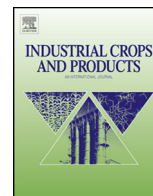




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Variation of essential oil composition of *Thymus pulegioides* in relation to soil chemistry

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ABSTRACT

Soil chemistry, as one of environmental factors, can influence both yield and chemical composition of essential oils, the natural industrial products, in essential oils bearing plants. Purpose of study was to evaluate effects of soil pH, humus, mobile phosphorus, mobile potassium and 14 chemical elements of soil on percentages of essential oil and their main chemical compounds in raw material of large thyme (*Thymus pulegioides* L.) that represents commercially important genus *Thymus* (Lamiaceae). Plant raw materials and soils were collected separately from spontaneous populations of *T. pulegioides*. Essential oils were isolated by hydrodistillation and analysed by gas chromatography/flame ionization (GC/FID) and gas chromatography/mass spectrometry (GC/MS) analytical methods. Amount of humus and mobile phosphorus in soil were estimated photoelectrocolorimetrically, mobile potassium – by flame photometry, soil pH – electrometrically, chemical elements – by energy-dispersive x-ray fluorescence analysis. Results showed that increase in amount of aluminium, copper, iron, potassium and manganese in soil of habitats was accompanied by decrease in amount of essential oils in raw material of *T. pulegioides*. Presence of higher amount of mobile phosphorus in soil can positively influence biosynthesis of α -terpinyl acetate. Increase of sulphur in soil was accompanied by increase in percentages of carvacrol and linalool and by decrease in percentage of *p*-cymene in *T. pulegioides* essential oils. Presence of higher amounts of manganese in soil can stimulate and inhibit the biosynthesis of main compounds in essential oils of *T. pulegioides* geraniol and carvacrol chemotypes, respectively.

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1. Introduction

Although the most commercial medicinal and aromatic species are cultured, the part of raw material of their yet being collected from spontaneous populations in many countries and regions. For example, wild raw material represents about 29% of total volume used in pharmaceuticals in Baltic States (Radušienė and Janulis, 2004; Balvočiūtė et al., 2010). Therefore, although wild raw material often is more heterogeneous in comparing with raw material of cultivated origin, the spontaneous populations of some species with potential industrial uses are yet as alternative for their field culture.

Thymus pulegioides L. (Lamiaceae), native and widely distributed in all Europe, is essential oil bearing medicinal and aromatic species. The strong intraspecific chemical polymorphism is typical

of species of genus *Thymus*: the chemical classification of individual plants within same species to chemotypes is based on chemical composition of essential oils; the names of distinguished chemotypes represent the main chemical compound (or compounds) of essential oils (Thompson et al., 1998). Thus the five chemotypes of *T. pulegioides* growing wild in Lithuania were defined according to the main essential oil compounds such as linalool, geraniol, thymol, carvacrol and α -terpinyl acetate (Mockutė and Bernotienė, 1999, 2001; Ložienė et al., 2002, 2003). These compounds amount the high percentages in essential oils and determine some properties and possibilities of the use of respective chemotypes: the monoterpenic phenols thymol and carvacrol has the strong antimicrobial and antioxidant activity and are widely usable in food and pharmaceutical industry (Yanishlieva et al., 1999; Dorman and Deans, 2000; Pina-Vaz et al., 2004; Ložienė and Venskutonis, 2007; Pirbalouti et al., 2013); the acyclic monoterpene alcohols geraniol and linalool are as a fragrance ingredients in cosmetic products, as a food flavouring in food industry, as insect repellents, and are usable in pharmaceutical industry (Mumcough et al.,

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1996; Radonić and Mastelić, 2008; Beier et al., 2013); α -terpinyl acetate has antimicrobial properties (Hugh and Tan, 2005; Zouaghi et al., 2015). Myrcene, *p*-cymene, γ -terpinene, β -caryophyllene, β -bisabolene, caryophyllene oxide also are frequent and rich chemical compounds of essential oils of *T. pulegioides*, however, different percentages of these compounds are found in all chemotypes of this species (Mockutė and Bernotienė, 1999; Radonić and Mastelić, 2008; Pavel et al., 2010; Tabrizi et al., 2010).

Although the chemotypes of plants in species of genus *Thymus* are determined genetically, the harvesting time and especially the environmental conditions such as temperature, rainfall, altitude, sun radiation, soil chemistry and other also can influence on quantitative composition of essential oils and percentage of chemical compounds of essential oils (Sangwan et al., 2001; Badi et al., 2004; Sotomayor et al., 2004; Hadeif et al., 2007; Nezhadali et al., 2014). Some studies about effect of environmental factors on composition of essential oil were done with *T. pulegioides*. Vaičiulytė and Ložienė (2015) investigated the effect of some meteorological conditions (temperature, rainfall, photosynthetically active solar radiation and sunshine duration) on phenolic and non-phenolic chemotypes of *T. pulegioides* cultured in the same locality of Lithuania. Senatore (1996) studied the influence of harvesting time on yield and composition of the essential oil of *T. pulegioides* growing wild in Campania (Southern Italy). The environmental factors of habitats also can effect on unlike distribution of different chemotypes of same species in space: the some environmental factor can be favourable for distribution of one chemotype but to eliminate from populations other chemotype. For example, the phenolic chemotypes of *Thymus vulgaris* occur predominantly at hot and dry sites (Gouyon et al., 1986; Amiot et al., 2005) the linalool chemotype of *T. pulegioides* – mostly at dry sites of natural populations with permeable soil (Mártonfi, 1992; Mártonfi et al., 1994). *T. pulegioides* can occur on different substrates with wide ranges of pH, humus content and contents of mobile phosphorus and mobile potassium (Mártonfi et al., 1994; Ložienė, 2006). The chemical elements of soil found in rhizosphere of plants enter into the composition of enzymes, which to share in biochemical processes of plants. Therefore the soil chemistry can influence both on the composition of essential oils and also on distribution of chemotypes. The objective of the present study was to evaluate the effects of soil pH, humus, mobile phosphorus, mobile potassium and 14 chemical elements of soil on the quantitative composition of essential oils, the percentage of chemical compounds in essential oils and distribution of different *T. pulegioides* chemotypes.

