The Change of *Cyclotrichium niveum* (Boiss) Manden & Scheng
Essential oils and their components at the different growth stages

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Cyclotrichium niveum (Boiss.) Manden.&Scheng. is an endemic species of Lamiaceae family spreading in different regions of Turkey. In order to determine the different growth stages in this species, the plant was harvested in its natural growing area for three times including the period of pre-flowering, full flowering and post-flowering. The highest rate of essential oil in plant was determined in post-flowering period (5.58 %), and this was followed by full-flowering (5.45 %) and pre-flowering periods (2.83%). In all harvest periods, the main component of the essential oil was pulegone, and depending upon the development of the plant, the rate of the essential oil increased, as well. The highest rate of pulegone was determined in full-flowering period as 74.37%.

Keywords: Cyclotrichium niveum, essential oil, ontogenetic variability, pulegone

In recent years, the traditionally used plants and medicinal plants have been commonly used due to their biological features and being natural sources [1]. Moreover, the secondary metabolites existing in plants are affected from the environmental factors and can show great changes due to the processes they are exposed to in periods after they are picked up, geographical location, altitude, season, maturity of the plant, the growing period, and the plant features [2, 3]. In studies carried out with different medicinal plants, it has been reported that the essential oil and components of a plant are affected by the harvest periods [4-9].

Cyclotrichium niveum (Bois.) Mandan & Scheng is a perennial species of Lamiaceae family that is endemic for Turkey and which has 20-50 cm plant length. In its natural growing areas, it has been used as a herbal tea for flu, nausea, and muscle pain complaints by the local people [10, 11]. In studies carried out upon this species, it has been reported that the plant included 1.5- 5.5% essential oil and the main components of the plants were pulegone (% 32.50-68.12), isomenthone (%6.53-35.4), isocamphonone (% 5.47- 11.03), 1,8- cineole (% 1.31- 4.49), and limonene (% 2.11- 3.26) [10 - 13]. These differences at essential oil and its components, as mentioned above, are derived from the factors such as geographical conditions of the areas where the plants are grown up, climate, harvest periods, and used plant organs. Especially the growing periods of the plant have a great effect upon the essential oil components. Reported that the rate of carvacrol increased to 38.20%, thymol to 15.27%, in harvests in pre-flowering period and the rate of carvacrol increased to 44.10% and thymol decreased at 10.16% after the flowering period [5].

In a study that was carried out upon *Melisa officinalis* species, it was reported that in pre-flowering period the rate of carvacrol increased to 2.04%, the geraniol rate was 25.03% and in post-flowering period the rate of carvacrol increased up to 37.62%, but the rate of geraniol decreased down to 4.58; on the other hand, the component of caryophyllene showed a constant increase (from 2.17% to 5.50%) up to the period after flowering [7]. In pre-flowering period, a component at the highest rate can decrease gradually as the development of the plant. The best example for this is the *gama*-terpinene component existing in *Thymbra spicata* essential oil. This component was determined as 19.45% in pre-flowering period, 16.18% in flowering period, and 14.29% in post-flowering period [14]. In some plants, the rate of essential oil increases up to the flowering period and then it decreases. Whereas the component of camphene existing in *Satureja hortensis* essential oil is at the highest level in flowering period (1.10%), it decreases (0.97%) in post-flowering period [15]. A similar case to this has been reported for hypericine existing in *Hypericum perforatum* essential oil. In this species, whereas the rate of hypericine increased its highest level (78.7%) in flowering period, it decreases (76.8%) after the flowering period [9].

When the studies carried out upon *C. niveum* have been analyzed, we notice that different growth stages and known ontogenetic variability of this species have not been determined. For that reason, this study was carried out to determine the ontogenetic variability of this plant and how the components existing in essential oil were affected from the ontogenetic variability.

