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PREFACE

Dear colleagues,

It is with great pleasure that I introduce the *Book of Proceedings* for the 15th International Scientific Agricultural Symposium "AGROSYM 2024." I trust that you will find it a valuable resource in your work. This year, around 800 contributions have been accepted for inclusion in the *Book of Abstracts*. The themes of AGROSYM 2024 span the full spectrum of agricultural sciences, divided into seven key sessions: 1) Plant Production, 2) Plant Protection and Food Safety, 3) Organic Agriculture, 4) Environmental Protection and Natural Resource Management, 5) Animal Husbandry, 6) Rural Development and Agro-Economy, and 7) Forestry and Agroforestry.

There is growing consensus among scholars and practitioners that technological innovations have the potential to boost agricultural production, enhance supply stability, and reduce the environmental impact of farming. Technology has been especially pivotal in increasing the productivity of annual crops such as maize, rice, soybeans, wheat, and cotton. However, with trees having longer growth cycles, breeding programs for these crops require more time. New plant breeding techniques promise improvements in productivity, but they have sparked an ongoing academic debate regarding their advantages and drawbacks.

While much of the focus in the past has been on the production side of agriculture (e.g., productivity and efficiency), there is an increasing emphasis today on the consumption side and the intermediate stages of the food chain, such as processing and distribution. This shift is driving the transition toward a "farm-to-fork" approach. Globally, consumers are sending clearer signals about their preferences for higher quality, healthier, safer, and more flavorful products. In response, many agri-food companies are exploring innovative ways to gain greater control over production processes and ensure the quality and safety of final products. Furthermore, changes in investment strategies hold the potential to reduce the environmental and social costs of agriculture. It has become increasingly clear to investors that companies that prioritize sustainability and social responsibility tend to yield better long-term returns.

Agri-food systems are at the heart of global discussions surrounding sustainable development and the achievement of the United Nations Sustainable Development Goals (SDGs) by 2030. These systems are deeply connected to numerous global challenges, including climate change, poverty, food insecurity, biodiversity loss, resource depletion, and ecosystem degradation. A key goal of the sustainable agriculture movement is to create farming systems that mitigate or eliminate the environmental harms associated with industrial agriculture. Additionally, it is critical to improve the resilience of food systems to crises, shocks, and pandemics.

I would like to extend my heartfelt thanks to all the authors, reviewers, and colleagues who contributed to the editing of the *Book of Abstracts*. Special thanks are due to our co-organizers and partners for their unwavering collaboration and comprehensive support throughout this endeavor.

Dušan Kovačević

East Sarajevo, 10 October 2024
Prof. Dušan Kovačević, PhD
Editor in Chief, President of the Scientific Committee of AGROSYM 2024

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EFFECTS OF HARVESTING TIME ON ESSENTIAL OIL YIELD AND COMPONENTS OF ORGANICALLY GROWN THYME (*Thymus vulgaris* L.)

Mehmet ARSLAN[1*], Sancar BULUT[2]

[1]Erciyes University Agricultural Faculty, Department of Agricultural Biotechnology, 38039-Kayseri, Turkey
[2]Kayseri University Safiye Cikrikcioglu Vocational Collage, Department of Plant and Animal Production, 38280, Talas-Kayseri, Turkey
*Corresponding author: mehmetarslan@erciyes.edu.tr

Abstract

Timing of essential oil-bearing plant harvest is a key determinant in achieving higher oil content and quality in the essential oil production. Thyme (*Thymus vulgaris* L.), an herbaceous perennial plant from the Lamiaceae family, is widely cultivated for its essential oil. Thyme essential oil has attracted much attention, due to its usage in medicine, pharmacy, perfumery-cosmetic and food industries. The aim of this study was to determine the effects of harvest time on essential oil content and composition of organically grown thyme under continental type of climate. The study was carried out in growing season of 2021 to determine the effects of harvesting hours on essential oil content and composition of thyme using a completely randomized design with four replications. Thyme was harvested at a two-hour intervals starting at 6:00 and ending at 18:00. Essential oils of freshly harvested and dried plants were extracted by hydro-distillation for three hours and essential oils were analyzed by gas chromatography to determine the composition and quality of the essential oil. Essential oil content of thyme varied slightly among the harvest time of the day. The highest essential oil yield was obtained from early morning harvest hours. More than 27 essential oil components were found in the essential oil of each harvest hour. Thymol, γ-terpinen, p-cymene and carvacrol were the main essential oil components of all harvest hours. The major essential oil components did not significantly fluctuate among the harvesting hours. The results of this study showed that the best harvesting hours of thyme for higher essential oil yield were between 6:00 and 10:00 AM in the morning.

