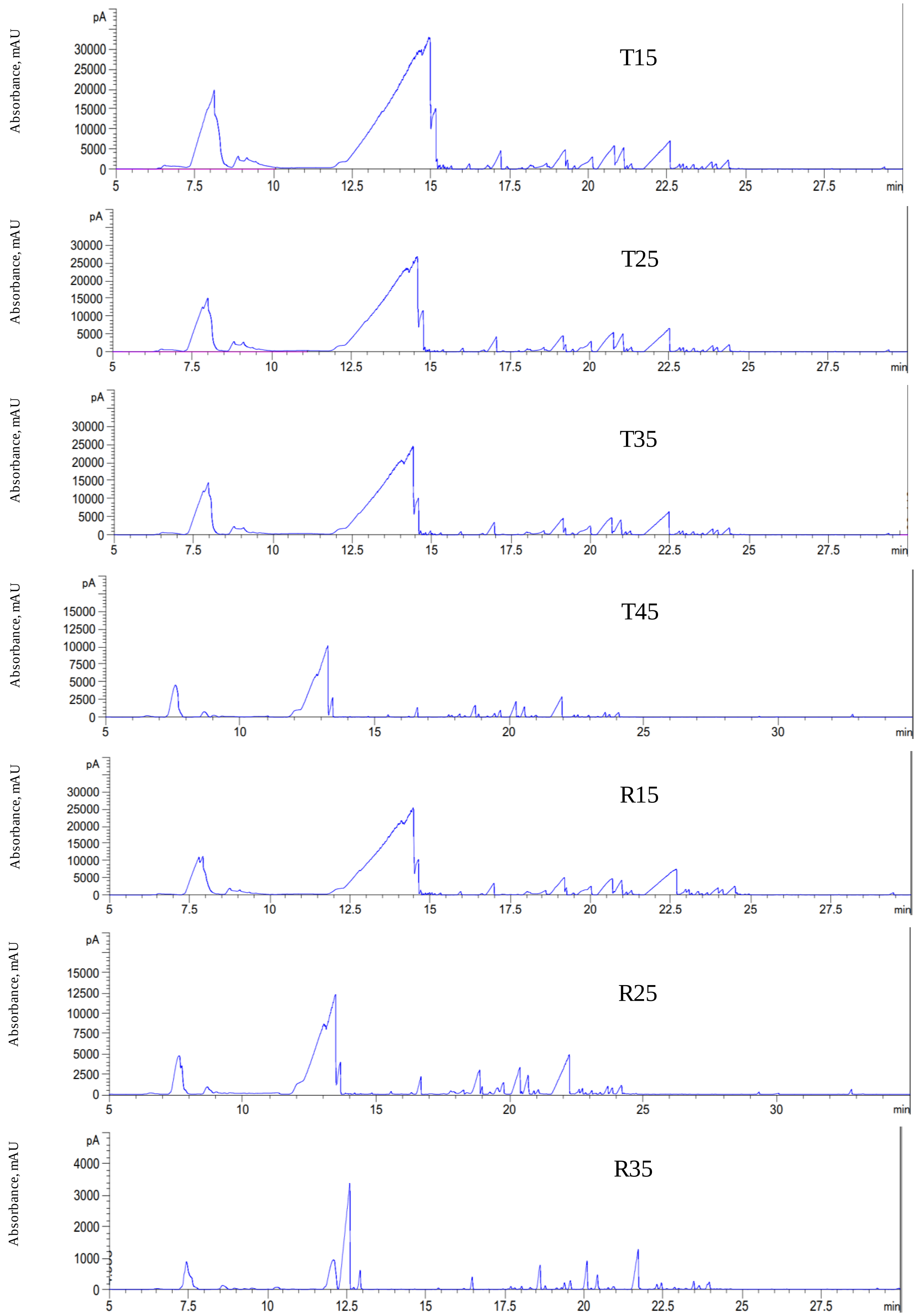

Fig. 3. Chromatogram of *Hyssopus officinalis* oil as affected by harvest height. The heights in basipetal order were designed as T15, T25, T35 and T45 while the residues were marked as R15, R25 and R35.

