World Applied Sciences Journal 27 (9): 1126-1129, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.27.09.716

Quantitative and Qualitative Assessment of Thymus Oil Field Accession of Cultivated Thyme (*Thymus vulgaris*) in Field Conditions

[1]*A. Zarezadeh,* [2]*Mahdi Jannati,*
[3]*Mahdi Mirza and* [3]*Ibrahim Sharifi ashourabadi*

[1]Scientific Board of Natural Resources and Agricultural research Center of Yazd Province
[2]Lecturer and head of department of Hort Science Iran-Yazd-Shahidan ashraf Blvd,
Mollasadra High Educational Center of Applied Science
[3]Scientific Board of Research Institute of Forests and Rangelands

Abstract: *Thymus* from Lamiaceae with 18 species of aromatic perennial, of which four species are endemic to Iran. This study was conducted during 2008-2011 to Phyto-chemical inspection of essences of different Thymus vulgaris Accession. At 50% full bloom Samples were collected from 5 cultivated Accession of YAANRR collection, dried and ground. Extractions were done base on the water distillation. Essential oils were analyzed using GC and GC/MS. The highest and the lowest yields of essential oil were obtained with XDRVGY and NJINJI with 2.8% and 9.0% respectively. The highest and the lowest yield of essence were for XDRVGY (87.5 $Kgha^{-1}$) and XDRXDR (5.9 $Kgha^{-1}$). Major components were: Parasymon, gamma-terpinene, borneol, thymol, carvacrol, limonene, linalool and E-Caryophyllene (27.8%, 2.3%, 3.11%, 3.7%, 48.5%, 7.7% 28.4%, 79% and 3.4%), respectively for XDRVGY, XDRVGYT, XDRXDR, XDRBHU, XDRXDR, NJINJI, MKOQ, XDRBHU and XDRVGY. Considering the efficiency, production and constituent of oils, Accession XDRVGY originated from Markazi province was introduced with yields 2.8 %, 87.5 $Kgha^{-1}$ of essential oil and 45.8% thymol and 27.8 % Parasymon as the elite from cultivated *Thymus vulgaris* accessions.

Key words: Garden thyme · Essential oils · Carvacrol · Thymol · Yazd

INTRODUCTION

Thymus genus belonging to the family Lamiaceae and over 215 different species from around the world to be found. The genus contains 18 species of aromatic perennial and among them; four species are endemic to Iran [1]. Thymus vulgaris plants with bushy form Grows between rocks, at the Mountain range of the dry areas of the Mediterranean countries, including France, Portugal, Spain, Italy and Greece [2]. In the semi-arid areas of New Zealand, there are several thousand hectares of self propelled thyme [3]. An increase is being witnessed in thyme cultivation in Iran. Thyme has a variety of uses in food, pharmaceutical, cosmetic and toiletry. Thyme essential oil has amazing properties such as: antispasmodic, carminative, anti-fungal, antiseptic, anti-worm, anti-rheumatic and expectorant and is known among dozens of important essence with anti-bacterial and anti-fungal attributes, anti-oxidants, food preservative and delay aging properties in mammalian as well as has a special position in the world trade of medicinal plants [4]. Garden Thyme plant, has a straight herbaceous or woody stems, ramous, height to 10 to 30 cm in some cases to 45 cm. Branched stems of this plant are covered with fine white slightly fragrant evergreen leaves, opposite, almost without petiole or short petiolate, elliptical leaves 5 to 15 mm long, lanceolate light gray. Sepals have fluff, gland-like and leave-like bracteas. The lateral branches in bundles and spiral purple flowers fade to white appears, either elliptical or spherical-shaped flowers are terminal. Tetrachen small fruits are surrounded by calyx of flowers [5, 6, 7]. Tests results on the effect of harvesting time and

Corresponding Author: Mahdi Jannati, Lecturer and head of department of Hort Science Iran-Yazd-Shahidan ashraf Blvd, Mollasadra High Educational Center of Applied Science.
Tel: +98-351-8249901, Fax: +98-351-8247439.

