Journal of Essential Oil Bearing Plants
ISSN Print: 0972-060X Online: 0976-5026
www.jeobp.com

Changes in Essential Oil Content and Composition of Catnip (*Nepeta cataria* L.) During Different Developmental Stages

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Received 12 August 2010; accepted in revised form 11 January 2011

Abstract: The variations in content and compositions of catnip (*Nepeta cataria*) essential oil were examined at different developmental stages (i.e. vegetative, floral budding, full flowering, and fruit set). The essential oil of the plant was extracted by hydrodistillation. The yield of essential oils (w/w %) were 0.3, 0.5, 0.9, and 0.4 % at vegetative, floral budding, full flowering, and fruit set stages, respectively. The essential oils were analyzed by GC and GC-MS. A total of 13, 12, 14, and 11 components were identified and quantified at the above mentioned stages, respectively. The essential oil content showed significant increase at full flowering stage. Three components, 4a-α,7-α,7a-β-nepetalactone (55 - 59 %), 4a-α,7-β,7a-α-nepetalactone (30 - 31.2 %), and α-pinene (2.7 - 4.6%) were the major oil constituents of all growth stages. The proportions of 4a-α,7-α,7a-β-nepetalactone and α4a-α,7-β,7a-α-nepetalactone, as major oil constituents were highest at fruit set and full flowering stages, respectively.

Key words: Growth stage, hydro-distillation, 4a-α,7-α,7a-β-nepetalactone, α4a-α,7-β,7a-α-nepetalactone, Lamiaceae.

Introduction: Aromatic plants represent a renewable source of flavoring substances which can be used in the food, perfumery, and pharmaceutical industries [18]. The family Lamiaceae includes large number of volatile oil plants that are wildly distributed in Europe, Asia, North Africa, and the mountains of tropical Africa [2]. Catnip (*Nepeta cataria*) an aromatic plant belonging to this family, is native to Asia and Southeast Europe and grows to a height of 90 cm. This plant has carminative, tonic, diaphoretic, refrigerant, and antispasmodic properties, and also is used for seasoning [10,12]. It has been reported that most essential oils of *Nepeta* species such as *N. cataria* comprise nepetalactones as the major compounds which show antibacterial, fungicidal, and antiviral activities. The essential oil mainly accumulated in the aerial parts of the plant [17].

Chemical and biological diversity of aromatic and medicinal plants differs significantly depending on factors such as cultivation area, climatic conditions, genetic modification, different plant parts and developmental stages [6,11,19]. In recent years, numerous publications have reported the chemical compositions of the essential oils of medicinal and aromatic plants demonstrating that growth stage and harvesting time have major impact on the essential oil content and composition [7,13,14,19,20]. Therefore, it is necessary to determine the proper time and plant growth phase for plant harvest by analyzing the essential oil and its composition during various growth and developmental stages. To the best of our

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knowledge literature pertaining to the essential oil content and composition of catnip during various growth stages is not available. It is therefore imperative to determine the appropriate time for plant harvest by analyzing the oil yield and composition of this plant.

The present study was initiated to assess the variations of essential oil content and composition of catnip at four different phenological stages (vegetative, floral budding, full flowering, and fruit set stages).

Experimental

Plant material: This study was carried out in Research Field Station of Faculty of Agriculture, Shiraz University, Iran. The station is located at 1810 m above mean sea level, latitude 29º 36´ north and Altitude 52º 32´ east. The minimum and maximum temperatures of the field in recent ten year periods were -10°C and 38°C, respectively. The daily climatic data during this study were obtained from the agro-meteorological station of Irrigation Department located in a state farm about 500 m far from the experimental site. The mean values for maximum and minimum temperature (°C) for the months of April, May, June, and July 2009 were 24.61 and 4.72, 29.68 and 8.7, 35.11 and 12, 35.65 and 15.2, respectively. The average relative humidity and total rainfall of the months of April, May, June, and July 2009 were 39.93% and 3.5 mm; 19.2 % and 0 mm; 28.76 % and 0 mm and 29.74 % and 0 mm, respectively.

Catnip seeds (obtained from Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Iran) were sown in 21 January 2009 in a sandy-loam textured soil with pH=7.5, EC=1.8 dS m^{-1}, 0.97 % organic matter, 0.094 % N, 24 ppm P, 250 ppm K, 4.5 ppm Fe, 0.42 ppm Zn, 20 ppm Mn and 0.94 ppm Cu. Plant samples were harvested at vegetative, floral budding, full flowering, and fruit set stages.

The plant species was identified and authenticated by A.R. Khosravi, a plant taxonomist, at Shiraz University, Herbarium, Shiraz, Iran. Voucher specimen (no. 24995) has been deposited in the herbarium.

Essential oil extraction: The aerial parts of the plants were harvested at vegetative, floral budding, full flowering, and fruit set stages, then air dried. Samples (30 g, three replicates for each stage) were hydro-distillated for 3 hr using an all glass Clevenger-type apparatus, to extract essential oils according to the method outlined by the European Pharmacopoeia [5]. The extracted essential oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography-mass spectrometric (GC-MS) analysis.

GC and GC-MS analysis: The oils were analyzed by GC-MS. The analysis was carried out on a Thermoquest- Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica capillary column (60 m × 0.25mm i.d., film thickness 0.25 μm). The oven temperature was programmed to increase from 60 to 250°C at a rate of 4°C min^{-1} and finally held for 10 min; transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1mL min^{-1} with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35 - 465 amu with an ionizing voltage of 70 eV and an ionisation current of 150 mA.