Keywords: *Harvesting time, Essential oil, Thyme, Organic farming.*

Introduction

Thymus vulgaris is a perennial plant from the Lamiaceae family native to southern Europe. It is about 15-30 cm tall and 30-40 cm wide. Thymus species are grown in most of the European countries, especially France, Spain, Italy, Bulgaria and the Portuguese Greece, Turkey, Israel, Morocco and North America (Stahl-Biskup and Sáez, 2002; Maghdi and Maki, 2003). Today there are approximately 250 taxa divided into eight divisions (214 species and 36 subspecies) are accepted.

Thyme, used for food and medical purposes, which has great economic importance due to its monoterpene derivatives such as thymol, γ-terpinen, p-cymene and carvacrol (Aeschbach *et al.*, 1994; Grigore *et al*., 2010).. Thyme was known to have hepatoprotective properties and its effectiveness as an expectorant, anti-acne agent, and fungicidal and antiviral effects. Thyme, used for food and medical purposes, has great economic importance due to its monoterpene derivatives such as thymol, γ-terpinen, p-cymene and carvacrol. Thyme was known to have hepatoprotective properties and its effectiveness as an expectorant, anti-acne agent, and fungicidal and antiviral effects (Bukovska et al. 2007). These numerous industrial

applications are mainly due to its antioxidant, antimicrobial, anti-inflammatory and anticancer effects (Miura and Nakatani, 1989; (Katsiotis, *et al*., 2009).

Essential oil content and components of thyme vary depending on the development stages in which they are harvested. Depending on the harvest periods, the essential oil content of the thyme varies between 0.4-4% (Ozguven and Tansi, 1998; Carlen *et al*., 2010). The major component of thyme, thymol, is active against enterobacteria and cocci bacteria. Thyme may also improve liver function and act as an appetite stimulant. Thyme used as a gargle is beneficial in the treatment of laryngitis and inflammation.

Most of the essential oil bearing plants is mostly harvested at the beginning of the flowering or during the flowering periods. (Bagdat, 2006; Karik et al., 2007; Hazrati et al. 2022). The content and composition of the essential oil varies with harvest hours of a day. Changes in the content and composition of essential oils at different hours of a day have been less studied (Salehi and Hazrati, 2017; Salehi et al., 2017; Kaya et al., 2013). Secondary metabolite content and composition are under influence of environmental factors such as soil profile, plant nutrition, light, photoperiod, temperature, day/night temperature ratio, precipitation, moisture, irrigation conditions and altitude (Silva et al., 1999; Rao et al., 2001; Figueiredo et al. 2008; Ramezani et al., 2009; Hassiotis et al., 2010; Bufalo et al., 2015). Therefore, to determine the most optimum harvest hour, it is necessary to consider essential oil content and essential oil composition to have maximum economic gain from the cultivated essential oil bearing plants. The purpose of this study was to determine an optimum harvest time (hour) in a day for higher essential oil yield and higher thymol content of thyme.

Material and Methods

The study was carried out at the Experimental Field of the Faculty of Agriculture, Erciyes University in Turkey, in 2021 growing seasons. One-year-old thyme plant was used for determining the best harvest time. At the beginning of the flowering stage, plants were harvested at two-hour intervals starting from 6:00 to 18:00 in 25 June 2021. Freshly harvested plant samples in each harvesting hour were sent for essential oil extraction and shadow drying to determine essential oil contend of both fresh and dry thyme plant. The fresh and shadow dried thyme plants were steam distilled for 3 h using a Clevenger type apparatus. The oil ration was expressed as w/v with respect to the fresh and dry herb of the initial material.

Analysis of the essential oil was carried out by using Thermo Scientific Focus Gas Choromatograph equipped with MS, auto sampler and Rtx®-5 capillary columns (60 m x 0.32mm, 0.25µm film thickness). Helium (99.9%) was the carrier gas at a flow rate of 1 mL/min. MS transfer line temperature was 250 °C, MS Ionization source temperature was 220oC, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1µL. The oven temperature was programmed to 50 °C to 220 °C at 3 °C /min. The structure of each compound was identified by comparison of their mass spectrum (Wiley) using the Xcalibur software, version 2.0.7.