method of Essence extraction on the quality and quantity of essential oils suggested that: different Harvest stages (vegetative, early flowering, full flowering), were significantly associated with the essence yields of thyme in 1% Probability level. The highest yield was for early flowering, which was equivalent to 1.18 percent. Constituent components analysis showed that all stages of harvesting and extracting method, thymol, Gamma Terpinene and Parasymon are the main Constituents of the essential oil [8]. The major compounds in the essential oil of thyme shoot Albanian origin, by steam distillation, Parasymon (43.75%-7.76%), Gamma Terpinene (27.62% -4.2%) and thymol (60.15% -21.38%) have been reported [9]. Thymol 39.1%, beta Caryophyllene 11.1% and Parasymon 10.5% has been Main components of the essential oil of Porlock Thymus [10]. Harvest time of Thyme is the critical point in the field management of the plant [3]. In general, the best time to collect vegetative organs to obtain the highest yields of essence is in the flowering stage of plant [11, 12]. In different places, the best time of Thyme Harvesting is different. In a study undertaken in climatic conditions of Karaj/Iran, suitable harvesting time, have been reported at the flowering stage and 10 cm height from ground level [13]. To prevent of decreasing of thyme odor, scent and its green color during the evaporation, plant should be dried at temperatures below 40°C [3]. Cross pollination in thyme genus is very routine. Whenever two or more species can be grown together, hybridization occurs between them. There are high morphological variations among the populations of Thymus species [14]. The major components of the essential oil of wild thyme from southern Italy including thymol, Gamma Terpinene, Parasimon, linalool, Myrsen, alpha-pinene, eugenol, carvacrol and alpha thozhan essential oil has been reported [15]. Khorrami *et al*, identified Essential oil composition of aerial parts of the flowering thyme grown in Estahban/Fars/Iran; They have reported yields 1.6% thymol, 63.1%, carvacrol 3.1%, Beta Caryophyllene 1.3%, 5 Parasymon 9.5% and Gama-tripinene 8.7% [16].

MATERIALS AND METHODS

Climatic Conditions of Research Station of Medicinal Plants in Yazd Province: Yazd Medicinal Plants Research Station[1] with about 40 hectares, coordinates of latitude and longitude 27 15 54 East and 55 31 49 north, elevation of 1210 meters above sea level in the region has been located in the Shahedyeh area, Yazd province, Iran. The average rainfall is about 70 mm; average temperature 18.8°C, the maximum is in July. The absolute maximum temperature zone 45.5°C and the minimum is - 15°C. As do marten classified, the climate is extra warm and dry, Soil conditions of the station, is Medium sandy- loamy soil textured and drainage of water is easily possible. Since the electrical conductivity of the soil was suitable and it is low in salt concentration and the quality of the water will not bring many restrictions to the growth and development of most plants, Alkaline soil, which was the amount needed for most plants is well tolerated. The soil fertility due to organic and chemical fertilizers were suitable was well. Except in the case of potassium, this was moderate. There is at least the possibility of nitrogen and phosphorus nutrition [17]. 5 accession planting operations in the second half of January 2007 through seeds, within the Jiffy Pots in greenhouse of Yazd Research Center for Agriculture and Natural Resources Was performed. After about 2.5 months, while they were 10-12 leaf, transplanted to plastic pots and then a month later they were transferred to the field station of Medicinal Plants Research. In the second year of planting for each sample to determine the amount of oil, at 50% full bloom, half maximum of 3 to 5 plants, 5 cm above the ground were harvested. After recording the fresh weight of the samples, were dried in the shade. After determining the oil yield based on the dry weight of plant, GC and GC / MS analysis was performed to identify the composition of the essential oils. In case of extraction of essential oils, 5 g of each sample prior to extraction of oil in the oven at 70°C for 48 h were exposed to moisture should be measured. Distillation method was used to extract the oil was based on the by BPCP[2] for 150 min. In this method, about 100 grams of dried plant specimens in the distillation flask and poured it on, add distilled water to two-thirds of the volume occupied by the balloon and then the balloon is attached to a Clevenger apparatus. Flask was heated and plant essences with the vapor were became into two-phase liquid in the refrigerant part of Clevenger. In most cases, the two-phase mixture, oil makes the top phase. Until Injection of the oil phase to the GC and GC / MS analyzers, the glasses of oils samples to be kept in the refrigerator.

