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THE EFFECT OF HARVESTING TIME ON ESSENTIAL OILS COMPOSITION OF *THYMUS PANNONICUS* L.

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Abstract: In this paper the authors investigating the possible effect of harvesting time on essential oils composition at *Thymus pannonicus* L., knowing that the harvesting date, time of day and weather conditions is very important for the quality and quantity of essential oils. *Thymus pannonicus* L. is a perennial herbaceous plant, distributed in central and eastern Europe. In Romania these plant is spread all over the country, including two subspecies *pannonicus* and *auctus*. The vegetal material was collected in 2 different phenophases (vegetative and anthesis), during 2 consecutive years. The chemical composition of the essential oil was established by GC-MS analysis with the help of a gas-chromatograph Agilent Technologies coupled to a mass detector. The main chemical components of essential oils are germacrene D (between 8.05% and 17.31%), nerolidol (between 7.74% and 18.49%), farnesol (between 12.95% and 14.77%) and α-terpinyl acetate (between 6.56% and 9.58%). Major differences were registered at the taxa collected in 2013 in anthesis stage, where the main components were carvacrol (42.32%) and thymol (13.98%).

INTRODUCTION

As part of the Lamiaceae family, comprising over 220 genera, *Thymus* is one of the most important genera, due to the number of species (about 350), species that vary greatly from taxonomic and biochemical point of view (Morales, 2002). The species of *Thymus* genus have been used for more than 2000 years as medicinal plants and many of them are still being used today (Rasooli and Mirmostafa, 2002; Tzakou and Constantinidis, 2005; Zarzuelo and Crespo, 2002). Their essential oils are utilized as flavour ingredients in a wide variety of food, beverage and confectionery products, as well as in perfumery for the scenting of soaps and lotions (De Martino et al., 2009). Because of their antiseptic, antispasmodic and antimicrobial properties, the essential oils of *Thymus* species are also used for medicinal purposes (Cosentino et al., 1999; El-Hela, 2007; Jirovetz et al., 2007; Maksimović et al., 2008; Safaei-Ghomi et al., 2009).

Thymus pannonicus L. is a perennial herbaceous plant, distributed in central and eastern Europe. It grows over open dry meadows, grasslands and rocks (Jalas, 1972). In Romania these plant is spread all over the country, including two subspecies *pannonicus* and *auctus* (Oprea, 2005). This species presents vigorous branched stems, covered with hairs with the same length of the axis diameter. The leaves are elliptic or prolonged, 6-12 mm in long and 3-5 mm wide, green in color, both epidermis are covered with hairs, nervures little proeminent. The inflorescence is capitate. The calyx is 3-4 mm long, the corolla is lilac-red, 6-7 mm long (Guşuleac, 1961). *Thymus pannonicus* is commonly used as herbal tea, flavoring agent and medicinal plant due to his biological active substances (such as thymol, carvacrol, geraniol, linalool and other compounds from the essential oil) (Stahl-Biskup and Saez, 2002).

The quality and the quantity of volatile oils from plants can be influenced by a series of environmental factors like the temperature, the radiations and the photoperiod (Yamura et al.,1989). Also, the nutritive material indispensable for plants’ growth, the water, the mineral elements and the nitrogen plays a primordial role on the metabolism products (Rajeswara et al.,1990). On the other hand, the seasonal gradient from the growth period is associated with changes of some environment parameters, like the photoperiod, the air temperature and the available water. The combination of these factors exerts a pressure on the plant that is expressed through influences of the morphology, anatomy, physiology and its productivity. The influence of such factors on the essential oils production was not widely investigated. Thus, the main objective of this paper is to highlight the possible effect of harvesting time on essential oils composition of *Thymus pannonicus*. For this purpose individuals of this species were collected in vegetative and full flowering phases, two consecutive years.

MATERIAL AND METHODS

Vegetal material

The vegetal material was represented by *Thymus pannonicus*, a species that grows wild in the Romanian flora. The species was collected in 2 different phenophases (vegetative and anthesis), during 2013 and 2014, from Fălticeni, Suceava

County, Romania. The identification of taxa was made by Dr. Ioan Sârbu from the Botanical Garden "Anastasie Fătu", Iasi. The collected material was registered and stored in „Alexandru Ioan Cuza" University's Herbarium from Iași.