GC-FID: Analyses of the oils were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow rate of 1.1mLmin^{-1}; the split ratio was the same as for GC/MS. The oven temperature was raised from 60 to 250°C at a rate of 4°C min^{-1} and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. Semi quantitative data were obtained from FID area percentages without the use of correction factors. Retention indices (RI)

were calculated using retention times of n-alkanes (C6–C24) that were injected after the oil at the same temperature and conditions.

Compounds were identified by comparison of their RI with those reported in the literature [1], and their mass spectrum were compared with the Wiley Library (Wiley 7.0).

Results and discussion: *N. cataria* has been identified as a plant with lesser amount of essential oil in comparison with the other plants of Lamiaceae family. Therefore, it is essential to harvest catnip plant at the stage that has the most amount of essential oil with the best quality.

The hydro-distillation of 30 g of aerial parts of catnip showed that essential oils at the vegetative, floral budding, full flowering, and fruit set stages were 0.3, 0.5, 0.9, and 0.4% based on dry weight, respectively (Table. 1). The oil content increased drastically (three times) from vegetative to full flowering and then decreased significantly (more than two times) at fruit set stage. However, at full flowering stage, the yield of essential oil was similar to the previous study [17] which extracted catnip essential oil at the same stage. The composition of essential oils at different growth stages are shown in Table 2 along with retention indices of the identified components that are arranged in the order of their elution from a DB-5 column. Comparing the composition of the essential oils during the growth stages revealed both quantitative and qualitative differences. A total of 13, 12, 14 and 11 compounds representing 93.6, 99.6, 99 and 98.5 % of the total were detected at vegetative, floral budding, full flowering, and fruit set stages, respectively. The results of GC and GC-MS analysis showed that the highest amount of components such as α-pinene (4.6 %), β-pinene (1.64 %), and 4a-α,7-β,7a-α-nepetalactone (31.2 %) were detected at full flowering stage. While, thymol (1.3 %), 4a-α,7-α,7a-β-nepetalactone (59 %), α-humulene (1.2 %), and 11-dodecenol (1.5 %) were maximum at fruit set stage (Table 2). However, three components, 4a-α,7-α,7a-β-nepetalactone (55 - 59%), 4a-α,7-β,7a-α-nepetalactone (30 - 31.2 %), and α-pinene (2.7 - 4.6 %) were the major oil constituents of all growth stages. Earlier report has shown that the same components were the major oil components of catnip from Kashan, Iran [17]. Nepetalactone isomers, which are the main compounds in this study, were determined in the essential oils of other *Nepeta* species [4,15,16].

There are no published reports regarding the evolution of the chemical compositions of essential oils of catnip during different growth stages. The results of the present study showed that some compounds such as spathulenol and caryophyllene oxide were not found at fruit set; however, the amounts of these components were highest at vegetative growth stage (Table 2). The analysis of the essential oil composition of catnip plants from Kashan Region of Iran revealed that α-pinene, sabinene, thymol, and α-humulene were not found in the oil [17] while these components were detected during different phenological stages of catnip in the present study (Table 2). However, ascaridole, α-copaene, β-caryophyllene, and β-bourbonene were only detected in the essential oil of Kashan catnip and not found in this investigation. It seems that such variations in quantity and quality of components in the same species might be due to extrinsic factors such as different developmental stages and harvesting times along with parameters such as edaphic and climatic factors, geographic origin, cultivation site [3,9] and/or the occurrence of a chemotype [8].

Conclusion: In conclusion, the results reported herein revealed that there are considerable differences in the content and composition of the essential oil of *N. cataria* during its developmental cycle. It is strongly believed that these differences are due to variations in the metabolic pathways and consequently modifications in secondary metabolism which coupled with the plant growth and development. Further studies are still required to explore the molecular and biochemical aspects of these variations during different maturation stages of the plant.

Acknowledgement: The authors would like to thank the staff of Department of Horticulture for their valuable assistance and Shiraz University for financial supports (grant no: 87-gr-agrst-98).

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Table 1. Effect of different harvest stages on the essential oil content (w/w%) of catnip (*N. cataria*)

Growth stage	Essential oil (w/w %)
Vegetative	$0.3 \pm 0.0055^{d*}$
Floral budding	0.5 ± 0.004^{b}
Full flowering	0.9 ± 0.0044^{a}
Fruit set	0.4 ± 0.0033^{c}

*Means followed by the same letter are not significantly different, as indicated by Duncan's multiple range test (DMRT) at $P < 0.05$

Table 2. Essential oil composition (%) of catnip (*N. cataria*) at four stages of harvest

Component	RI*	Vegetative	Floral budding	Full flowering	Fruit set
α-Pinene	936	2.7	3.1	4.6	3.3
Sabinene	957	-	-	0.2	0.3
β-Pinene	978	0.8	1.13	1.6	-
1-Cyclohexen-1-yl-methyl ketone	980	0.5	0.6	0.7	0.5
Triplal	1023	0.1	0.4	0.4	0.2
Thymol	1294	0.4	0.4	0.6	1.3
4a-α,7-α,7a-β-Nepetalactone	1332	55.0	58.0	55.0	59.0
4a-α,7-β,7a-α-Nepetalactone	1342	30.1	31.1	31.2	30.0
trans-Caryophyllene	1430	1.1	2.7	2.1	1.1
α-Humulene	1446	0.8	0.9	0.9	1.2
11-Dodecenol	1500	0.8	0.7	1.1	1.5
Spathulenol	1580	0.6	0.4	0.3	-
Caryophyllene oxide	1569	0.5	0.2	0.1	-
6,10-dimethyl-2-undecane	1907	0.2	-	0.3	0.2

* The retention Kovats indices were determined on DB-5 capillary column.