Analysis by Gas Chromatography (GC): Gas chromatography system is a very reliable means for separating compounds and provides important

[1]Yazd Research Center for Agriculture and Natural Resources

[2] British Pharmacopoeia Clevenger plan

information about the percentage of the separated components. Gas Chromatography Thermo-UFM (Ultra Fast Model) made in Italy and processing Chrome-Card A / D, Capillary columns with commercial Ph-5 (non polar) manufactured by Thermo, length of 10 m and an inner diameter of 0.1 mm thick o.4 mm, the inner surface was covered with stationary phase material, Dimethyl siloxane phenyl 5%. Column temperature was 60°C programmed for stating. To reach a final temperature of 285°C, 80°C per minute was added. Stopped at this temperature for 3 minutes. Type of used FID was of the detector and helium as the carrier gas to the column, inlet pressure is set equal to 0.5 kg/cm. Detector and injection chamber temperature Were adjusted 280 and 290°C respectively.

Analysis by Gas Chromatography Connected to a Mass Spectrometer (GC/MS): Varian 3400 gas chromatograph connected to a mass spectrometer device model Saturn II, the ion trap system with ionization energy of 70 eV with a semi-polar column DB-5 column (length 30 m, inner diameter of 0.25 mm and the thickness of the phase resident of 0.25 mm) have been used. Gas pressure 35 pounds per square inch, column temperature 40 to 250°C with an increasing rate of 3°C per minute, the injection chamber temperature 260°C and. The temperature of the transfer line was set 270°C. Spectra identified using their retention indices and by injection of normal hydrocarbons (C7-C25) under the same conditions and with the injection of oils were determined by a computer program and the Basic language. Also, comparing them was performed with various resources [2, 18, 19] and with using of mass spectra standard compounds and information of the library of GC / MS analyzer. To calculate the moisture content of 5 grams of the sample taken at least 24 hours at 45°C and placed in the oven. Dried samples were weighed accurately after dehumidifier. Essential oil distillation method was performed. After determining the oil yield based on the dry weight of oil by gas chromatography and gas chromatography linked to mass spectrometry analysis and identification of essential oils was performed by the Research Institute of Forests and Rangelands.

RESULTS AND DISCUSSION

Survey conducted shows that for 5 accessions planted; the highest yields in flowering time for the accession XDRVGY with2.8% essential oil and the lowest 0.9% for the accession NJINJI. The highest and the lowest oil yield per hectare, respectively accessions XDRVGY and XDRXDR with 87.5 and 5.9 kg oil per hectare. The maximum amount of essential oil compounds were Parasymon, Gamatripinene, Borneol, thymol, carvacrol, limonene, linalool and E-Caryophyllene respectively 27.8%, 2.3%, 11.3%, 3.7%, 48.5%, 7 / 7%, 28.4%, 79% and 3.4%, respectively accession of XDRVGY, XDRVGYT, XDRXDR, XDRBHU, XDRXDR, NJINJI, MKOQ, XDRBHU, XDRVGY. Considering the efficiency and production of oil and composition of constituent oils; accession XDRVGY origin Markazi province with yields 2.8 percent and 87.5 kg/ha essential oil and 45.8% thymol and 27.8 Parasymon are introduced as top accession of Thymus vulgaris species grown. All accession contains thymol, carvacrol, linalool and E-Caryophyllene. Thymol was the predominant compound in all accession and from 3.4% to 48.5% was variable. Thymol in accession XDRXDR was highest and the lowest was related to accession XDRBHU. The maximum carvacrol (7.7%) to accession JINJI and the lowest was

Table: Efficiency, production rate and percentage of accession essential component of garden thyme, during flowering

Scientific name	T. vulgaris	T. vulgaris	T. vulgaris	T. vulgaris	T. vulgaris
Accession code	MKOQ	NJINJI	XDRBHU	XDRVGY	XDRXDR
production rate kg/ha	14	9.8	20.1	87.5	5.9
Efficiency)	0.5	0.9	1.5	2.8	1.8
E-Caryophyllene (%)	1.5	2.8	0.8	3.4	1.9
Linalool (%)	4.2	2.9	79	1.3	4.2
Limonene (%)	28.4	-	0.4	-	-
Carvacrol (%)	5.2	7.7	3	4.6	7.3
Thymol (%)	38.8	45.9	3.4	45.8	48.5
Borneol (%)	1.4	1.3	3.7	2.1	1.3
µ-terpinene (%)	1.6	5.8	-	0.7	11.3
1,8-cineole (%)	-	-	1.4	2.3	1.4
p-cymene (%)	-	23.3	0.6	27.8	14.6

