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Wild Habitat Altitude and Harvest Time Influence Volatile Oil Content and Composition of *Thymus kotschyanus* Boiss. & Hohen.

M. Hajihassani[1], A. Hassani[1,2], M. B. Hassanpouraghdam[3*], M. H. Meshkatalsadat[4], A. Pirzad[5], M. Heidari[6] and Ali Reza Eivazi[7]

[1]Scholar of Seed and Plant Improvement Research Department, West Azerbaijan Agricultural and Natural Resources Research And Education Center, AREEO, Urmia, Iran

[2]Department of Medicinal and Industrial Plants, Institute of Biotechnology, Urmia University, Urmia, Iran

[3]Department of Horticultural Sciences, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran

[4]Department of Chemistry, Faculty of Basic Sciences, Lorestan University, Khoram Abad, P. O. Box: 465, Iran

5Department of Agronomy and Plant Breeding, Faculty of Agriculture, Urmia University, Urmia, P. O. Box: 165, Iran

[6]Scientific Board of Forests and Rangelands Research Department, West Azerbaijan Agricultural and Natural Resources Research And Education Center, AREEO, Urmia, Iran

[7]Assistant Professor of Seed And Plant Improvement Research Department, West Azerbaijan Agricultural And Natural Resources Research And Education Center, AREEO, Urmia, Iran

*Corresponding Author E-mail: maryam_hajihasani@yahoo.com

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ABSTRACT

Essential oil content and composition of wild growing Thymus kotschyanus Boiss. & Hohen. were evaluated in response to different harvest time (pre-flowering or vegetative, flowering and post-flowering stages) and wild habitat altitude (1400, 1600 and 1800 m above sea level). The results showed that the highest essential oil content (2.9%) belonged to the flowering stage at 1600 m altitude. GC and GC/MS analysis of essential oil revealed that carvacrol (4.2- 49.1%) and thymol (2.6-13.3) were the predominant constituents of volatile oils. Other common major components were α-terpineol, p-cymene, 1,8-cineole, borneol, camphor, γ-terpinene and linalool. Geraniol (17.8%) had considerable amounts possessed by plants harvested from 1400m during vegetative stage. Taking into account the essential oil content and, carvacrol and carvacrol + thymol percentage amounts it seems that flowering stage at 1600m is the most appropriate harvest time for this plant from its wild habitat. However, considering other major components, any of nine treatment combinations have the potential suitability for the utilization of their volatile constituents in the related industries.

Key words: *Thymus kotschyanus Boiss. & Hohen., Lamiaceae, altitude, Flowering stage, GC/MS, Carvacrol, Thymol*

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INTRODUCTION

Thymus species (Fam. Lamiaceae or Labiatae) have a wide distribution pattern especially in temperate regions of the world[1]. Fourteen species have been reported for this genus from Iran. These species are mainly distributed in North and West of Iran[1]. *Thymus kotschyanus* Boiss. & Hohen., as an important aromatic herb is a well known species with frequent occurrence in high altitude wild habitats in Northwest Iran[2]. *T. kotschyanus* is a hardy perennial small herb with branched growing pattern. Leaves of *T. kotschyanus* are covered with peltate glandular trichomes as red colored spots[3].

Pharmaceutically, this plant and its preparations have been used as carminative, anti-cancer, anti-tussive, anti-convulsant, moderate diuretic and digestive[4]. Furthermore, anti-fungal activities have been detected for the essential oil of this plant describes its application in food industry as flavoring, aromatic and preservative agent[4,5].

Several intrinsic and extrinsic factors influence essential oil content and composition of aromatic plants. Apart from species, genetic basis and biochemical potential of essential oil bearing plants, volatile components biosynthesis in those plants is closely related with photosynthesis, primary metabolism and other basic biological activities within the plants. These routine activities are greatly influenced by the geographical and climatological factors of the growing habitats [6,7]. Some extrinsic factors such as temperature, humidity, soil characteristics and growing habitat altitude have been considered to have inevitable impact on essential oil profile [6-8]. Harvest time and plant growing stage are other factors with appreciable effects on essential oil content and composition as well [9].