Isolation and analysis of essential oils

The dried aerial parts of the plant (100g) were subjected to hydro-distillation, for 3 hours, using a NeoClevenger apparatus, according to the method recommended by the European Pharmacopeia (1997). The yield of essentials oils was 0.2% for vegetative phase, 0.8% for anthesis phase and 0.5% for fruiting phase. The obtained essential oils were stored at

+4°C until analysis. The chemical composition of the essential oil was established by GC-MS analysis with the help of a gas-chromatograph Agilent Technologies 6890N coupled to a mass detector (MSD) of the 5975 inert XL Mass Selective Detector type. The conditions for chromatography were: column HP 5MS, mobile phase Helium – discharge: 1 mL/min, injector temperature: 250°C, detector temperature: 250°C, temperature regime from initial 40°C (10 degrees/min.) to 280 degrees, injected volume: 0.1-0.3 µl, splitting ratio-1:100. The DB5 chromatographic column has a length of 30 m an interior diameter of 0.25 mm and a film diameter of 0.25 µm. The separated compounds were identified by means of the NIST spectrum database, and the peak position was confirmed by the Kovats retention index.

RESULTS AND DISCUSSION

Following our analysis of essential oils, a total of 58 compounds were identified, representing between 92.26% and 97.58% of the total number of identified compounds (Tabel 1). The highest number of chemicals (46 compounds) was identified in the volatile oil derived from individuals collected in 2013, in the vegetative stage. The lowest number of compounds (31) was identified in the volatile oil derived from plants collected in 2013 in the anthesis stage. The main chemical components are germacrene D (between 8.05% and 17.31%), nerolidol (between 7.74% and 18.49%), farnesol (between 12.95% and 14.77%) and α-terpinyl acetate (between 6.56% and 9.58%). Major differences were registered at the taxa collected in 2013 in anthesis stage, where the main components were carvacrol (42.32%) and thymol (13.98%).

Carvacrol and thymol are phenolic compounds specific to the *Thymus* genus, known for their wide spectrum of antimicrobial activity (Dorman and Deans, 2000; Lambert et al., 2001; Adam et al., 1998; Manohar et al., 2001). They possess multiple biological properties such as anti-inflammatory, antioxidant, hepatoprotective and anti-tumoral activities (Aeschbach et al., 1994; Alam et al., 1999; Robledo et al., 2005; Skold et al., 1998; Weber and De Bont, 1996; Zeytinoglu et al., 2003).

The geraniol, a monoterpenoid, it was found in higher amounts (13.45%) on the taxa collected in 2013, in anthesis stage. This compound was found in smaller amounts on the other taxa (between 0.48% and 2.21%). The monoterpene geraniol, which is emitted from flowers and herbs (Mockute et Bernotiene, 1999) of many species, has an important role in their overall flavor and aroma. Is also use as repellent (Barnard and Xue, 2004).

α-Terpinyl acetate, nerolidol and farnesol are 3 compounds identified in large quantities in analyzed taxa, except taxa collected in 2013 in anthesis stage. These 3 compounds are important natural flavours and usually are used in perfumes and soaps. Farnesol is also a natural pesticide for mites and is a pheromone for several other insects (Wang et al., 2011).

In generally, a high chemical variability and diversity is observed in the essential oils of *Thymus* species: at least 20 different chemotypes in the genus have been established until now (Tepe et al., 2005). According to Karuza-Stojaković et al., the principal constituents of *Thymus pannonicus* essential oil from southern parts of Vojvodina province were terpinyl acetate, terpinen-4-ol, thymol, carvacrol and geranyl acetate (listed in order of descending quantity). Maksimovic and collaborators in 2008 identified in the volatile oil *Thymus pannonicus*. All., harvested in northern Serbia, a total of 33 constituents, the main being geranial (41.42%) and neral (29.61). Other researchers have identified in the volatile oil of this species large amounts of

thymol (25-41%) and p-cimen (17-38%) (Pluhar et al., 2007).

