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## Effect of different harvesting time on growth, yield and quality of Thyme (*Thymus serpyllum* L.) under the agro-climatic conditions of Doon valley, Uttarakhand

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**ABSTRACT**

*A field experiment was conducted under the agro-climatic conditions of Doon valley, Uttarakhand during 2008 to assess the performance of four harvesting times ($H_1$-115 days, $H_2$-135 days, $H_3$-155 days and $H_4$-175 days after planting) of Thymus serpyllum L. The number of branches/ plant found maximum in $H_1$ harvesting. The parameters i.e. fresh and dry herbage (leaves and small twigs) yield showed greater response in 115 days after planting, whereas fresh oil yield maximum in $H_3$ harvesting. The quality of Thyme essential oil was evaluated at different harvesting times using GC/MS. Thymol content was rich in all the four harvestings (34.61-49.53%), though it was maximum in $H_2$ (49.55%) followed by $H_1$ (42.87%), $H_3$ (42.51%) and $H_4$ (34.61%) harvesting times.*

**Key words:** *Thymus serpyllum* L., harvesting times, essential oil composition, thymol.

## INTRODUCTION

*Thymus* L. (Lamiaceae), a genus of variable aromatic, prostrate and evergreen perennial herb is distributed in temperate zones. One species, *Thymus serpyllum* L., occurs in India, and *T. vulgaris*, a native of Europe, has been introduced [1]. *T. serpyllum* also known as wild Thyme, banajwain is found in western temperate Himalaya from Kashmir to Kumaon and western Tibet at the altitudes of 1500-4500 m [2]. In India, Thyme is used in culinary herb for flavouring. Thyme is considered to possess antiseptic, antispasmodic, expectorant, carminative, anthelmintic and stimulant properties and also used in mouth washes, gargles, cough and colds etc. [1,3-5]. Many studies have been conducted to determine essential oil content and composition of wild Thyme [6-11]. However, information about harvesting stages, herb and oil yield of wild Thyme is limited.

In Uttarakhand Himalaya, Thyme is wildly grown in forest lands, but due to deforestation, over-exploitation, overgrazing the availability of this important traditional medicinal and aromatic herb is limited. The commercial cultivation of this herb is also not in trends, although it is cultivated some parts of India. Keeping in view, the aim of present investigation is to conduct the agronomical studies and to determine suitable harvesting time for maximum herbage, oil yield and quality of essential oil of wild Thyme.

## EXPERIMENTAL SECTION

### Experimental site and climatic conditions

The study was conducted during October, 2008 in Doon valley agro-climatic conditions of Uttarakhand at the experimental field of Centre for Aromatic Plants (CAP), Selaqui, Dehradun to evaluate the performance of four harvesting/clipping times of Thyme viz. $H_1$-115 days of planting (DAP), $H_2$-135 DAP, $H_3$-155 DAP and $H_4$-175 DAP. The experimental site is located at $30^0$ 9' N latitude and $78^0$ 2' E longitude at 680 m. above sea level. The climate of the study area is sub-tropical with mild summer and cold winter. The temperature often reaches up to $40^0$C during summer with desiccating wind and in winter it goes down to $2.6^0$C. The Soil of experiment field compositions were: sand 68.8% (fine sand 62.2%, course sand 6.6%), silt 25.6%, clay 5.6%, pH 7.4, available K 103 ppm, available P 26.3 ppm, total N 0.03%, organic carbon 0.465% and electronic conductivity (EC) 0.159 $dsm^{-1}$.

### Growth and developmental studies

During the experiment 16 plots were prepared and 24 rooted plants were transplanted in each plot with a spacing (plant to plant and row to row distance) of 45x45 cm (total 384 rooted plants). The Design of experiment was in RBD manner with four replications. In each replication, 5 plants were randomly selected for recording of different parameters. For the estimation of production, one $mt^2$ quadrat was used. The crop was irrigated as and when required. During the experiment growth attributes viz. plant height (cm), number of branch/plant, fresh and dry herbage yield (qt./hectare), fresh and dry oil yield (lt/ha), fresh and dry oil content (%) was recorded time to time during the experiment.

### Qualitative and Quantitative analysis

***Extraction of essential oil:*** Shade dried leaves and small twigs (aerial parts) of each sample (300g) of different harvesting times were separately hydro distilled for 4 hours using a Clevenger-type apparatus. The collected oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at $4^0$C until analyzed.

***Gas chromatography-mass spectrometry:*** The GC/MS analyses were carried out on a Perkin Elmer *Clarus 500* gas chromatograph equipped with a split-splitless injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 m x 0.32mm, 0.25µm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 ml/min. The GC was interfaced with (Perkin Elmer *Clarus 500*) mass detector operating in the $EI^+$ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as follows: initial temperature of $60^0$ C (hold: 2 min) programmed at a rate of $3^0$C /min to a final temperature of $220^0$C (hold: 5 min). The

temperatures of the injector, transfer line and ion source were maintained at $210^0$C, $210^0$C and $200^0$C, respectively.

Identification of the individual components was made by matching their recorded mass spectra with the library (NIST/ Pfleger /Wiley) provided by the instrument software, and by comparing their calculated retention indices with literature value [12].