Compositional analysis of *T. kotschyanus* essential oil has been the subject of previous works. Several studies reported thymol and carvacrol as the major components of *T. kotschyanus* volatile oil from different origins[5,10-13]. At the same time, Semnani *et al* [14] reported that pulegone and cavacrol were the main essential oil components of this plant from North Iran. Linalool and α-terpinene have been characterized as the main volatile oil components from Iran as well [15]. There is considerable research interest towards the impact of growing habitat altitude on essential oil biosynthesis and accumulation of aromatic plants. Mazandarani and Rezaei[16] reported that altitude had significant effect on essential oil profile of *T. carmanicus* plant. Geographical and climatological factors had substantial effect on *Thymus vulgaris* L. essential oil composition [8]. *Tanacetum polycephalum* Sczhult-Bip ssp. *Duderanum* (Boiss.) pod, *Origanum vulgare* ssp. Hirstum and *Nepeta macrosiphon* essential oil contents and composition were affected by altitude of growing region as well[17-19].

Harvest time is another main factor influences secondary metabolites content and composition of medicinal and aromatic plants [20-22]. Various studies conducted on *T. kotschyanus* revealed the significance of appropriate harvest time on herbage yield and essential oil content and composition of this plant[5,23].

The aim of the present experiment was to evaluate the essential oil content and composition of an endemic *T. kotschyanus* plant affected by growing habitat altitude and harvest time from Northwest Iran for the first time.

MATERIAL AND METHODS

Plant material: Aerial parts of *Thymus kotschyanus* plants were collected from Forest Reservoir of Ghasemlou Valley located at North of Urmia in Northwest Iran. Plant materials were harvested during three growing stages (vegetative or pre-flowering stage, flowering stage and post-flowering stage) from three altitudes (1400, 1600 and 1800 meters above sea level). A sample of specimen was deposited in the Herbarium of Urmia University, Iran.

Ghasemlou valley is a small (577 hectares) district with typical semi-dry cool climatic conditions, 270 mm annual precipitation and 19.5^0C and 7.2^0C mean maximum and minimum temperatures during growing season.

Essential oil extraction: 50 grams of air dried and grinded plant materials were subjected to hydrodistillation by an all-glass Clevenger type apparatus for 2 hrs. Extracted yellow colored essential oils were dried over anhydrous Na_2SO_4 and kept at refrigerator in sealed dark glass vials until analysis. Essential oil content was expressed as volume per weight (v/w) based on plant material air-dried weight. Extraction was carried out in triplicate and mean data were subjected to statistical analysis.

GC and GC/MS analysis: The analysis of the oils were carried out using a Shimadzu 17A gas chromatograph equipped with an apolar DB-5 (95% dimethylpolysiloxane) capillary column (30m × 0.25mm i.d. and 0.25µm film thickness). Oven temperature was set at 40^0C for 3 min, then programmed until 200^0C at a rate of 4^0C/min and finally increased at the rate 40^0C/min to 280^0C, isothermal at the temperature for 3 min. The carrier gas was helium with constant flow rate of 0.8 ml/min. Injection mode, split: split ratio 1:40, Injector and detector temperatures were 240^0C and 260^0C, respectively. GC/MS analysis were performed on a Shimadzu 17A GC interfaced with a Shimadzu QGF 5050 mass selective detector. The chromatographic conditions were the same as described above. The MS operating parameters were as follows: Ionization potential, 70 eV, interface temperature 200ºC and acquisition mass range; 50-450 amu.

Identification and quantification of components: Relative percentage amounts of the essential oil constituents were evaluated from total peak area (TIC) by apparatus software. Identification of components in the volatile oil was based on the comparison of their retention indices and mass spectral data with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the mass spectral data with those reported in the literature[5,10-15,22-24].

Statistical analysis: Analysis of variance for essential oil content was carried out by MSTATC software based on completely randomized block design as factorial combination. Mean comparisons were evaluated by Duncan's multiple range test. Graphs were drawn by Excel (Microsoft Office 2003).