Experimental part
Materials and methods
Plant Material

C. niveum plants were collected from Adiyaman/ Kahta/ Degirmenba Malatya (West of Mount Nemrut) road at 950 m altitude. In order to determine the ontogenetic variability, the plants were picked in three different periods as pre-flowering (10.06.2013), full-flowering (12.07.2013), and post-flowering (09.08.2013) from the same location and as being harvested from the 10 cm above of the soil surface. The diagnosis of plants was provided by Ahmet Zafer Tel (Adiyaman University, Faculty of Arts and Science, Department of Biology) and their herbariums were recorded in aforementioned faculty (Voucher No: Tel 8881). After each harvest, the plants were dried in a natural

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	Retention Index		Pre-Flowering	Full-Flowering	Post-Flowering
		Essential Oil (%)	2.83	5.45	5.58
		Components (%)			
1	936	α- pinene	0.35	0.57	0.31
2	970	sabinene	0.14	0.32	0.17
3	976	β- pinene	0.44	1.12	0.41
4	991	β- myrcene	0.17	0.27	0.21
5	1020	pseudolimonene	0.03	0.03	0.03
6	1026	**limonene**	-	**2.46**	**0.31**
7	1033	**1,8- cineole**	**3.23**	**1.93**	**2.40**
8	1038	β-ocimene	-	0.25	-
9	1040	β-methylene	0.05	-	-
10	1043	trans-sabinene	0.06	0.03	-
11	1081	terpinolene	-	0.12	-
12	1099	**linalool**	-	**0.21**	**0.20**
13	1130	cyclobutene	0.03	0.16	0.11
14	1164	**isomenthone**	**18.48**	**6.61**	**9.86**
15	1182	isopulegone	1.87	1.47	1.23
16	1237	**pulegone**	**50.55**	**70.22**	**74.37**
17	1241	carvomenthenone	0.15	-	-
18	1248	isopiperitenone	0.08	-	-
19	1272	naphthalene	0.04	0.35	0.04
20	1290	**thymol**	**0.03**	**0.18**	**0.09**
21	1302	piperitenone	0.76	0.82	0.66
22	1356	**eugenol**	0.20	0.05	-
23	1406	β-elemene	0.17	0.03	-
24	1421	hexenone	**0.82**	-	**0.07**
25	1437	**carvenone**	**15.04**	**6.19**	**5.27**
26	1484	germacrene	0.36	0.59	0.15
27	1486	**bicylogermacrene**	**0.36**	**1.52**	**0.60**
28	1576	**spathulenol**	**2.75**	**0.28**	**0.19**
29	1580	isospathulenol	0.14	0.21	0.04
30	1586	**caryophyllene**	**0.20**	**0.08**	-
31	1590	octahydroazulene	0.42	0.26	0.36
32	1594	hexadecanoik acid	0.11	0.05	0.68
Total			**97.03**	**96.38**	**97.76**

Table 1
THE RATE AND COMPONENTS OF ESSENTIAL OIL IN PRE-FLOWERING, FULL-FLOWERING AND POST-FLOWERING PERIODS IN *C. NIVEUM* PLANTS

environment with shadow and air stream. Within 10-12 days, leaf-stalk separation was performed and separated leaves were made ready for the analysis.

Essential Oil Analysis

The dried leaves obtained from each harvest were weighted as 3-recursive and were boiled in Clevenger type water vapor distillation device for 3 h. At the end of this period, the device was cooled and average rate of essential oil for each harvest was determined. The obtained essential oils were put into different bottles for the GC/MS analysis and stored at +4 ^{0}C temperature. After taking the essential oils of the latest harvests, the essential oils obtained from 3 periods were exposed to analysis in GC/MS device to provide the same conditions.

Gas Chromatography Mass Spectrometry (GC/MS) Analysis:

Analysis of the essential oils of *C. niveum* carried out by using Agilent 7890A GC System equipped withAgilent 5795C MS, and HP-5 MS (0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250^{o}C, MS Ionization source temperature was 230^{o}C, the injection port temperature was 250^{o}C. The samples were injected with 250 split ratio. The injection volume was 1μL. Oven temperature was programmed in the range of 50 to 250 ^{o}C at 3^{o}C /min. The structure of each compound was identified by comparison with their mass spectrum (Nist05 and Wiley7 library).