Table 1. Chemical composition of the essential oil of *Thymus pannonicus*, collected in various phenophases in two consecutive years (2013-2014), from Fălticeni, Suceava County, Romania

Compound	Vegetative stage		Anthesis stage	
	Year		Year	
	2013	2014	2013	2014
α-Pinene	0.21	0.23	0.16	0.91
Camphene	0.21	0.25	0.22	-
Octen-3-ol	0.18	0.26	0.30	0.28
Myrcene	**3.29**	**3.15**	**5.20**	**2.53**
o-Cymene	0.37	0.33	0.25	0.62
Limonene	0.93	0.50	0.09	0.58
Eucalyptol	0.90	0.98	0.27	-
cis-β-ocimene	2.31	1.26	-	2.15
γ-Terpinene	0.33	-	0.68	-
cis- Sabinene hydrate	0.78	0.85	0.39	0.58
Linalool	3.05	-	0.31	2.55
Octen-3-ol- acetate	0.17	0.31	-	0.41
Camphor	0.42	1.09	-	0.54
Borneol	-	0.41	0.45	0.33
Terpinen-4-ol	0.45	0.317	0.13	-
α-Terpineol	0.69	0.261	-	0.62
Nerol	0.46	0.47	0.51	0.26
Linalyl acetate	-	-	-	3.73
Neral	0.46	0.51		0.32
Methyl thymol	-	-	2.50	-
Geraniol	2.21	0.74	**13.45**	0.48
Geranial	0.67	-	0.15	-
Thymol	-	-	**13.98**	-
Carvacrol	-	-	**42.32**	-
α-Terpinyl acetate	**8.52**	**6.56**	-	**9.58**
Neril acetate	0.32	0.65	-	0.33
Linalil acetate	0.45	-	-	-
Geranyl acetate	-	1.37	4.21	--
β-Burbonene	0.79	**4.11**	0.14	5.11
α- Cariophyllene	-	1.50	1.14	2.10
Alloaromadendren	0.44	-	-	-
β- Cariophyllene	1.30	-	-	-
Farnesene	0.18	-	-	-
τ-Murolen	0.30	0.51	-	0.74
Germacrene D	**17.08**	**17.31**	**8.05**	**14.51**
β-Elemene	0.39	-	-	-
γ-Elemene	2.10	1.03	-	1.32
β-Bisabolene	2.56	1.53	0.12	2.72
γ-Cadinol	0.89	-	-	2.32
τ-Cadinol	0.45	-	-	-
τ-Cadinene	0.36	-	0.35	-
γ-Cadinene	1.27	-	0.61	0.54
Elemol	4.30	2.59	-	1.59
Nerolidol	**7.74**	**18.49**	-	**12.51**
Spathulenol	**7.70**	1.18	0.31	-
Caryophyllene oxide	-	2.36	0.73	2.79
Leden	1.36	-	0.13	0.35
Cubenole	0.47	-	-	-
γ-Eudesmol	0.85	-	-	-

Spatulenol	-	-	0.14	1.48
τ-Muurulol	3.68	-	0.15	-
Aromadendrene epoxyde	-	1.31	0.14	1.13
Eudesmol	-	0.55	-	
τ-Murolol	-	1.47	-	1.27
Cis-Trans-Farnesol	-	2.03	-	1.06
Farnesol	**14.18**	**14.77**	-	**12.95**
Farnesal	0.49	0.68	-	1.23
Farnesil acetate	-	0.35	-	0.14
TOTAL %	**96.26**	**92.26**	**97.58**	**92.66**

The yield of plant material, the essential oil content and quantitative composition of plants can be influenced by harvest time, ecological and climatical conditions (Cabo et al., 1982; Putievsky and Basker, 1977; Inan et al., 2011). Regarding the harvesting time, in generally, thyme is most aromatic during the period of blooming (or at the beginning of full bloom); the blooming period being considered the best time for harvesting (Venskutonis, 2002). How-ever, period of vegetation and blooming can be different in various geographical zones depending on their climatic conditions. Also, weather conditions during the day of harvest are very important. In generally, sunny days should be preferred. The plants harvested after rain are difficult to dry and they deteriorate much faster, became inferior from chemical point of view (Kauniene and Kaunas, 1991). Our studies show that the chemical composition is relatively similar for vegetative and anthesis stage (except the taxa collected in anthesis, during 2013); registering differences in the percentages of chemical compounds.