RESULTS AND DISCUSSION

The results showed that the effects of harvest time and interaction of harvest time and altitude were significant ($p\leq 0.01$) on essential oil content. However, altitude had no significant effect on this trait. Mean comparisons for ineraction of harvest time and altitude showed that the highest (2.9%) essential oil content belonged to the flowering period at 1600m altitude. There was significant ($p\leq 0.01$) difference between this treatment and other treatment combinations. The least amount (0.7-1%) for this trait was held by all harvesting times at 1800m altitude. There were statistical differences between different times at 1400 altitude as well (figure 1). Habibi *et al*. [15] and Jamshidi *et al*. [10] reported that essential oil content of *T. kotschyanus* was 1.3-2.6 and 1-1.9 percent, respectively. Considering volatile oil content, there is some difference between our findings and reports of above scientists. It seems that the appropriate condition for maximum essential oil acquiring is harvesting of plants from 1600m altitude during flowering period. This is similar with the findings of Sefidkon and Askari [23] and Sefidkon *et al*. [5]

The results obtained from the chemical analysis of volatile oils of *T. kotschyanus* are reported in table 1. In total, fifty seven components were identified in the essential oils of nine treatment combinations accounting for 96.2 to 98.6% of the total oils. Table 1 shows that there are considerable differences between treatment combinations regarding type and percentage amounts of essential oil constituents. Twenty compounds were common between treatment combinations. Carvacrol (4.2-49.1%) and thymol (2.6-13.3) were the highlighted common components of volatile oils consistent with the previous reports [5, 10-13]. Other common components with substantial amounts were α-terpineol (0.5-

15.3%), p-cymene (4.4-9.6%), 1,8-cineole (3.2-9.5%), borneol (2.3-9.2%), caryophyllene oxide (1.2-7.3%), camphor (0.5-7.3%), γ-terpinene (0.4-6.6%), linalool (1.2-5.8%), (E)-ocimene (0.5-4.5%), α-pinene (1.9-4.5%), (E)-caryophyllene (1.1-4.2%), (E)-sabinene hydrate (1.7-3.2%) and camphene (1.3-2.6%). Some other compounds had appreciable amounts exclusive of special treatment combinations such as; geraniol (17.8%) and geranyl acetate (6%) possessed by H_1T_1 treatment combination and nerolidol (5.8%) related to H_3T_1 treatment combination. Taking into account the chemical profile and major constituents of volatile oils, it appears that there are some discrepancy and/or similarity between our results and reports of other scientists from elsewhere [5, 10-15]. Alongside, with our past work on *Thymus migricus* we realized that α-terpineol (3.4-21.8%), thymol (3.4-19.4%), 1,8-cineol (1.9-14.5%) and carvacol (2.5-16.1%) were the major oil components[22].

Economic and pharmaceutical significance of *Thymus* species essential oils is due to the high occurrence of thymol and carvacrol [10,23]. Figures 2 and 3 show that thymol, carvacrol and thymol + carvacrol content of treatment combinations had regular patterns regarding same harvesting time or altitude. In both conditions, like with essential oil content, carvacrol and carvacol + thymol content had the highest percentage during flowering period at 1600m altitude. In contrast, this trend was reversed for thymol. However, thymol (2.6%) content of plants in H_2T_2 treatment combination was not comparable with carvacrol (49.1%) content. This is likely that the observed chemical variations are due to the diverse climatological and geographical conditions especially altitude related (slope side, slope percent, altitude itself, soil characteristics) factors. Furthermore, the effects of different plant growing stages on these differences are inevitable. Besides aforementioned reasons, effects of divergent plant genetic, biochemical and physiological potential at different growing stages and wild growing habitats on the compartmentalization of different biochemical pathways is worthy of great consideration.

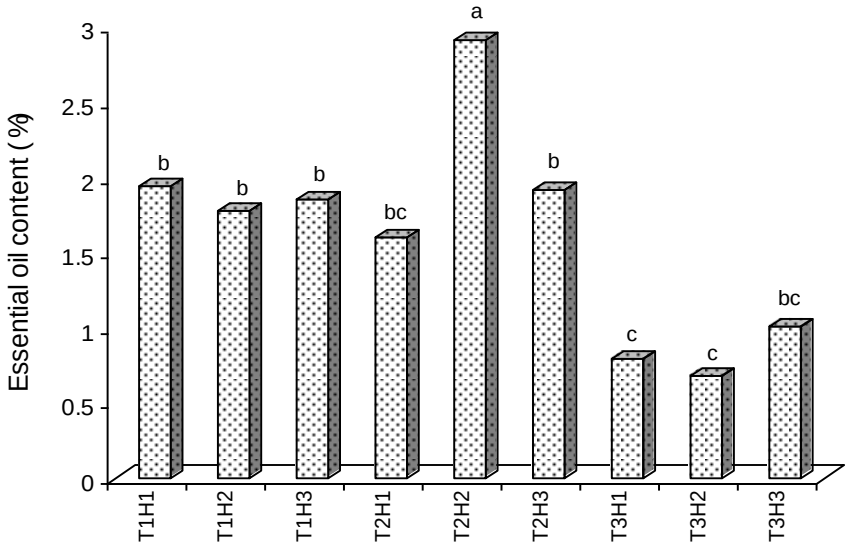

Fig. 1: Mean comparisons for interaction effects of harvest time and altitude on essential oil content of *Thymus kotschyanus*