## RESULTS AND DISCUSSION

The results of different growth parameters are given in Table 1. The parameters i.e. plant height and number of branch/plant were found non-significant. However, no. of branch was maximum in $H_1$ harvesting (316.62). The fresh and dry herbage production was also higher in $H_1$ (147.13 and 17.35 qt/ha, respectively). It should be noted that increase in herbage yield per hectare (fresh and dry) in I$^{st}$ harvesting was due to maximum no. of branches/plant. The plant height in general increased with the advancement of crop age. In different harvesting times ($H_1$, $H_2$, $H_3$, $H_4$), the essential oil content showed some differences. Oil content, obtained by dry yield was maximum in 4$^{th}$ harvesting (0.74%), the stage of seed set whereas, on fresh weight basis, it was more or less similar in 3$^{rd}$ (full flowering stage) and 4$^{th}$ harvesting, i.e. 0.14% and 0.13%, respectively. The maximum oil yield in fresh herb was found by harvesting the crop at full flowering stage in 155 days after planting.

A total number of 22 components were identified by GC/MS analysis (Table 2). Among the constituents of oil, thymol (34.61-49.53%) was found as the major component followed by *p*-cymene (7.43-11.45%), γ-terpinene (8.26-9.30%), thymol methyl ether (5.25-6.47%) and carvacrol methyl ether (3.94-4.67%). Thymol which was found in abundance in the essential oils of all the four harvestings, recorded maximum after 135 days of planting. The maximum (49.52%) and minimum (34.61%) thymol content has moderate difference among the harvesting times. The maximum thymol content in the essential oil was recorded at the 2$^{nd}$ harvest i.e. at the flowering stage and the minimum thymol content was observed at the 4$^{th}$ harvest i.e. at the seed ripening stage. Our results are agreements with Omidbaigi *et al.* (2008). They reported the maximum thymol content in the oil of the herb harvested before flowering stage, and the lowest amount at the seed ripening stage.

**Table 1: Effect of harvesting on plant height, no. of branches, herb and oil yield of Thyme (*Thymus serpyllum* L.)**

| Harvests | Plant height (cm) | No. of branch/ plant | Herb yield (Q./ha) | | Oil yield (lt/ha) | | Oil content (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | Fresh wt. basis | Dry wt. basis | Fresh | Dry | Fresh | Dry |
| $H_1$ | 35.25 | 316.62 | 147.13 | 17.35 | 11.77 | 9.02 | 0.08 | 0.52 |
| $H_2$ | 31.71 | 174.98 | 122.49 | 14.64 | 8.57 | 9.37 | 0.07 | 0.64 |
| $H_3$ | 41.00 | 168.86 | 102.54 | 13.06 | 14.35 | 8.88 | 0.14 | 0.58 |
| $H_4$ | 42.00 | 137.99 | 98.54 | 13.47 | 12.85 | 9.97 | 0.13 | 0.74 |
| CD 5% | NS | NS | 17.97 | 1.94 | 1.97 | 1.20 | - | - |
| H1 - 115 days after planting (DAP), H2 - 135 DAP, H3 - 155 DAP, H4 - 175 DAP | | | | | | | | |

**Table 2: Chemical composition of Thyme oil cultivated in Uttarakhand (India)**

| Components | RI | Harvesting times | | | |
|---|---|---|---|---|---|
| | | $H_1$ | $H_2$ | $H_3$ | $H_4$ |
| α-thujene | 931 | 1.06 | 1.29 | 0.54 | 1.57 |
| α-pinene | 939 | 0.87 | 1.24 | 0.47 | 1.38 |
| camphene | 953 | 0.88 | 1.15 | 0.54 | 1.60 |
| β-myrcene | 991 | 1.45 | 1.60 | 1.08 | 1.53 |
| α-terpinene | 1018 | 1.45 | 1.87 | 1.43 | 2.14 |
| *p*-cymene | 1026 | 8.70 | 7.43 | 9.78 | 11.45 |
| limonene | 1030 | 0.28 | 0.47 | 0.39 | 0.56 |
| γ-terpinene | 1062 | 8.74 | 8.26 | 9.30 | 9.26 |
| trans-sabinene hydrate | 1097 | 0.43 | 0.61 | 0.72 | 0.75 |
| camphor | 1141 | - | 0.96 | - | 0.82 |
| borneol | 1165 | 2.15 | 2.83 | 2.81 | 3.55 |
| terpinen-4-ol | 1177 | - | 1.03 | - | - |
| thymol methyl ether | 1235 | 5.40 | 6.47 | 5.25 | 6.00 |
| Z-citral | 1240 | 1.32 | - | 0.02 | - |
| carvacrol methyl ether | 1241 | 3.94 | 4.67 | 4.22 | 4.42 |
| thymol | 1290 | 42.87 | 49.53 | 42.51 | 34.61 |
| thymyl acetate | 1352 | 0.63 | - | 0.52 | 0.35 |
| neryl acetate | 1359 | 0.28 | - | - | - |
| trans-caryophyllene | 1422 | 1.80 | 2.38 | 2.09 | 1.83 |
| α-bisabolene | 1504 | 3.54 | - | 3.01 | 2.79 |
| β-bisabolene | 1509 | 2.40 | - | 4.05 | 2.35 |
| γ-cadinene | 1513 | 0.01 | - | 0.01 | 0.01 |
| monoterpene hydrocarbons | | 23.43 | 23.31 | 23.53 | 29.49 |
| oxygenated monoterpenoids | | 57.02 | 66.1 | 56.05 | 50.5 |
| sesquiterpenes hydrocarbons | | 7.75 | 2.38 | 9.16 | 6.98 |
| **Total identified** | | 88.2 | 91.79 | 88.74 | 86.97 |
| *$H_1$ - 115 days after planting (DAP), $H_2$ - 135 DAP, $H_3$ - 155 DAP, $H_4$ - 175 DAP* | | | | | |

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