CONCLUSIONS

Our studies have shown that in the case of *Thymus pannonicus* the main chemical components of essential oils are germacrene D, nerolidol, farnesol and α-terpinyl acetate. The changes due to the harvesting period are found only in percentage variations of the compounds, except the plants collected in anthesis in 2014.

REFERENCES

Aeschbach, R., Loliger, J., Scott, B. C., Murcia, A., Butler, J., Halliwell, B., Aruoma, O. I., (1994): *Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol*. Food Chem. Toxicol, 32, 31–36.

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., (1998): *Antifungal activity of Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia and Salvia fruticosa essential oils against human pathogenic fungi*. J. Agric. Food Chem, 46, 1739–1745.

Alam, K., Nagi, M. N., Badary, O. A., Al-Shabanah, O. A., Al-Rikabi, A. C., Al-Bekairi, A. M., (1999): *The protective action of thymol against carbon tetrachloride hepatotoxicity in mice*. Pharmacol. Res, 40, 159–163.

Barnard, D.R., Xue, R., (2004). *Laboratory evaluation of mosquito repellents against Aedes albopictus, Culex nigripalpus and Ochlerotatus triseriatus (Diptera: Culicidae)*. J. Med. Entomol, 41(4): 726–730.

Cabo, J., M.E. Crespo, J. Jimenez, Navarro, C., Risco, S., (1982): *Seasonal variation of essential oil yield and composition of Thymus hyemalis.* Planta Medica, 380-382.

Cosentino, C.; Tuberoso, C.I.G.; Pisan, B.; Satta, M.; Arzedi, E.; Palmas F., (1999): *In-vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils*. Lett. Appl. Microbiol. 29, 130–135.

De Martino, L., Bruno, M., Formisano, C., De Feo V., Napolitano, F., Rosselli, S., Senatore F., (2009): *Chemical Composition and Antimicrobial Activity of the Essential Oils from Two Species of Thymus Growing Wild in Southern Italy*. Molecules, 14, 4614-4624.

Dorman, H. J. D., Deans, S. G., (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol, 88, 308–316.