Different letters on bars show significant difference (P≤ 0.01) between treatment combinations based on Duncan's multiple range test.
T_1: Pre-flowering or vegetative stage, T_2: Flowering stage and T_3: Post-flowering stage
H_1: 1400m altitude, H_2: 1600m altitude and H_3: 1800m altitude

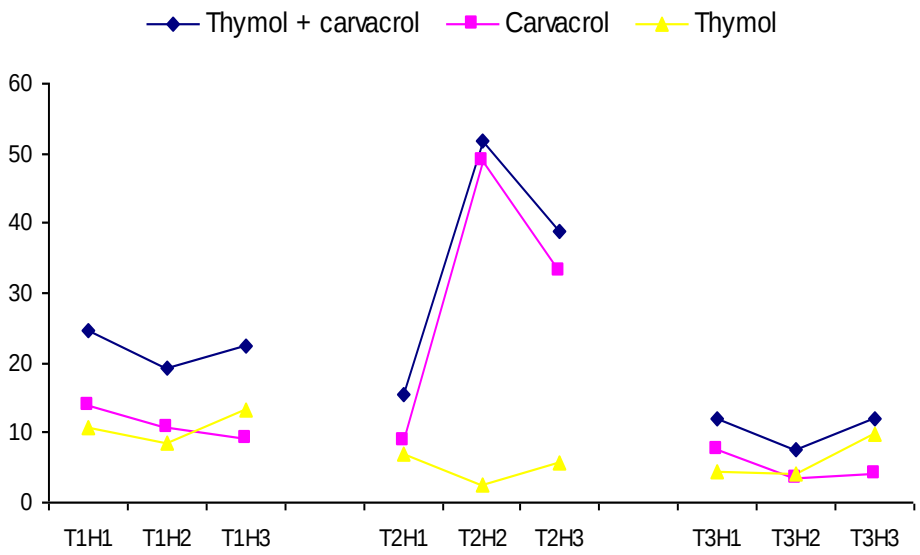

Fig. 2: Thymol, carvacrol and thymol + carvacrol content of *Thymus kotschyanus* related to altitude variation in same harvest time or growing stage

T_1: Pre-flowering or vegetative stage, T_2: Flowering stage and T_3: Post-flowering stage
H_1: 1400m altitude, H_2: 1600m altitude and H_3: 1800m altitude

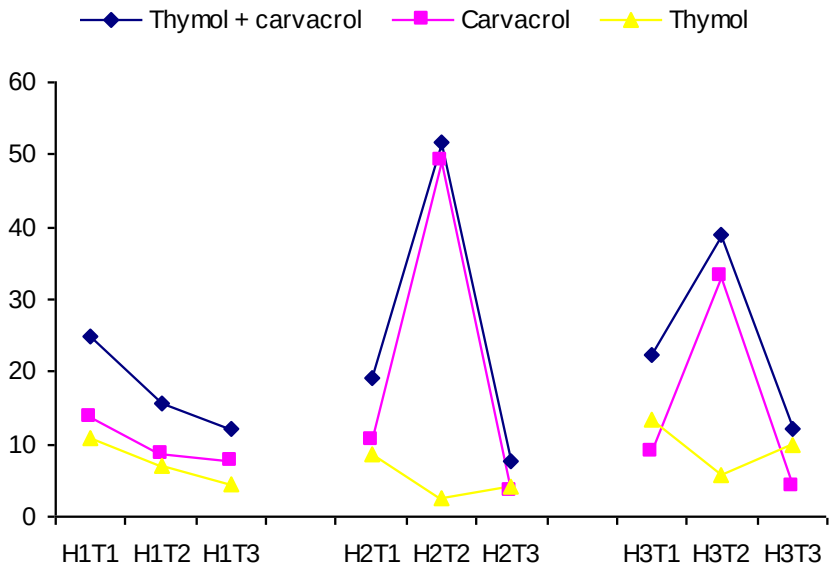

Fig. 3: Thymol, carvacrol and thymol + carvacrol content of *Thymus kotschyanus* related to harvest time variation in same growing habitat altitude

