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Effects of meteorological conditions and plant growth stage on the accumulation of carvacrol and its precursors in *Thymus pulegioides*

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ABSTRACT

The effects of meteorological conditions (temperature, rainfall, photosynthetically active solar radiation (PAR) and sunshine duration) and plant growth stages on the quantitative composition of a secondary metabolite – essential oil and its main compounds, in the carvacrol chemotype of *Thymus pulegioides* L. (Lamiaceae) cultivated in open ground were studied under the same micro-edaphoclimatic environmental conditions for six years. The essential oil was isolated by hydrodistillation, the analysis of monoterpenic phenol carvacrol and the biogenetic precursors (monoterpene hydrocarbons p-cymene and γ-terpinene) were carried out annually using GC-FID and GC-MS. In the carvacrol chemotype investigated in this study, the yield of essential oil varied from 0.72% to 0.98% (CV = 12%) at full flowering stage. Regression analysis showed a significant negative relationship between the amount of essential oil and both temperature and rainfall during *T. pulegioides* flowering (July) and the period from April (beginning of vegetation) to July, but a strong positive relationship with photosynthetically active solar radiation during April–July (*beta* = 0.658, $p < 0.05$). The percentage of carvacrol, p-cymene and γ-terpinene ranged between 16.88 and 29.29% (CV = 18%), 5.54–11.33% (CV = 23%) and 20.60–24.43% (CV = 6%) respectively. Regression analysis showed the significant positive relationship between the percentage of carvacrol and sunshine duration at the flowering stage (in July) (*beta* = 0.699, $p < 0.05$); while the negative relationship was established between the percentages of precursors of carvacrol and photosynthetically active solar radiation and sunshine duration. The accumulation of p-cymene, the percentage of which varied most strongly from all investigated chemical compounds, showed significant positive relationships with temperature and rainfall during the period April–July and temperature in July (*beta* = 0.617, *beta* = 0.439 and *beta* = 0.429 respectively, $p < 0.05$). The analysis of plant growth stages showed that the highest amount of essential oil in carvacrol chemotype of *T. pulegioides* accumulated at ripening/seed maturity stage. The percentage of carvacrol increased gradually from the stem elongation to the post-flowering/seed maturity stage, overtaking the full flowering stage by 22%. The highest carvacrol content in the *T. pulegioides* carvacrol chemotype coincided with the highest oil yield during the fruiting stage. The amount of essential oil was also measured at the second full flowering stage, because after cutting the aerial part of the plant at the full flowering stage *T. pulegioides* often bloom for a second time: the carvacrol chemotype accumulated by 21% lower yield of essential oil at this stage compared to the first flowering, however, only traces of carvacrol and its precursors were found at the second full flowering stage.

1. Introduction

Species of the genus *Thymus* (Lamiaceae) are essential oil bearing perennials characterized mostly as medicinal plants, but among them are also aromatic plants and potherbs. Both *thymi herba* (leaves and flowers separated from the previously dried stems) and *thymi aetheroleum* (thyme oil) are used as pharmaceutics (European Pharmacopoeia, 2008b). The pharmacological properties of thyme depend mostly on monoterpenic phenol thymol, which has strong antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Burt, 2004; Dorman and Deans, 2000; Ložienė et al., 2008; Pauli and Schilcher, 2004;

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Yanishlieva et al., 1999). However, species of the genus *Thymus* are characterized as plants with an intraspecific chemical polymorphism, and more different chemotypes have been defined in the *Thymus* species according to the dominance of one or more compounds of essential oil (Thompson et al., 1998). One of the most frequent chemotypes of thyme is the carvacrol chemotype, the main compound of which, as its name indicates, is carvacrol which is attended by two precursors – p-cymene and γ-terpinene (Sáez and Stahl-Biskup, 2002). Carvacrol (5-isopropyl-2-methylphenol) is a monoterpenic phenol isomeric with thymol (5-methyl-2-isopropylphenol); both phenols have the same molecular weight ($Mr = 150$), but differ in the position of the hydroxyl group at the phenolic ring and in their physical occurrence (thymol is crystalline, whereas carvacrol is liquid, with respective boiling points of 233 °C and 238 °C, and carvacrol is more volatile than thymol). Carvacrol has a wide spectrum of biological and pharmacological activity (Ahmad et al., 2011; Baser, 2008; Burt, 2004; Dorman and Deans, 2000; Landa et al., 2009; Yanishlieva et al., 1999; Zheng and Wang, 2001).

Seven different chemotypes of *Thymus pulegioides* L., commonly found throughout Europe, are defined according to their main essential oil compounds (linalool, geraniol, thymol, carvacrol, α-terpenyl acetate, fenchone and cis-sabinene hydrate), the most frequent of which is the carvacrol chemotype (Groendahl et al., 2008; Mártonfi, 1992; Mockutė and Bernotienė, 2001; Ložienė et al., 2003). Published data suggest that the amounts of carvacrol among the essential oils of *T. pulegioides* growing wild in different regions and countries varied widely (Table 1). High variation in the amount of carvacrol may result from different climatic conditions as well as genetic factors. Cultivation of plants of the same genotype, i.e. vegetatively propagated from one individual plant, grown in the same location enables assessment of the influence of meteorological conditions on the accumulation of carvacrol.