Results and Discussion

The essential oil content and components of fresh and dry herbage of thyme were given in Table 1, Table 2 and Table 3. The essential oil content significantly varied among the harvesting hours (Table 1).

Essential oil contents of fresh herbage varied between 0.75 and 0.98%. The lowest essential oil content was obtained from 12:00 PM harvesting hour, while the highest was obtained from 6:00 o'clock harvesting hour. When dry herbage was in consideration, essential oil content varied between 1.92 and 2.40 %. Like fresh herbage, the highest essential oil content was

obtained from early morning harvest (6:00 AM), and the lowest was obtained at 2:00 PM harvesting hour. Our results are in good agreement with the findings of Ozguven and Tansi, (1998); Carlen *et al*., (2010) that the essential oil content of the thyme within the reported limits.

Table 1. The effect of harvest hours of thyme on essential oil content.

Harvesting hour	**Essential oil content (%)**	
	Fresh herbage	**Dry herbage**
6:00 AM	0.98±0.01	2.40±0.02
8:00 AM	0.84±0.01	1.98±0.02
10:00 AM	0.86±0.02	2.23±0.02
12:00PM	0.75±0.01	1.95±0.01
2:00 PM	0.77±0.01	1.92±0.03
4:00 PM	0.79±0.02	1.97±0.01
6:00 PM	0.96±0.03	1.97±0.01

Table 2. The effect of harvest hours of fresh thyme on essential oil components

Components	RT	Harvesting hours of fresh herbage						
		6:00 AM	8:00 AM	10:00 AM	12:00 PM	2:00 PM	4:00 PM	6:00 PM
α-Pinene	3.63	0.90	0.74	0.65	0.62	0.85	0.68	0.69
α-Thujene	3.69	1.34	0.96	0.88	0.92	1.17	0.77	0.97
Camphene	4.34	0.67	0.65	0.51	0.42	0.68	0.78	0.46
β-Pinene	5.13	0.25	0.23	0.18	0.20	0.25	0.19	0.22
β-Myrcene	6.48	1.52	1.31	1.08	1.06	1.43	1.09	1.22
δ-4-Carene	6.88	1.20	1.06	0.91	0.86	1.05	0.93	1.20
Bornylene	7.40	0.41	0.41	0.32	0.29	0.41	0.32	0.37
Eucalyptol	7.59	0.83	0.78	0.57	0.90	0.72	0.63	0.72
β-Phellandrene	7.67	0.14	0.11	0.11	0.67	0.07	0.11	0.16
γ-Terpinene	7.96	9.40	10.10	11.72	9.60	7.38	8.85	8.71
p-Cymene	10.28	15.31	18.05	19.12	17.14	16.21	15.13	15.09
1 Octen 3 ol	16.13	0.44	0.42	0.45	0.51	0.42	0.45	0.42
Cis-Sabinene hydrate	16.54	0.86	0.87	1.03	1.04	0.88	0.94	0.80
Camphor	18.31	0.39	0.40	0.44	0.42	0.34	0.36	0.71
Linalool	19.87	1.92	2.12	1.30	1.70	1.19	1.12	1.44
Linalyl acetate	20.24	0.19	0.12	0.17	0.06	0.13	0.21	0.22
Caryophyllene	21.35	1.55	1.32	1.98	1.40	2.47	4.04	2.41
Thymyl methyl ether	21.62	0.63	0.77	0.25	0.20	0.62	0.25	0.22
Terpinene 4-ol	21.74	0.68	0.50	1.44	0.54	1.06	1.75	1.81
Carvacrol methyl ether	22.00	0.23	0.15	0.18	0.21	0.36	0.25	0.17
β-Selinene	24.02	0.09	0.05	0.18	0.08	0.20	0.23	0.15
Borneol	25.33	1.79	1.51	1.00	1.42	1.96	1.36	1.50
Caryophyllene oxide	34.79	0.76	0.74	0.58	0.62	0.60	0.50	0.33
Thymol	41.73	46.30	45.42	44.90	43.19	38.63	35.20	32.74
Carvacrol	32.09	2.95	5.11	5.19	9.43	12.37	15.27	23.22