Table3. Effect of harvest height on *Hyssopus officinalis* essential oil constituents[a] (%)

NO.	Compound[b]	RI[c]	T15(cm)	T25(cm)	T35(cm)	T45(cm)	R15(cm)	R25(cm)	R35(cm)
1	sabinene	977	1.17±0.29	0.77±0.03	0.3±0.11	0.27±0.09	0.67±0.03	0.67±0.03	0.13±0.03
2	β-pinene	982	15.33±0.38	14.7±0.25	14.4±0.53	12.6±0.65	13.8 ±0.3	13.17±0.29	12.5±0.21
3	sylvestrene	1032	1.73±0.03	1.83±0.03	2.6±0.15	3.03±0.35	2.7±0.05	2.8 ±0.63	2.87±0.24
4	*cis*-sabinene hydrate	1070	0.33±0.03	1.13±0.07	2.53±0.12	2.67±0.31	1.47±0.14	1.77±0.14	1.9±0.26
5	linalool	1089	0	0	0	0.53±0.03	0.3±0.05	0.63±0.14	0.67±0.06
6	*trans*-pinocamphone	1167	12.2 ±0.26	11.27±0.18	10.43±0.33	8.63±0.27	9.37±0.09	9.3±0.65	8.17±0.32
7	*Cis* -pinocamphone	1175	44.6±0.3	46.03±0.93	48.7±0.58	53.93±1.19	46.8 ±0.7	48.23±1.53	48.83±1.49
8	myrtenol	1196	1.03±0.03	1.13±0.03	1.3±0.17	1.4±0.3	2.6±0.35	2.7±0.65	2.97±0.35
9	*trans*-2-hydroxy-pinocamphone	1250	0.5±0.29	0.57±0.12	0.7±0.23	0.87±0.03	0.5±0.25	0.57±0.12	0.67±0.18
10	methyl myrtenate	1294	2.17±0.33	1.13±0.12	0.83±0.12	0.43±0.14	0.63±0.09	0.57±0.23	0.27±0.03
11	β-bourbonene	1388	1.37±0.12	1.17±0.12	1±0.06	0.63±0.06	0.53±0.03	0.3±0.06	0.3±0.06
12	caryophyllene	1429	0.57±0.09	0.83±0.03	0.93±0.14	1.47±0.12	1.43±0.09	2.57±0.03	3.13±0.13
13	allo-aromadendrene	1473	1.53±0.09	1.87±0.07	2.2 ±0.09	2.33±0.18	0.63±0.07	1.33±0.58	1.57±0.08
14	germacrene	1495	5.9±0.35	5.53±0.03	3.9±0.06	2.93±0.12	4.23±0.09	3.97±0.46	3.6±0.11
15	elemol	1560	4.2 ±0.46	3.63±0.09	3.4±0.1	3.3±0.23	3.97±0.14	3.67±0.42	3.6±0.11
16	spathulenol	1589	4.63±0.55	4.37±0.18	4.17±0.12	2.87±0.07	4.23±0.14	3.83±0.54	3.03±0.09
17	caryophyllene oxide	1594	0.27±0.07	0.33±0.03	0.87±0.09	1.17±0.09	0.53±0.14	0.83±0.2	2.07±0.09
	Total		97.5±0.23	96.33±0.68	98.2 ±1.3	99.03±0.3	94.37±0.48	95.9±0.43	95.9±0.38

[a] percentage obtained by FID peak-area normalization

[b] results are compound percentages in essential oil expressed as means ± standard errors (n=3)

[c] Linear retention indices (DB-5 column) using normal n-alkanes series (C6-C24)

The heights in basipetal order were designed as T15, T25, T35 and T45 while the residues were marked as R15, R25 and R35.

The amount of main chemical groups in different harvest treatments was within the 94.37-99.03% range. This can be also noticed in monoterpene hydrocarbons with 15.5-18.23%, oxygenated monoterpenes with 60.83-68.73%, sesquiterpene hydrocarbons with 6.8-7.33%, and oxygenated sesquiterpenes with 7.33-9.1%. The variations and the trend in each group, as well as the four main chemical groups, are given in Figure 4. However, β-pinene (12.5-15.33%) and germacrene (2.93-5.9%) were the dominant hydrocarbons. Other compounds were below 5% in different treatments.

