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Decomposition profiles of leaf essential oils in the soil environment

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ABSTRACT

In this study, the changes that essential oils undergo during the decomposition process of the plants producing them are examined. This was done for peppermint, spearmint, and rosemary, the shoots of which were added as soil amendments at a concentration of 4% (w/w). We sampled at 0, 30 and 60 days after the plants' incorporation into the soil. We distilled the soil mixtures and found the essential oil content to be reduced, in the spearmint and peppermint treatments, by approximately 90% after 30 days. In the rosemary treatment, it was reduced only at the last sampling to about 50% of its initial value. Essential oil composition changed dramatically with time. The relative contribution of monoterpenoids, initially about 90% in spearmint and peppermint, fell to 45% and 20%, respectively, after 60 days. Sesquiterpenes and sesquiterenoids increased both in number and relative contribution. Percent participation of β-caryophyllene increased more than 15-fold in the spearmint and peppermint essential oils, and compounds that were not detected at first appeared at later stages of the decomposition process (after 30 or 60 days). In contrast, monoterpenoids made the bulk of rosemary oil at all sampling times, with only a minor reduction (about 5%) at the end of the experimental period. In a second experiment, where rosemary was let to decompose for one year, quantitative and qualitative analysis confirmed persistence of its oil for long in the soil environment. These results can explain the different effects on crop plants that have been reported for rosemary and spearmint soil mixtures and provide further insight for the potential of aromatic plants to find novel uses in agriculture.

1. Introduction

One of the features characterizing the Mediterranean environment is its fragrance. In fact, abundance and diversity of aromatic plants maximize in regions with Mediterranean-type climate (Ross and Sombero, 1991). Essential oils produced by aromatic plants are mixtures of a high number of compounds. Structural relationships of essential oil constituents allow them to easily convert to each other by oxidation, isomerization, cyclization, or dehydrogenation reactions, triggered either chemically or enzymatically (Turek and Stintzing, 2013). Due to the high diversity of their constituents, essential oils have a multifaceted bioactivity. Effects on microbes and on plant germination and growth are its most studied aspects (Vokou, 2007).

Assessing 47 constituents of essential oils, Vokou et al. (2003) found hydrocarbons to be less active than oxygenated compounds in inhibiting germination and plant elongation. Carvone, menthol, and camphor and cineol, which are major constituents of spearmint (*Mentha spicata*) (Kokkini and Vokou, 1989), peppermint (*Mentha piperita*) (Grulova et al., 2015) and rosemary (*Rosmarinus officinalis*) (Karamanoli et al., 2000), respectively, are among the most inhibitory oxygenated compounds (Vokou et al., 2003; Argyropoulos et al., 2008). Similarly, the oxygenated compounds proved more active than hydrocarbons with microbes (Karamanoli et al., 2000; Santoyo et al., 2005). Although the antimicrobial activity of essential oils is the most commonly assessed property (Vokou, 2007), stimulatory effects were also observed (Vokou and Liotiri, 1999; Kadoglidou et al., 2011; Vokou et al., 1984, 2002, 2006; Simas et al., 2017). Of these effects, most pronounced is the activation of soil metabolism and soil bacteria (Vokou et al., 1984, 2002).

Because of the importance of soil conditions in maintaining sustainability and environmental quality (Ghorbani et al., 2008), and of the positive effects of essential oils and their constituents on soil metabolism, aromatic plants have been assessed as soil amendments and alternative crop-stimulating/protective agents. For *Mentha spicata*, it was found that, parallel to stimulating soil microbes, it also stimulated growth of tomato seedlings (Chalkos et al., 2010; Kadoglidou et al., 2011), whereas *Rosmarinus officinalis* (rosemary) negatively affected tomato growth (Bouzoukla, 2017).

The scope of the present work is to examine the quantitative and qualitative changes that essential oils undergo in the soil environment,

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in an attempt to explain the above contrasting effects and further explore the potential of aromatic plants and their natural products to find novel applications in agriculture.

2. Material and methods

2.1. Soil and plant material

The aromatic plants examined in this study are all members of the Lamiaceae family. They are the co-generic peppermint (*Mentha piperita)* and spearmint (*Mentha spicata)*, both being perennial herbs with similar essential oil features, and also the very different rosemary (*Rosmarinus officinalis)*, an evergreen shrub. All three are native to the Mediterranean region and are also cultivated. Plants were purchased from a commercial supplier. For peppermint and spearmint, the whole aboveground biomass was used, whereas for rosemary the leafy upper part of the shoots. These were cut into small pieces, air-dried in the dark till moisture content was approximately 5–7%, and stored in a cool (12 °C), dark and dry place until use.

Soil was obtained from a field left in fallow for a 10-year period; its properties are described in Kadoglidou et al. (2014). Briefly, soil consisted of 32% clay, 56% silt, 12% sand, 3.1% organic matter, and 1.7% $CaCO_3$ with C:N equal to 6.8 (analyses performed at the Soil Science Institute of the National Agricultural Research Foundation).