related to accession XDRBHU (3%). The maximum rate of Linalool (79%) and the lowest thymol and carvacrol was observed with accession XDRBHU. In general, there were various types of accession efficiency, yield and essential oil constituent material. Changes are due to genetic diversity between different accessions in their habitats from different climates. Major component identified in this study match with the compounds identified by other researchers [8-10, 15, 16]. In accessions: NJINJI, XDRVGY, XDRXDR. Except for a composition E-Caryophyllene that Khorrami and Mirza Instead of it, reported beta–Caryophyllene [10, 16] and is in contrast to major components of accessions MKOQ and XDRBHU lacking Parasymon and gamma-terpinene respectively (Table 1).

REFERENCE

1. Jamzad, Nice, 2008. Iranian Thymes and Savories. Research Institute of Forests and Rangelands Publications. Tehran, 171 pages [In Persian].
2. Davis, N.W., 1990.Gas chromatographic retention indices of monoterpense and sesquiterpenes on methyl silicone and carbowasx 2oM phaseses.
3. McGimpsey, J.A., M.H. Douglas, J.W. van Klnk, D.A. Beauregard and N.B. Perry. 1994. Seasonal Variation in essential oil yield and composition from naturalized Thymus vulgaris L. in Newzealand. Flavors and Fragrance J., 9: 347-52.
4. Leung, A.Y. and S. Foster, 1996. Encyclopedia of common natural ingredients: used in food, drugs and cosmetics. A Wiley Interscience publication. John Wiley & Sons, Inc., pp: 649.
5. Burnie, D., 1995. wild flowers of Mediterranean. Dorling Kindersley, pp: 320.
6. Letchamo, W.R., J. Marquaed, Holzl and A. Gosselin, 1995. effect of water supply and light intensity on growth and essential oil of two thymus vulgaris selections. Hort. Absts, 65: absts: 11028.
7. Morton, J.F., 1977. Major medicinal plants, botany, culture and uses. Charles C. Thomas publisher, Banner stone House, pp: 431.
8. SEFIDKON, F. and F.E. Sharifi Nikkhah, 2000. Effect of harvesting time and methods of measurement of the quantity and quality of essential oil of Thymus vulgaris. Medicinal Plants Research 3(45): 320-309 [In Persian].
9. Asllani, U. and V. Toska, 2003. Chemical/composition of Albanian thymus oil (thymus vulgaris).Journal of essential oil research, 15: 165-1670.
10. Mirza, M. and Z. Baher, 2003. chemical composition of essential oil from thymus vulgaris hybrid. Journal of Essential oil research, 15: 329-330.
11. OmidBeigi, Reza, 2000. Production and processing of medicinal plants. Volume III. Second edition. Astan Quds Razavi Publications [In Persian].
12. Zargari, A., 1990. Medicinal plants. Sixth edition. Tehran University Press, Volume IV, pp: 28-38 [In Persian].
13. Naqdibadi, H.A. and S.A. Yazdani, 2002. Seasonal variation of essential oil yield and composition at different planting densities. Journal of Medicinal Plants, 5: 6-51. [In Persian].
14. Thompson, J.D., 2002. Population structure and the spatial dynamics of genetic polymorphism in thyme in The Genus Thymus, pp: 76-122.
15. Shabnum, S. and W.M. Wagay, 2011 Essential oil composition of thymus vulgaris and Their uses, Journal of research & Developmentroii, pp: 83-95.
16. Khorrami, M. *et al*., 2011. Constituent compounds compared daenensis and thyme essential oil. New ideas Sixth National Conference on Agriculture, Islamic Azad University, Faculty of Agriculture [In Persian].
17. Zare Zadeh, A. *et al*., 2005. Yazd Medicinal Plants Collection Final Report. [In Persian].
18. Shibamoto, T., 1987, retention indices in essential oil analysis in capillary Gas chromatography in essential oils analysis. Edits., P. Sandra and C. Bicchi, Dr. Alfred Huethig Verlag, New York, pp: 259-274.
19. Adams, R.P., 1989. Identification of essential oil by ion Trap mass spectroscopy. New York. : Academic press McLaffety, F.W. and Stauffer, D. B. (1989). The Wiley NBS registry of mass spectral data. New York: John Wiley, pp: 302.