Considering the results of the present study, it can be suggested that the main components of Hyssop essential oil are the group of oxygenated monoterpenes (cis-pinocamphone, trans-pinocamphone) and that harvesting at the 15 cm (T15) in basipetal order led to higher quality of the essential oil in Hyssop.

Correlation Analysis

Correlations between the properties and components of the essential oil (Tables 4 and 5) at different harvest heights and residues of unharvested parts were analyzed. Different harvest height levels (Table 4) indicated a significant correlation between the essential oil content and its yield (-0.577*). Besides, a highly significant correlation (-0.862**) was found between the essential oil content and the total hydrocarbons. The essential oil content had a highly significant correlation with the three most frequent components (-0.874**, 0.771** and 0.947** for cis-pinocamphone, ß-pinene and trans-pinocamphone, respectively). Regarding essential oil yield, there was a significant correlation only between cis-pinocamphone (0.695*) and trans-pinocamphone (-0.663*). Cis-pinocamphone (0.876**) and trans-pinocamphone (0.904**) showed a highly significant correlation at total hydrocarbons whereas ß-pinene was -0.651*.

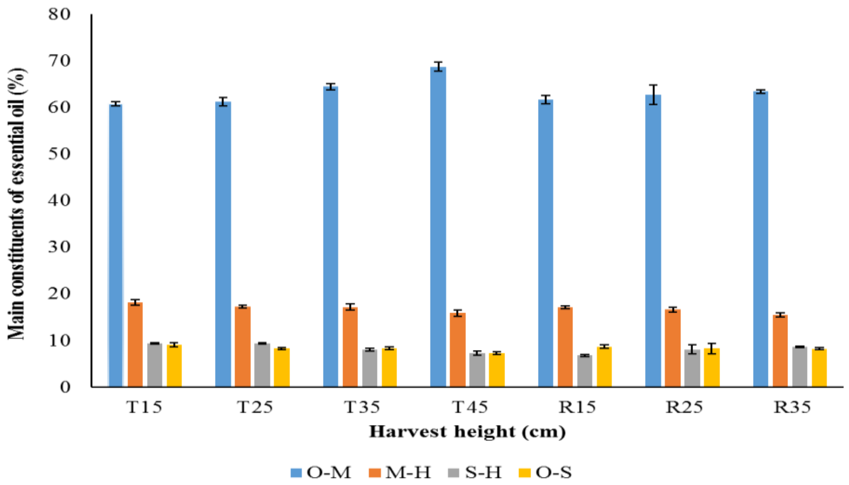

Fig. 4. Average values for the percentages of all groups of chemical constituents of *Hyssopus officinalis* oil as affected by harvest height. (O-M) Oxygenated monoterpenes, (M-H) Monoterpene hydrocarbons, (S-H) Sesquiterpene hydrocarbons and (O-S) Oxygenated sesquiterpenes. The heights in basipetal order were designed as T15, T25, T35 and T45 while the residues were marked as R15, R25 and R35. The bars indicate the standard errors of the means

Table 4. Correlation coefficients between the amount of essential oil and its main constituents in the harvested portion of the Hyssop plant.

Characteristic	Essential oil (%)	Essential oil yield (g/m²)	Total hydrocarbon compounds	Total oxygenated compounds	*cis*-pinocamphone	ß-pinene	*trans*-pinocamphone
Essential oil (%)	1						
Essential oil yield(g/m²)	-0.577*	1					
Total hydrocarbons compounds	-0.862**	0.534	1				
Total oxygenated compounds	0.171	0.251	-0.379	1			
cis-pinocamphone	-0.874**	0.695*	0.876**	-0.162	1		
ß-pinene	0.771**	-0.430	-0.651*	0.427	-0.659*	1	
trans-pinocamphone	0.947**	-0.663*	-0.904**	0.195	-0.952**	-0.662*	1

*P < 0.05, **P < 0.01

Table 5. Correlation coefficients relating percentage of essential oil and its main constituents in the residual portion of the Hyssop plant