T_1: Pre-flowering or vegetative stage, T_2: Flowering stage and T_3: Post-flowering stage
H_1: 1400m altitude, H_2: 1600m altitude and H_3: 1800m altitude

Table 1: Essential oil composition (% of peak area) of *Thymus kotschyanus* Boiss. & Hohen. plant affected by different harvest time and wild habitat altitude

No	Compound	RI	H_1T_1	H_1T_2	H_1T_3	H_2T_1	H_2T_2	H_2T_3	H_3T_1	H_3T_2	H_3T_3
1	α-Thujene	930	1	0.6	0.6	0.7	1.1	0.2	0. 9	0.8	0.9
2	**α-Pinene**	**939**	**3.4**	**2.6**	**3.8**	**2.9**	**1.8**	**1.9**	**3.8**	**2.3**	**4.5**
3	**Camphene**	**954**	**2.1**	**1.8**	**2.6**	**1.7**	**1.3**	**1.6**	**1.5**	**1.8**	**2.4**
4	Sabinene	975	0.7	0.4	0.5	0.8	-	0.3	1	0.3	0.9
5	β-Pinene	979	1.3	0.9	1	1.1	0.6	0.5	1.3	0.8	1.2
6	Myrcene	991	2.1	1.2	0.8	2.4	1.7	0.7	2.3	1. 4	2.1
7	α-Phellandrene	1003	-	-	-	-	0.3	-	-	-	0.2
8	α-Terpinene	1017	0.9	0.5	0.2	0.5	1.7	0.1	0.9	0.9	0.6
9	**ρ-Cymene**	**1025**	**5.3**	**4.4**	**9.6**	**4.5**	**6.8**	**5.2**	**4.7**	**7.9**	**5.8**
10	Limonene	1029	0.8	0.6	-	-	-	-	0.5	-	-
11	**1,8-Cineole**	**1031**	**8.9**	**5.8**	**12**	**7.2**	**3.2**	**6.5**	**9.1**	**6.2**	**9.4**
12	**(E)-Ocimene**	**1050**	**4.5**	**2.2**	**0.8**	**5.1**	**0.6**	**0.5**	**4.3**	**0.7**	**2.9**
13	**γ-Terpinene**	**1060**	**6.6**	**3**	**1.4**	**3.5**	**5.5**	**0.4**	**5.5**	**3.5**	**3.6**
14	**(E)-Sabinene hydrate**	**1070**	**1.8**	**2.1**	**3.4**	**2.1**	**2**	**2.8**	**1.8**	**2.2**	**2.1**
15	Terpinolene	1089	-	-	-	-	0.2	0.1	-	-	-
16	**Linalool**	**1097**	**3.8**	**2**	**2.9**	**2.9**	**3.2**	**5.8**	**4.3**	**1.2**	**2.3**
17	(Z)-Sabinene hydrate	1098	0.5	0.5	0.9	0.4	0.3	0.9	0.3	0.5	0.7
18	α-Campholenal	1126	-	-	0.2	-	-	-	-	-	0.2
19	(E)-Pinocarveol	1139	-	0.2	1.1	-	-	1.1	-	-	0.6
20	Verbenol	1145	-	0.4	2.4	-	-	-	-	-	-
21	**Camphor**	**1146**	**3.6**	**2.9**	**5.3**	**2.9**	**0.5**	**7.3**	**2.5**	**3.4**	**5.6**
22	Pinocarvone	1165	-	-	0.3	-	-	0.3	-		0.1
23	**Endo-Borneol**	**1169**	**5.3**	**4.6**	**9.2**	**4.3**	**2.3**	**9**	**4**	**4**	**7.2**
24	4-Terpineol	1170	0.8	0.6	-	0.6	1.2	-	0.5	1.1	-
25	ρ-Cymene-8-ol	1183	-	0.3	0.9	-	-	-	-	-	-
26	**α-Terpineol**	**1189**	**9.1**	**4.6**	**5.4**	**13.1**	**0.6**	**15.5**	**10.4**	**11.2**	**12.3**
27	(Z)-Citral	1222	-	0.6	-	-	-	-	-	-	-
28	(Z)-Carveol	1230	-	-	0.4	-	-	-	-	-	-
29	Nerol	1241	-	1.1	-	-	-	-	-	-	-
30	Carvacrol methyl ether	1245	1.7	0.7	1.7	1.1	0.6	1.8	0.8	-	1.4
31	**Geraniol**	**1253**	**-**	**17.7**	**-**	**4.6**	**3.7**	**2.2**	**1.4**	**3.7**	**1.9**
32	Geranial	1267	-	1.6	-	1	-	-	-	-	-
33	Bornyl acetate	1289	-	0.7	0.7	-	-	1.6	-	-	0.7
34	**Thymol**	**1290**	**10.9**	**6.9**	**4.5**	**8.5**	**2.6**	**4.1**	**13.3**	**5.6**	**9.9**
