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Short communication

Effect of foliar nutrition on the essential oil yield of Thyme (*Thymus vulgaris* L.)

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ABSTRACT

Thyme (*Thymus vulgaris* L.) is an important cultural aromatic plant, whose essential oils (EOs) have been used in many industrial applications. EO yields are often negatively influenced by various factors, so it is important to keep finding new growing procedures that increase the quantitative content of EOs. Therefore, this paper is focused on the effects of applying foliar nutrition, including N, P and K in combination with salicylic acid (SA), on selected yield characteristics related to the quantitative and qualitative content of EOs in *T. vulgaris* plants.

As shown by our field experiments, targeted pre-harvest application can significantly increase the yield of EOs, resulting in a quantitative increase in the EO content of the plants, ranging from 18.76% to 42.47% compared to untreated plants. In addition, EOs obtained from the treated plants were found to contain 62.1–64.1% thymol; this range was more stable compared to untreated plants, where the thymol content ranged between 53.1% and 62.7%.

1. Introduction

Essential oils (EOs) are synthesized through secondary metabolic pathways of plants as communication and defence molecules. In addition to their important roles in direct and indirect plant defences against herbivores and pathogens, reproduction (through the attraction of pollinators and seed disseminators), and plant thermotolerance, EOs are responsible for the specific taste and aroma of plants. These characteristics, together with their diverse biological activities, have made them highly attractive for industrial purposes, food processing, perfumery and medicine, including the development of plant protection products (Pavela and Benelli, 2016).

Thyme (*Thymus vulgaris* L.) is a plant of the Lamiaceae family, originally from the Western Mediterranean. This small sub-shrub is currently one of the most important aromatic plants, and is grown not only in Southern and Central Europe, but also in Southeast Asia, North America and Africa. Thyme is traditionally used in the medical, cosmetics and food industries for its singular scent and taste (Guine and Goncalves, 2016).

In addition to the use of dried *T. vulgaris* as a spice and medicinal herb, various solvents and methods are also used to obtain extracts from the plant, including EOs that have found many uses in industry (Stahl-Biskup and Saez, 2002). The content of EOs in dry *T. vulgaris* herbs ranges from 0.3% to 4.0%. The content of the EOs depends on several factors, the most important being genetic characteristics, stage of development, environmental and agronomic factors, and drying and storage conditions (Ozguven and Tansi, 1998; Badi et al., 2004; Calin-Sanchez et al., 2013).

Although several chemotypes of *T. vulgaris* exist, with EOs of different chemical compositions, thymol and its isomer carvacrol are the typical major substances found in thyme EOs (Stahl-Biskup and Saez, 2002). Thanks to these phenols, EOs from thyme often exhibit a higher biological efficacy compared to EOs from other aromatic plants (Pavela, 2011; Matusinsky et al., 2015). This is why thyme EOs have found applications in medicine, where they are traditionally used in treating bruises, various types of dermatitis and rheumatic types of pain, reducing seborrhoea, regenerating capillary glands, and improving the condition of the hair. On account of its expectorant, spasmolytic and antiseptic properties, it is notably used to treat a variety of illnesses of the respiratory tract, such as flu, colds, sinusitis, chronic and acute bronchitis, tuberculosis, calming convulsive coughs, and irritable and spasmodic coughs; due to its stimulant properties, it also acts as a nervous tonic and is used in asthenic states (Marinelli et al., 2016). Besides medicine, thyme EOs are also used in the food industry as food flavours and aromas, antioxidants or substances that extend the shelf-life of foods (Stahl-Biskup and Saez, 2002). In addition, the benefit of this EO in the production of botanical pesticides is not negligible (Pavela, 2011, 2016).

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For thyme growers, it is therefore important to obtain sufficient quantities of good-quality biomass, with a high EO content. Although some factors having a negative impact on EO yields (Letchamo et al., 1995) cannot really be affected (e.g. climatic and pedological conditions, the course of the weather), others can be influenced by growers when the culture is first established (e.g. by choosing a suitable variety, chemotype, and planting density), and later by choosing proper agrotechnical interventions (Thompson et al., 2003). In addition, although *T. vulgaris* is considered an agrotechnically undemanding plant, according to the findings of recent years its EO content can be increased considerably, e.g. through nitrogen fertilization (Sharafzadeh et al., 2011; Juárez-Rosete1 et al., 2014), and some substances (such as citric acid) may have a positive impact on the EO yield (Jaafari and Hadavi, 2012).

Nevertheless, most existing studies have been conducted under controlled conditions and/or under conditions significantly different, in terms of climate and pedology, from temperate climate zone conditions. As we know, no tests have yet been performed regarding the effect of applying foliar nutrition, including nitrogen, phosphorus and potassium in combination with salicylic acid, which is known for its ability to induce in the plants the synthesis of defensive metabolites, including the group of EOs (Kleinwächter et al., 2015).

For this reason, the Crop Research Institute developed a special foliar fertilizer that has been tested in field experiments for three consecutive years. We observed selected yield characteristics, related to the quantitative and qualitative content of EOs in the plants of *Thymus vulgaris*.