Twenty-five essential oil components was obtained in the essential oil of fresh herbage (Table 2). Thymol, carvacrol, p-Cymene and γ-terpinene were the major essential oil components. When major essential oil components were compared among the harvesting hours, they

fluctuated among the harvesting hours. Thymol which is the most important essential oil components of thyme oil varied among harvesting hours. The highest thymol rate was obtained early morning harvest hours with 46.30%. On the other hand, carvacrol content was low in the early morning harvest hours. Thymol content negatively correlated with carvacrol content. p-Cymene and γ-Terpinene ratios also fluctuated among harvesting hours.
The analysis of dry herbages resulted in detection of 26 essential oil components (Table 3). Like fresh herbage, the major essential oil components were thymol, carvacrol, p-Cymene and γ-terpinene. Essential oil components varied among harvest hours. The highest thymol rate was obtained for the early morning harvest hours. Thymol contains decreased with the midday and late harvest hours. Senatore (1996) stated that the best time for thyme harvest was during or immediately after the full flowering stages. In the present harvesting study, thyme plant was harvested at the beginning of flowering stage.

Table 3. The effect of harvest hours of dry thyme on essential oil components.

Components	RT	Harvesting hours of dry herbage						
		6:00 AM	8:00 AM	10:00 AM	12:00 PM	2:00 PM	4:00 PM	6:00 PM
α-Pinene	3.63	0.14	0.70	0.61	0.59	0.46	0.67	0.54
α-Thujene	3.69	0.18	0.93	0.96	1.66	0.71	1.18	0.96
Camphene	4.34	0.05	0.56	0.41	0.52	0.27	0.44	0.34
β-Pinene	5.13	0.07	0.22	0.20	0.17	0.15	0.21	0.19
Sabinene	5.43	0.08	0.03	0.47	0.00	0.14	0.30	0.19
β-Myrcene	6.48	0.36	1.23	1.15	1.03	0.94	1.31	1.28
δ-4-Carene	6.88	0.41	1.04	1.26	0.94	1.12	1.92	1.77
Bornylene	7.40	0.11	0.36	0.38	0.29	0.30	0.44	0.44
Eucalyptol	7.59	0.28	0.80	0.68	0.56	0.40	0.47	0.43
β-Phellandrene	7.67	0.00	0.11	0.16	0.11	0.16	0.25	0.25
γ-Terpinene	7.96	11.23	9.45	13.70	8.10	9.87	9.96	10.14
p-Cymene	11.20	16.35	18.20	16.61	15.42	16.01	15.22	14.83
α-Terpinolene	10.02	0.15	0.29	0.06	0.07	0.00	0.05	0.32
1 Octen 3 ol	16.13	0.37	0.43	0.21	0.51	0.47	0.30	0.40
Cis-Sabinene hydrate	16.54	0.85	0.97	0.51	0.89	0.96	0.57	0.96
Trans-sabinene hydrate	19.69	1.95	1.97	0.88	0.60	1.04	1.53	1.67
Linalool	19.87	1.24	1.57	0.46	1.65	1.46	1.12	1.42
Linalyl acetate	20.24	0.61	0.33	0.22	0.12	0.32	0.11	0.31
Caryophyllene	21.35	3.36	2.78	2.69	1.60	2.50	2.72	1.99
Thymyl methyl ether	21.62	0.18	0.07	0.00	0.95	0.14	0.33	0.56
Terpinene 4-ol	21.74	2.81	2.94	1.35	0.89	2.16	2.99	2.89
Borneol	25.33	1.31	1.60	1.06	1.51	1.23	1.30	0.68
Farnesol	26.34	1.83	0.43	0.32	0.16	0.13	0.95	0.31
Caryophyllene oxide	34.79	0.54	0.48	0.88	0.66	0.54	0.69	0.49
Thymol	41.73	33.50	35.42	36.90	34.16	32.21	30.23	32.29
Carvacrol	31.78	23.72	18.60	16.61	20.24	22.80	23.30	24.11

Conclusions

Different harvesting times were evaluated to understand the importent parameters for producing the highest quantity and quality of thyme essential oil. Harvesting time schedules in day for the thyme (6:00 AM, 8:00 AM, 10:00 AM, 12:00PM, 2:00 PM, 4:00 PM and 6:00

PM) greatly affected essential oil content and composition of thyme. Both fresh and dry herbages, the highest essential oil content was obtained from 6:00 AM harvesting hour with 2.40 % and the lowest essential oil content was obtained from 2:00 PM with 1.92 %. The essential oil contents in midday harvests were lower than the early and late harvesting hours due to increased air temperature. The major essential oil component ratios varied among harvesting hours. The high thymol content is in trend to associate with the early morning harvest hours optimum harvesting hours for thyme.

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