35	**Carvacrol**	**1299**	**13.9**	**8.7**	**7.6**	**10.6**	**49.1**	**3.4**	**9.1**	**33.3**	**4.2**
36	Eugenol	1359	-	-	-	-	-	0.1	-	-	-
37	Neryl acetate	1362	-	0.5	-	-	-	.08	-	-	-
38	**Geranyl acetate**	**1381**	**-**	**6**	**-**	**2.7**	**3.5**	**0.8**	**1.6**	**2.2**	**1**
39	α-Bourbonene	1388	-	0.3	0.6	-	-	0.9	0.2	-	0.6
40	β-Bourbonene	1388	-	-	-	0.3	-	-	-	-	-
41	**(E)-Caryophyllene**	**1419**	**3.7**	**3.2**	**1.9**	**4.2**	**1.8**	**1.6**	**4.1**	**1.1**	**2.5**
42	Alloaromadendrene	1460	-	-	0.3	0.2	-	0.1	0.2	-	0.1
43	α-amorphene	1485	-	-	-	-	-	-	0.2	-	0.2
44	Germacrene D	1488	1	0.9	0.3	0.9	-	0.3	1.3	-	0.8
45	Bicyclogermacrene	1497	1.3	1.1	0.4	0.5	0.2	0.2	1.4	-	0.5
46	Farnesene	1506	0.5	0.4	-	0.5	-	-	0.5	-	0.4
47	Germacrene A	1509	-	-	0.3	-	-	-	-	-	-
48	δ-Cadinene	1523	0.5	0.5	0.6	0.3	-	0.4	0.5	-	0.3
49	**Nerolidol Z and E**	**1563**	**-**	**-**	**1.3**	**-**	**-**	**-**	**5.8**	**-**	**-**
50	Elemol	1566	-	-	-	-	-	-	0.9	-	0.7
51	Nerolidol	1573	-	-	-	1.9	-	-	-	0.4	-
52	Spathulenol	1578	1.2	1.1	-	1.6	0.4	0.5	1.3	0.6	-
53	**Caryophyllene oxide**	**1583**	**1.2**	**1.8**	**7**	**2**	**1.5**	**7.5**	**1.2**	**1.3**	**4.7**
54	Carotol	1595	-	-	-	-	-	0.7	-	-	-
55	δ-Cadinol	1649	0.3	1	2.2	0.3	-	2.2	0.6	0.3	0.9
56	β-Eudesmol	1651	-	0.2	-	0.3	-	0.6	0.4	-	1.2
57	Juniper camphor	1652	-	-	2.5	-	-	1.5	-	-	-
Total			98.5	97.2	97	98	98.4	96.2	98.6	98.5	96.9

Compounds are reported according to their elution order on apolar column

T_1: Pre-flowering or vegetative stage, T_2: Flowering stage and T_3: Post-flowering stage

H_1: 1400m altitude, H_2: 1600m altitude and H_3: 1800m altitude

CONCLUSION

In brief, the chemical composition of volatile oil of wild *T. kotschyanus* from Northwest Iran was characterized by the high occurrence of carvacrol and thymol. Flowering stage at 1600m altitude had the highest essential oil, carvacrol and thymol + carvacrol content, and could be considered as the suitable criteria for deciding the optimum harvest time and altitude of *T. kotschyanus* from its wild habitats. But, in the light of other major volatile oil constituents, essential oil derived from all treatment combinations have the potential applicability in the related industries. Accordingly, *T. kotschyanus* plants studied in the present experiment can be a promising and easy-to-access source of carvacrol, thymol and other principle constituents for submitting the great demands of pharmaceutical, food and hygienic industries for these high-valued volatile compounds.

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