2. Materials and methods

2.1. Plant and soil material

Forty four natural habitats of *T. pulegioides* were investigated in the east and south-east part of Lithuania (Fig. 1). According to LTDK-99 classification of soils, there dominate arenosols (about 80% of all investigated territory) with small insertions of podzols (in Vilnius region only) in these parts of Lithuania (Buivydaite et al., 2001).

The raw materials of *T. pulegioides* have been collected from investigated habitats separately at the full flowering stage in July. The plant raw material of habitat was collected in the following way: same selected weight of aerial part of *T. pulegioides* was cut from each individual plant growing in the habitat and mixed. The weight was selected depending on each habitat abundance and/or size of individual plants in habitat: in 10 g of aerial part from each individual plant of *T. pulegioides* were cut in abundant and big habitats, but in 30–50 g – in small habitats. Subject to habitat abundance the final weights of fresh (not dried) mixes were different, but no less than 150 g. Thus in total 44 mixes were prepared and one mix

represented the raw material of *T. pulegioides* of one habitat. The plant material of each habitat was dried separately at room temperature in room protected from direct sunlight. Thus prepared and dried plant raw material of each habitat was kept in separate bags for further analysis.

The samples of soil were collected from each habitat separately and dried at room temperature. Each sample of topsoil was prepared in the following way: in 5–9 subsamples (subject to area of habitat; each subsample ~ 100 g) were taken from the depth of 10–15 cm (from plant rhizosphere) by the envelope principle with the distance of 1 m from the central point of habitat and mixed (homogenised).

2.2. Isolation and analysis of essential oils

The essential oil was isolated from each mix (as described above one mix represented the raw material of one *T. pulegioides* habitat) separately by hydrodistillation in the Clevenger apparatus during 2 h (European Pharmacopoeia, 2008). The distillation of essential oil from each mix was carried out in 3–5 repeats, all repeats of one mix collected in one bottle (in total were 44 bottles and one bottle represented one *T. pulegioides* habitat), dried with anhydrous sodium sulfate and stored in freezer until further analysis.

Essential oil solutions of 1% were prepared in the mixture of diethyl ether and *n*-pentane (1:1) for further investigations. The identification of the compounds of essential oil were based on a GC-2010 Plus instrument equipped with a GC-QP 2010 Plus (Shimadzu) series mass selective detector in the electron impact ionisation mode at 70 eV. Separation of compounds was performed on fused silica (100% dimethyl polysiloxane) column (30 m \times 0.25 mm ID \times 0.25 μ m film thickness) (Restek, USA), splitless injection; helium as carrier gas at a flow rate of 1.6 ml/min, injector and detector temperatures 250 °C. The GC oven temperature programmed as follows: initially temperature 50 °C (isothermal for 7 min) increased to 250 °C at the rate 4 °C/min to (isothermal for 5 min) and further increased at the rate 30 °C/min to 300 °C, the final temperature kept for 2 min. The identification of investigated compounds was based on the comparison of retention indices (RIs) (Adams, 2007), computer mass spectra library (NBS75K) and analytical standards of these (Sigma-Aldrich). The retention indices were determined relative to the retention times of a series of *n*-alkanes (C₇–C₃₀) with linear interpolation. The quantitative analysis of main compounds were carried out using a FOCUS GC (Thermo Scientific) gas chromatograph with a flame ionisation detector (FID) on the silica capillary column TR-5MS (30 m \times 0.25 mm ID \times 0.25 μ m film thickness) (Thermo Electron Corporation, USA) under the same chromatographic conditions. The percentage amounts of the investigated compounds were recalculated according to the areas of the FID chromatographic peaks assuming that all constituents of the essential oil comprise 100%. Only compounds, the percentage of which was $\geq 4\%$ though in one habitat, were included in the further analysis.

2.3. Analysis of soil material

The content of humus and mobile phosphorus (P₂O₅) was estimated photoelectrocolorimetrically, the content of mobile potassium (K₂O) – by flame photometry, the soil pH – electro-metrically using 1 M KCl solution; the soil analysis was done at the Agrochemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry.

The collected samples of soil were prepared for the energy-dispersive x-ray fluorescence analysis in the following way: each sample of topsoil was sieved out through 2 mm sieve, subdivided in two independent subsamples and analysed separately. Each subsample (each had 5 g mass) was milled by MM 400 mill with

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