2.2. Experimental procedure

To study the persistence of the essential oils into the soil environment, soil mixtures were prepared by adding the aromatic plant materials to soil samples at a rate of 4% w/w. Each mixture was placed in pots of 2 kg, which remained at 16–24 °C and 45–60% relative humidity for the whole duration of the experiment. There were three replications per treatment. Pots were irrigated every other day. At 0, 30 and 60 days after the establishment of the experiment, which is the time when the aromatic plants were mixed with soil, the soil mixtures of three pots per treatment were subjected to a 3-h hydrodistillation in a Clevenger apparatus. GC–MS analysis followed, performed in a Thermo Trace Ultra GC equipped with ISQ MS and TriPlus RSH autosampler (Switzerland). Samples of one microliter were injected with a split ratio of 50:1. GC separations were carried out on a TR-5MS capillary column 30 m × 0.25 mm × 0.25 µm. Temperature of the injector was 220 °C, of the ion source 230 °C and of the interface 250 °C. The carrier gas was helium flowing at a constant rate of 1 ml min^{-1}. The GC temperature program was held at 70 °C for 5 min, then increased to 240 °C at a rate of 8 °C min^{-1}, and held at final temperature for 15 min. After 5 min of solvent delay, mass range of *m/z* 50–600 was recorded. The mass spectra were acquired in electron impact ionization (EI) mode. The peak area integration and chromatogram visualization were performed using Xcalibur processing program. Peak identification and mass spectra tick evaluation was performed using NIST11 database. All analyses were made in triplicate.

Because rosemary exhibited a remarkable delay in its decomposition, we carried out an additional experiment with only the rosemary:soil mixture. This new experiment lasted for one year. The soil mixture was prepared as above. Samples were taken at 0, 90, 180, 270 and 360 days after the establishment of the experiment and were analyzed as described above.

2.3. Statistical analysis

Both experiments were established in a randomized design. Data of the first experiment were subjected to two-way ANOVA (three aromatic plants × three time points), whilst data of the second were subjected to one-way ANOVA using SPSS version 17.0 package. Different letters indicate statistically significant differences according to Duncan's multiple range test; $P < 0.05$.

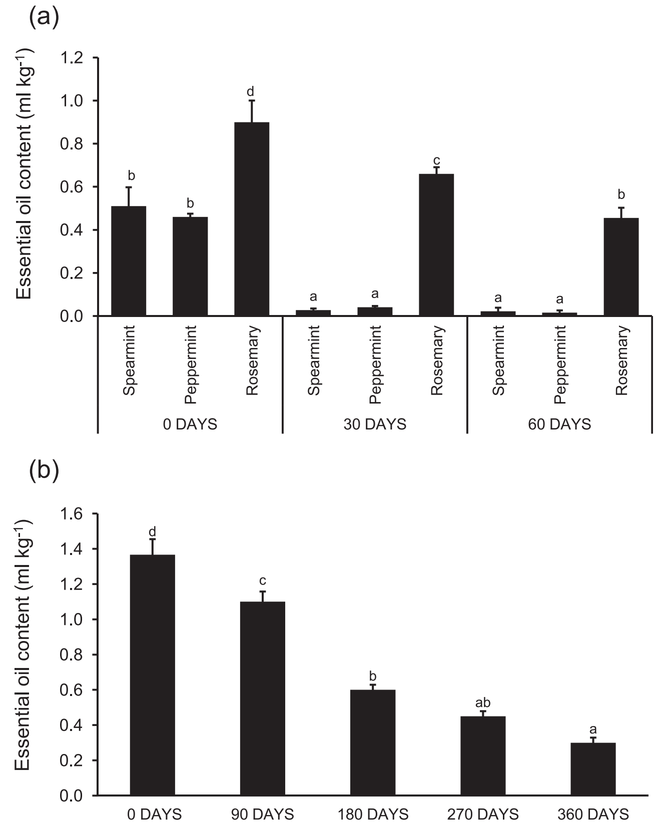

Fig. 1. Essential oil content (ml kg^{-1}) of soil mixtures containing (a) spearmint, peppermint and rosemary, at a rate of 4%, after 0, 30, and 60 days from the beginning of the experiment, and (b) rosemary, at the same rate, after 0, 90, 180, 270, and 360 days. Values are means ± SE. Statistical tests were run separately for (a) and (b), so different letters indicate significant differences for values of (a) or (b), not for both (Duncan's multiple range test; $P < 0.05$).

3. Results and discussion

The essential oil content of the spearmint and peppermint soil mixtures rapidly decreased, by approximately 90% after 30 days. In contrast, in the rosemary mixture, the essential oil content decreased much more slowly, by about 27% and 50%, after 30 and 60 days, respectively (Fig. 1a).

GC–MS analysis revealed that spearmint and peppermint oils exhibited remarkable differences compared to their initial composition. Monoterpene/monoterpenoids that made up more than 90% of the two *Mentha* oils in the beginning of the experiment were reduced by approximately 65% and 80%, after 30 and 60 days, respectively, whereas the higher-molecular-weight sesquiterpenes/sesquiterpenoids exhibited opposite trends (Fig. 2a). More specifically, menthol in peppermint oil fell from 40% to 3.2% after 60 days, whereas carvone in spearmint oil almost disappeared: it fell from 28% to only 0.4% after 60 days (Table 1). During the same period, β-caryophyllene increased from around 1.5% to about 27% in peppermint oil and to 15% in spearmint oil (Table 1). Similarly, the contribution of oxygenated sesquiterpenes, such as acetates and oxides, also increased, indicating oxidative reactions taking place in the soil environment. Additionally, differences were also observed in the number of constituents that were identified. Compounds like α-copaene, γ-cadinene, α-muurolene that were not detected in the initial oils appeared after 30 or 60 days of decomposition (Table 1). Overall, 51 compounds were identified at the last sampling in spearmint oil when 42 compounds were identified in the beginning of the experiment. Similarly for peppermint, the respective numbers are 42 and 39 (Table 2). Almost all of these new constituents are sequiterpenes/sesquiterpenoids (Table 1).