Characteristic	Essential oil (%)	Essential oil yield (g/m²)	Total hydrocarbon compounds	Total oxygenated compounds	*cis*-pinocamphone	ß-pinene	*trans*-pinocamphone
Essential oil (%)	1						
Essential oil yield(g/m²)	0.917**	1					
Total hydrocarbons compounds	0.407	0.345	1				
Total oxygenated compounds	-0.202	-0.202	-0.872**	1			
cis-pinocamphone	-0.333	-0.178	-0.226	0.236	1		
ß-pinene	-0.881**	0.832**	0.085	-0.074	-0.320	1	
trans-pinocamphone	0.629	0.562	0.072	0.055	-0.258	0.543	1

*P < 0.05, **P < 0.01

According to the results, shown in Table 4, none of the Hyssop essential oil compounds had a significant correlation with oxygenated compounds. Among the three compounds, the correlation between cis-pinopamphone and trans-pinocamphone had the highest significant level with -0.952**. On the other hand, the correlation between cis-pinocamphone and ß-pinene was -0.659*, and -0.662*. The residues from unharvested parts at 25, 25 and 35 cm heights (Table 5) showed a very significant correlation (0.917**) between essential oil content (%) and yield. However, ß-pinene was the only compound that had a high correlation with essential oil content (-0.881**) and yield (0.832**). There was a highly significant correlation (-0.872**) between total hydrocarbons and total oxygenated compounds for residues from different height treatments.

Discussion

Analysis of essential oil content and yield

In the present study, increase in dry matter was due to the natural increase in plant mass (Misra and Sharma, 1991). To obtain the highest amount of essential oil, the spring harvest was selected due to its best environmental conditions such as light, temperature, and nutrient condition (Govahi et al., 2015). For essential oil content and yield, results was indicative of an inverse relationship between the amount of biomass and essential oil content, which is similar to the results reported by Misra and Sharma (1991). It was also revealed that the essential oil yield had a direct relationship with the amount of biomass. This also agreed with the results obtained by Misra and Sharma (1991). Considering the essential oil yield results in Figure 5, the effect of harvest height on essential oil yield was significant as it ranged from 2.17 ± 0.13 g/m^2 for T15 to 5.7 ± 0.57 g/m^2 for T45 (i.e. a 260% increase). This result supported the possibility of the existence of a direct relationship between biomass and essential oil yield. The analysis of the effect of harvest height on essential oil content and prediction about other heights showed the highest amount of essential oil in T15 with 1.02 ± 0.01% and its lowest amount was in T45 with 0.75 ± 0.01%. The response of essential oil content to harvest height followed a declining linear equation ($*p \leq 0.05$). This indicates a uniform reduction in essential oil content when moving down from the upper branches, which can help in planning and precisely determination of the optimum harvest height (Fig. 5). The response of the essential oil yield's response to the increase in harvest height followed a quadratic equation that showed the highest yield in T45. This suggests that, despite the increase in essential oil yield at higher harvest heights, the increase in essential oil content compensated the loss of yield because of the positive effect of the harvesting height on the quantity of essential oil in the upper branches.

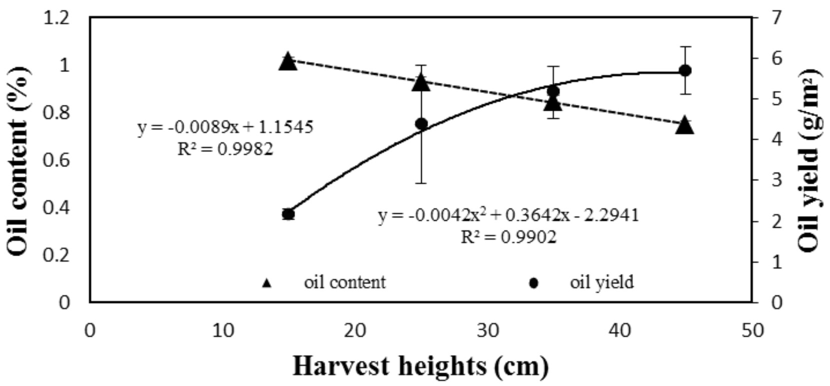

Fig. 5. Essential oil content and yield as function of harvest heights in the Hyssop plant. The error bars represent standard error