El-Hela, A.A.A., (2007): *Chemical composition and biological studies of the essential oil of Thymus decussatus Benth growing in Egypt*. Egypt. J. Biomed. Sci., 23, 146–153.
European Pharmacopoeia. 3rd ed.1997 (Ph. Eur. 1997).
Guşuleac, M., 1961, *Thymus*, *In Flora Republicii Populare Române*, VIII, Ed. Acad. RPR, Bucureşti, pp. 301-334.
İnan, M., Kirpik M., Kaya A., Kirici S., (2011): *Effect of Harvest Time on Essential Oil Composition of Thymbra spicata L. Growing in Flora of Adıyaman*. Adv. Environ. Biol, 5(2): 356-358.
Jalas J., (1972): *Thymus* L., in: Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., et al., Flora Europaea, Vol. 3, Cambridge University Press, Cambridge, 172-182.
Jirovetz, L.; Wlcek, K.; Buchbauer, G.; Gochev, V.; Girova, T.; Stoyanova, A.; Schmidt, E., (2007): *Antifungal activity of various Lamiaceae essential oils rich in thymol and carvacrol against clinical isolates of pathogenic Candida species*. Int. J. Essent. Oil Therap. 1, 153–157.
Karuza-Stojaković, Lj., Pavlović, S., Živanović, P., Todorović, B., (1989): *Količina i sastav etarskih ulja različitih vrsta roda Thymus L.* Arh. Farm, 39:105-111 (in Serbian).
Kauniene, V., Kaunas, E., (1991): *Medicinal Plants*. Varpas, Kaunas, Lithuania.
Lambert, R. J. W., Skandamis, P. N., Coote, P. J., Nychas, G. J. E., (2001): *A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol*. J. Appl. Microbiol, 91, 453–462.
Maksimović, Z., Milenković, M., Vučićevic, D., Ristić M., (2008): *Chemical composition and antimicrobial activity of Thymus pannonicus All. (Lamiaceae) essential oil*. Cent. Eur. J. Biol, 3 (2): 149-154.
Manohar, V., Ingram, C., Gray, J., Talpur, N. A., Echard, B. W., Bagchi, D., Preuss, G. (2001). *Antifungal activities of origanum oil against Candida albicans.* Mol. Cell. Biochem. 228, 111–117.
Mockute, D., Bernotiene, G., (1999): *The main citral geraniol and carvacrol chemotypes of essential oil of Thymus pulegioides L. growing wild in Vilnius district (Lithuania).* J. Agric. Food Chem, 47 (9), 3787-3790.
Morales, R., (2002). *The history, botany and taxonomy of the genus Thymus, The genus Thymus*. Ed. Taylor and Francis. p. 1-44.
Oprea, A., (2005): *Lista critică a plantelor vasculare din România*. Ed. Univ. Al. Cuza, Iaşi, pp. 306-311.
Pluhár, Z., Héthelyi, É., Kutta, G., Kamondy, L., (2007): *Evaluation of environmental factors influencing essential oil quality of Thymus pannonicus All. and Thymus praecox Opiz*. J. Herbs Spices Med. Plants, 3:23-43.
Putievsky, E., D. Basker, (1977): *Experimental cultivation of marjoram oregano and basil*. J. Hortic. Sci, 52: 181-188.
Rajeswara R., Bhaskaruni R., Kakaraparthi P. Sastry, (1990): *Variation in Yields and Quality of Geranium, under Varied Climatic and Fertility Conditions,* J. Ess. Oil Res., 2:73-79.
Rasooli, I., Mirmostafa S.A., (2002): *Antibacterial properties of Thymus pubescens and Thymus serpyllum essential oils*, Fitoterapia, 73:244–250
Robledo, S., Osorio, E., Munoz, D., Jaramillo, L. M., Restrepo, A., Arango, G., Velez, I. (2005): *In vitro and in vivo cytotoxicities and antileishmanial activities of thymol and hemisynthetic derivatives*. Antimicrob Agents Chemother, 49, 1652–1655.
Safaei-Ghomi, J.; Ebrahimabadi, A.H.; Djafari-Bidgoli, Z.; Batooli, H., (2009): *GC/MS analysis and in vitro antioxidant activity of essential oil and methanol extracts of Thymus caramanicus Jalas and its main constituent carvacrol*. Food Chem. 115, 1524–1528.
Skold, K., Twetman, S., Hallgren, A., Yucel-Lindberg, T., Modeer, T., (1998): *Effect of a chlorhexidine/thymol-containing varnish on prostaglandin E2 levels in gingival crevicular fluid*. Eur. J. Oral Sci, 106, 571–575.
Stahl-Biskup, E. and Saez, F., (2002): *Thyme*. Taylor and Francis. London. p. 293
Tepe, B., Munevver, S., Akpulat, H. A., Daferera, D., Polissiou, M., Sokmen, A., (2005): *Antioxidative activity of the essential oils of Thymus sipyleus subsp. sipyleus var. sipyleus and Thymus sipyleus subsp. sipyleus var. rosulans*. J. Food Eng, 66:447-454.
Tzakou, O., Constantinidis, T., (2005): *Chemotaxonomic significance of volatile compounds in Thymus samius and its related species Thymus atticus and Thymus parnassicus.* Biochem. Syst. Ecol, 33:1131-1140.
Venskutonis, P.R., (2002): *Harvesting and post-harvesting handling in the genus Thymus, In Thyme, The genus Thymus*. Ed. Taylor and Francis, p. 197-223.
Zarzuelo, A., Crespo, E., (2002). *The medicinal and non-medicinal uses of thyme. In: Stahl-Biskup E, Sáez F, editors*. *Thyme: The genus Thymus*. London: Taylor & Francis; p. 263.
Zeytinoglu, H., Incesu, Z., Baser, K. H., (2003): *Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human NRAS oncogene*. Phytomedicine, 10, 292–299.
Wang, C., Kim, J.Y., Choi, E.S., Kim, S.W., (2011): *Microbial production of farnesol (FOH): Current states and beyond*. Process Biochemistry, 46 (6):1221–1229.
Weber, F. J., De Bont, J. A., (1996): *Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes*. Biochim. Biophys. Acta, 1286, 225–245.
Yamaura T., Tanaka S., Tobana M., (1989): *Light Dependent Formation of Glandular Trichomes and Monoterpenes in Thyme Seedlings*. Phytochemistry, 28: 741-744.

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