2. Materials and methods

2.1. Plant material

The Switzerland hybrid cv. Varico 2 of *Thymus vulgaris* L. (obtained from mediSeeds sàrl, Route des Vergers 18, 1964 Conthey, Switzerland) were used in the study. The experiment was conducted in the Crop Research Institute in Prague (50°5′32″ N, 14°18′2″ E) during three growing seasons (2012, 2013 and 2014). Weather data (rainfall and average temperature) were recorded daily and are reported as mean monthly data for both years (Table 3). The experimental design was a completely randomized block (micro-plots of 10 m^{-2}, clay loam soil, pH 6.5, N 102 mg kg^{-1}, available P 0.48 mg kg^{-1}, available K 121 mg kg^{-1}). Thyme was sown manually on 10 April 2012 in rows of 20 cm distance. After emerging, the plants were thinned out manually to obtain a final distance of 15 × 20 cm (about 300,000 plants/ha). Basic fertilization was applied every year in the spring (30 kg ha^{-1} N, 30 kg ha^{-1} P_2O_5 and 120 kg ha^{-1} K_2O). The plots were kept weed-free by hand hoeing. No pesticide protection was applied.

2.2. The fertilizer

Foliar fertilizer developed at the Crop Research Institute, Czech Republic was used in the experiments; the fertilizer contained a combination of acceptable nutrients (N 93 g kg^{-1}, P_2O_5 402 g kg^{-1} and K_2O 262 g kg^{-1}) and salicylic acid (20 g kg^{-1}).

2.3. Experiments

2.3.1. Effect of the application dose on the quantitative increase in EO content

In the first year (2012), the thyme plants were initially small (15–20 cm); the first flower buds were observed only in the second half of August. The fertilizer was therefore applied in the second half of August, at the beginning of the flowering stage. The fertilizer was dissolved in 10 g L^{-1} and 20 g L^{-1} of water and was applied onto the plant leaves using a manual sprayer in a dose of 60 mL m^{-2}, corresponding approximately to a fertilizer dose of 6 kg ha^{-1} and 12 kg ha^{-1}. The control plants were treated only with water.

Samples from 10 randomly selected plants were taken before application and on day 1, 3, 5, 8, 10 and 12 after application. The samples were dried at 35 ± 1 °C and were kept at 18 ± 1 °C until further processing. The experiment was done using randomized complete block designs with four replications.

2.3.2. Effect of the foliar fertilizer on the yield rate and essential oil composition

In two consecutive seasons (2013 and 2014), the fertilizer was applied 5 days before the harvest each time, using a dose of 6 kg ha^{-1} and the above-described method (Section 2.3.1.). Two harvests were done in every season (harvest 1 in the second half of June and harvest 2 at the end of September). At the time of the harvest, when the EO content of the plants is at its highest, the flowering rate of the plants was at least 50% (Salehi et al., 2014). The plants were harvested manually by cutting the plants at 8–10 cm above ground. The samples were dried at 35 ± 1 °C and were kept at 18 ± 1 °C in the dark until further processing. The experiment was done using randomized complete block designs with four replications.

2.3.3. Isolation of essential oil

The samples of wild thyme (about 30 g) were subjected to hydrodistillation for 2 h using a Clevenger-type apparatus, and the oils obtained were dried over anhydrous sodium sulfate and kept in a dark glass bottle at 4 °C for the analyses.

2.3.4. Essential oils analysis

GC–MS analyses were performed on a Finnigan GCQ instrument, column Rxi–5 ms (Restek Co., Bellefonte, PA, USA), 30 m × 0.25 mm × 0.25 μm, temperature program: 60 °C, hold for 1 min, then gradient 4 °C min^{-1}–180 °C, then gradient 10 °C min^{-1} to 275 °C, and hold 5 min at this temperature. Temperature of the transfer line 275 °C, ion source 200 °C, ionization energy 70 eV. Linear velocity of the carrier gas (helium) was 40 cm s^{-1}. Full scan spectra in the range of relative mass m/z 50–450 Da were compared with mass spectra of authentic standards.

2.4. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using a randomized block design. The significance of the differences among different studied characteristics was determined with Tukey's test (P = 0.05) using STATGRAPHICS Plus 4.0 statistical software. Data are presented as mean ± standard deviation (S.D.) calculated from each replication.

3. Results and discussion

The effect of foliar nutrition in combination with salicylic acid on the quantitative content of the essential oil in the thyme plants is shown in Fig. 1. Application of both different doses resulted in an increased EO production compared to untreated plants as early as one day from application. However, a significantly higher yield ($p < 0.05$) of the EOs was found only between days 5 to 8 from application. Later, the difference compared to untreated plants was again no more significant. Similarly, no significant difference was found between the two tested doses (6 kg ha^{-1} and 12 kg ha^{-1}).

Therefore, in the two subsequent years, the application was performed 5 days before harvesting the plants and using only a dose of 6 kg ha^{-1}. Selected yield parameters are shown in Table 1. *Thymus vulgaris* cv. Varico 2 was found to be able to provide 1.00 kg m^{-2} to 1.28 kg m^{-2} of dry biomass in total. The treated plants contained significantly more EOs compared to the untreated plants, and this quantitative increase ranged from 18.76% to 42.47%. In 2013 and 2014, the potential EO yield from the treated plants was 313.7 kg ha^{-1} and