After the cutting the aerial part of the plant at the full flowering stage, *T. pulegioides* often blooms twice (second full flowering stage), making it possible to collect an additional yield of plant raw material. Variation in the quantity of essential oil and the predominant compounds at different phenological stages is characteristic of essential oil bearing plants. The highest amounts of essential oils in species of the genus *Thymus* are often found at full flowering (Ebrahimi et al., 2008; Gholami Takaloo et al., 2012; Golparvar et al., 2015; Jamali et al., 2013; Senatore, 1996). Therefore, the assessment of the quantitative composition of essential oil, carvacrol and its precursors in raw material from a second yield of the *T. pulegioides* carvacrol chemotype and comparison with other plant growth stages is topical.

The objective of this study was to evaluate the effects of meteorological conditions (temperature, rainfall, photosynthetically active solar radiation and sunshine duration) and plant growth stage on the amount of essential oil and metabolomics of carvacrol and its precursors in carvacrol chemotypes of *T. pulegioides* cultivated in the same locality.

2. Results and discussion

2.1. Effects of meteorological conditions on the amount of essential oil

The yield of essential oils at full flowering stage in the investigated carvacrol chemotype varied from 0.72% to 0.98% (CV = 12%) during 2008–2013 (Fig. 1). The amount of essential oils in carvacrol chemotype of *T. pulegioides* that grows wild in different locations may vary strongly: for example, in Romania it varies from 0.7% to 1.1% (Pavel et al., 2010), and in Lithuania from 0.6 to 0.9% (Ložienė, 2009). These variations have been attributed to both the genetic characteristics of the plants and the environmental conditions under which they grew (Ložienė and Venskutonis, 2005). The plant material investigated in this study was of the same genetic origin and grew under the same micro-edaphoclimatic environmental conditions throughout the experimental period. This ensured that annual differences in meteorological conditions were the main source of variation in yield of the essential oils and/or the percentage of carvacrol and its precursors in the investigated carvacrol

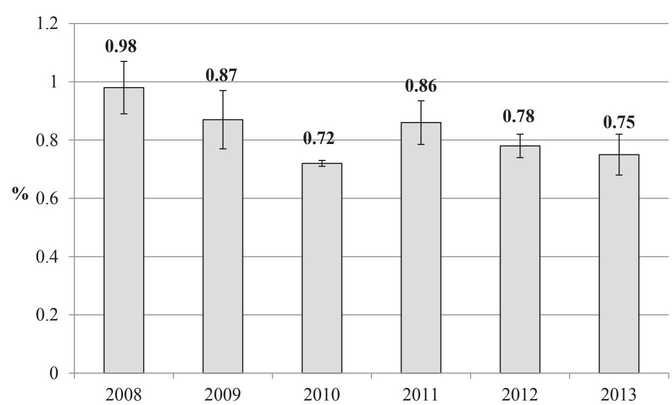

Fig. 1. The amount of essential oil (%) in carvacrol chemotype of *Thymus pulegioides* L. at full flowering stage in 2008–2013. The parent plant of *T. pulegioides*, belonged to the carvacrol chemotype, was vegetatively propagated (five clones in 1.5 m^2), and grown in the open ground under the same micro-edaphoclimatic environmental conditions for six years. Because the parent plant was propagated vegetatively, all five plants (clones) investigated were genetically identical. The amount of essential oil in separate clones ($n = 5$ in 2008–2012 and $n = 3$ in 2013, because two plants died in exposure; error bars represent ± standard deviations of the amount of essential oil between the investigated clones) was tested annually during July in the aerial part of the plants (free from stems).

Table 1
Percentage of carvacrol (≥10%) in essential oils of *Thymus pulegioides* L. estimated in various European countries. Very different percentages of carvacrol were determined in essential oils of *T. pulegioides* growing wild in different regions and countries. High variation in the percentage of carvacrol may arise from different climatic conditions as well as genetic factors of plants.

Country	Carvacrol, %	Source
Norway	35.2	Stahl-Biskup, 1986
Lithuania	0.7–32.8	Mockutė and Bernotienė, 1999; Ložienė et al., 2003
Poland	4.9–31.0	Kowal and Krupinska, 1979
Germany	4.9–32.5	Messerschmidt, 1965
Slovakia	24.0–58.1	Mártonfi, 1992; Mártonfi et al., 1994
former Yugoslavia	10.0–30.0	Kuštrak et al., 1990
Bosnia-Herzegowina	4.3–11.1	Karuza-Stojakovic et al., 1989
Romania	17.9–62.6	Rădulescu et al., 2009; Pavel et al., 2010; Rădulescu et al., 2011; Boz et al., 2015
Croatia	13.2–29.8	Kuštrak et al., 1990
Portugal	21.0	Pinto et al., 2006
Italy	0.4–4.2	Senatore, 1996

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