In point to point comparison of different harvest heights, the essential oil yield showed a 62% increase with the height from T15 cm to T45 cm; whereas its quantity reduced by 36%. Yield was increased from T15 to T35 by 58% and its amount decreased by 20%. A 50% increase in yield and a 10% reduction in the quantity observed when harvest height increased from T15 to T25. The interesting point was the small variation at higher heights against sharp changes at lower plant heights. Finally, it can be concluded that, at lower heights, a substantial amount of biomass yield consisted of lower levels of substances with nutritional/medicinal value, making their harvest unjustifiable due to increased processing requirements.

Essential oil composition and groups of chemical constituents

As prior studies demonstrated, the most important compositions for Hyssop plant are cis-pinocamphone, β-pinene and trans-pinocamphone (Said-Al Ahl et al., 2015). Reports on the dominant components of essential oils support the findings of present study. Accordingly, cis-pinocamphone (43.3%) and limonene (12.2%) were the dominant components in Italy (Mazzanti et al., 1998), pinocarvone (36.3%) was the dominant component of Hyssop in Turkey (Özer et al., 2005), and thymol (18.95%), ß-bisabolol (16.62%) and carvacrol reported as dominant components in Iran (Dehghanzadeh et al., 2012). In a similar work in Serbia, the amount of cis-pinocamphone and pinocarvone found to be 44.7% and 14.1%, respectively (Mitić and Đorđević, 2000). Hyssop in Indian settings reported to have cis-pinocamphone (38.1%), pinocarvone (20.3%), 1.8-cineole (12.2%) and ß-pinene (12.2%) (Jankovský and Landa, 2002). The effects of the harvest season, extraction methods, geographical resources and soil on the quantity and quality of essential oils have been previously reported (Burt, 2004).

In other studies, about groups of chemical constituents, oxygenated monoterpenes have reported to be as the main chemical component of the Hyssop essential oil. These findings are consistent with those of Džamić et al. (2013). In a study on Hyssop components, Khan et al. (2012) reported that hydrocarbons form 27.66% and oxygenated compounds form 70.90% of the essential oil. The oxygenated compounds were identified as dominant in this study due to the presence of cis-pinocamphone (44.6-53.93%) and trans-pinicamphone (8.17-12.2%), which agreed with several previous studies (Afsharypuor et al., 1996; Azadbakht et al., 2003; Ghani et al., 2008; Ghasemi Pirbalouti, 2017; Turkmenoglu et al., 2015). The analysis of properties of the four essential oil component groups showed that monoterpene-containing compounds are volatile terpenoids emitted at warm temperatures (>20°C) (Demasi, 2018). Sesquiterpenes have the most diverse classes of terpenoids (Holopainen et al., 2013). They are more in herbal products than oils and used effectively in aromatherapy (Cavanagh and Wilkinson, 2002). The increase in aromatic compounds—phenols in general—and the lower content of hydrocarbons can be the result of environmental conditions (here, altitude) (Giuliani et al., 2013). In general, oxygenated monoterpenes have reported to possess better antimicrobial properties than hydrocarbon monoterpenes (Giuliani et al., 2013).

Finally, for correlation analysis, Khan et al. (2012) reported that hydrocarbons made up 27.66% and oxygenated compounds made up 70.90% of Hyssop compounds (Khan et al., 2012; Farmanpour-Kalalagh et al., 2020), showing close similarity to the results of the present study.

Conclusion

Amount of essential oil at different heights of medicinal plants is different. Based on the obtained results, the essential oil content and yield are affected by the harvesting height. The highest percentages of active compounds and essential oil were observed in the highest parts of the plant and decreased when moving in basipetal order. Therefore, harvesting from T15 cm leads to improved quality and best availability of the active compounds in the Hyssop plant. This indicates the importance of precision harvesting of medicinal plants for optimizing the yield of essential oil and active compounds while minimizing the processing requirements.

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Conflict of interest
The authors declare that they have no conflict of interest.

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