Soil and Temperature

Soils of the experimental locations contained low organic matter (0.32%), salt content of 0.017%, *p*H of 7.90% and the texture was clay [16]. According to the long-term meteorological record, the average temperature values of June, July and August were 26.8, 31.0 and 30.5 ^{0}C, respectively.

Results and discussions

Essential Oil

The rates of essential oils obtained from harvests performed in different periods in *C. niveum* plants were presented in table 1. When table 1 has been analyzed, it has been noticed that the highest essential oil rate (5.58 %) was obtained from the harvests performed in post-flowering period. Whereas the lowest essential oil rate was 2.83 % in pre-flowering period, the essential oil rate in full-flowering period was determined as 5.45 %.

The essential oil rate obtained from the pre-flowering period was in compliance with the essential oil rates reported in studies carried out with the same species, the essential oil rates obtained in full flowering and post-flowering periods were higher than the results reported by other researchers [10, 11, 13]. However, it should be considered that the results reported by other researchers were obtained only from the flowering period. It was reported in the studies with *Thymbra spicata* [14] and with *Satureja hortensis* species [15] that the highest essential oil rate in plants was in full-flowering period. However, in the study with *Melisa officinalis* species [7] reported that the highest essential oil rate was determined in pre-flowering period. In our study, the highest rate of essential

oil was determined in post-flowering period. And this proves that the rate of essential oil can vary according to each plant species in different growth periods of the plant.

Chemical Composition of the Essential Oil

The rates of essential oils obtained from harvests performed in different periods in *C. niveum* plants were presented in table 1. There were determined 28 components in harvests performed in pre-flowering and full-flowering periods and 21 components in post-flowering period. These components create the 97% of the essential oil. The components such as pulegone, isomenthone, carvenone 1,8 cineole (eucalyptol) were determined as the main components in each harvest periods.

Whereas some components were not existing in essential oil before the flowering period (such as limonene, linalool), the others were not present in periods after the flowering (such as *trans*-sabinene, eugenol, *beta*-elemene, caryophyllene). *Beta*-methylene, carvomenthenone, isopiperitenone components were determined only in pre-flowering period. It has been mentioned in studies carried out in different plants that essential oil components varied according to the period of harvest, turned into a different component or could not be determined according to the development of the plant [5, 7, 14].

In our study, we noticed that as the plant development proceeded, the main component pulegone amount increased proportionately. According to this, the lowest pulegone rate (50.55%) was determined in pre-flowering period, and it was determined as 70.22% in full-flowering period and 74.37% in post-flowering period. In essential oils obtained from the pre-flowering period, the results we specified related to pulegone component were lower than the results reported by some other researchers, and the amounts of pulegone we determined in full-flowering and post-flowering periods were found as higher [10, 12] .

Another important component determined in the essential oil was isomenthone. Depending upon the harvest time, the proportional rate of this component in plants was remarkable. The rate of isomenthone in pre-flowering period was 18.48%, it decreased down to 6.61% in flowering period and increased up to 9.86% in post-flowering period. The same was also determined for 1.8-cineole component. The rate of this component in essential oil was 3.23% in pre-flowering period and was determined as 1.93% in flowering period and 2.40% in post-flowering period. Whereas the results we determined for isomenthone were lower than the results of some researchers [10, 12]. The aforementioned researchers reported lower values for 1.8-cineole.

The rate of some components in essential oil decreases in parallel with the development of the plant. The components such as isopulegone, carvenone, spathulenol, and caryophyllene are good examples of this. Especially the rate of carvenone component in pre-flowering period was 15.04% and it decreased down to 5.27% in post-flowering period. The highest sabinene, *beta*-pinene, *beta*-myrcene, limonene, linalool, naphthalene, thymol, piperitenone, germacrene, and bicylogermacrene rates were determined in harvests performed in full-flowering period.

Conclusions

The rate of the highest essential oil and pulegone as the main component essential oil in *C. niveum* species were determined in full-flowering period and post-flowering period. For that reason, full-flowering period should be waited for and this period should be exceeded with 10-15 days for the yield of the essential oil.

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