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Variability in essential oil composition of wild populations of Labiatae species collected in Spain

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ABSTRACT

Essential oils of 11 populations of *Thymus mastichina* (L.) L., 10 populations of *Salvia lavandulifolia* Vahl and 12 populations of *Lavandula latifolia* Medik., collected in full bloom during 3 years (2009–2011) were analyzed by gas chromatography (GC)-flame ionization detector (FID) and mass spectrometry (MS) to study the variability among populations and the influence of the year of harvest in the essential oil composition. One factor ANOVA for population origin and year of harvest, and Principal Component Analyses (PCA) using the main compounds as set of observations were carried out for each species. For *T. mastichina* all the samples were 1,8-cineol chemotype (58.52–68.82%), however the linalool content showed a great range of variation (1.16–10.24%). 1,8-Cineol (6.21–33.69%), camphor (2.85–22.44%) and β-pinene (5.11–19.85%) were the main compounds for *S. lavandulifolia* and 1,8-cineol (30.57–54.09%) and linalool (15.82–45.94%) for *L. latifolia* essential oils. Populations from *T. mastichina* and *S. lavandulifolia* from different years appeared mainly grouped in the PCA figures while *L. latifolia* populations showed no clustering. *T. mastichina* was the least environmentally influenced species, showing mainly differences among populations. *S. lavandulifolia* also had small differences among campaigns and higher differences within populations. Conversely, *L. latifolia* showed a higher percentage of differences in the volatile composition depending on the year of harvest but genotypic variability was also observed. In conclusion, the variability of the essential oil composition is largely dependent on the population studied having genetic factors a greater influence than the environmental factors. However, environmental factors are also influencing the essential oils composition and must be taken into account.

1. Introduction

Lamiaceae is a family of great diversity which includes the following species: *Thymus mastichina* (L.) L. (Spanish marjoram) distributed over most of the Iberian Peninsula (Spain and Portugal), *Salvia lavandulifolia* Vahl (Spanish sage) characteristic-growing plant of Spain, SE France and NW Africa and *Lavandula latifolia* Medik. (spike lavender), growing wild in the Iberian Peninsula, France, Italy and the West-Balkan States (Morales et al., 2010). The essential oils or dry leaves of these three species are used for the perfumery, cosmetic and aromatherapy industry, or for phytotherapy and as food preservative, respectively. *S. lavandulifolia* and *L. latifolia* essential oils are among the 20 most traded in the world with a global annual production of 50–100 t (Lubbe and Verpoorte, 2011).

These species have been harvested from nature since ancient times, but wild collection presents a set of problems such as: misidentification of the material, lack of rural labor, heterogeneous and insufficient production, impossibility to certify the material as organic products and absence of awareness of the chemical composition. In addition, consumers sometimes prefer wild plants because they are considered more natural and crop parameters to cultivate this plant material are often unknown. However, cultivation of these species results in a homogeneous production, protecting them and preventing the threat of the habitat that occurs with wild collection. Cultivation also enables the development of rural areas where climatic conditions allow the growth of these species. Nowadays, they are already domesticated, but still an important part of the production comes from wild plants. Especially, *T. mastichina*, since 90% of the production in the

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Iberian Peninsula is harvested from its natural habitat. As industry requires material with a specific quality, some standards have been established. Achieving quality parameters established in the pharmacopeias and ISO quality standards is not possible if the material is not cultivated and previously selected.

Essential oils are complex mixtures of volatile compounds produced by plants as secondary metabolites. The production of these secondary metabolites can be influenced by several factors. According to Figueiredo et al. (2008) these factors include: physiological variations, environmental conditions, geographic variations, genetic factors, evolution, storage, etc.

Many studies have been conducted to analyze how these various factors influence the production and composition of essential oils, with the aim of optimizing the culture conditions, knowing the adequate time of harvest and obtaining the quality essential oils required by the industry. With respect to physiological variations, different organs of the plant may produce differences in the composition of the essential oils. Miguel et al. (2004a) found different composition in flowers and leaves of *T. mastichina* and Schmiderer et al. (2008) obtained differences within the volatile fraction of calyx, corolla and anthers of *S. lavandulifolia*.

Environmental factors are also a parameter to be taken into account, both in cultivated (Jamali et al., 2014; Kleinwächter et al., 2015) and natural environments (Delgado et al., 2014; Llorens et al., 2014; Rajabi et al., 2014). Salgueiro et al. (1997) found a correlation between the linalool content of *T. mastichina* and Atlantic humidity. Other authors have proved that seasonal variation influence the essential oil composition as showed Miguel et al. (2004b) for Spanish marjoram. There are three known chemotypes for this species: 1,8-cineol, linalool and an intermediate chemotype (García Vallejo et al., 1984), and Salgueiro et al. (1997) found that the geographical variation of *T. mastichina* was related to the different chemotypes.

On the other hand, storage condition can produce chemical transformation of the terpenoids (Misharina et al., 2003) and different extraction methodologies can lead to different volatile composition of the same sample (Méndez-Tovar et al., 2015).

The above mentioned studies refer to examples that may cause changes in the chemical composition. However, no long term assays have been conducted to study which chemical compounds remain stable during different harvesting campaigns and which compounds can be influenced by weather conditions in each year of harvest.

In order to study the influence of the year of harvest and the geographical origin of population in the variability of the essential oils of *T. mastichina*, *S. lavandulifolia* and *L. latifolia* species, several wild populations were collected in the field during three consecutive years (2009–2011) for further analysis.

2. Materials and methods

2.1. Plant material

Three species of Lamiaceae family were chosen for this study, belonging to three of the most representative genera those grown in the Iberian Peninsula, aiming to select adapted species, which would be able to be cultivated, or so are already, in different environments of the Central region of Spain.

Representative samples of 11 populations of *T. mastichina*, 10 population of *S. lavandulifolia* and 12 populations of *L. latifolia*, were collected during full blossom phase between June and August for three consecutive years (2009–2011) in Castilla y León, Spain.

All the samples were air-dried at room temperature and kept from light. Flowers and leaves were separated from the stems and used for further analysis. Collection data of the populations are given in Table 1. Voucher specimens from all the samples were deposited in the herbarium of the botanic area (PALAB) in the Campus of Palencia, University of Valladolid.

2.2. Hydrodistillation

The essential oils were isolated from 180 g of dried material of each population by hydrodistillation in 2 L of water for 150 min, using a Clevenger-type apparatus.

2.3. GC–MS and GC-FID analysis

Essential oils were analysed with a GC–MS (gas chromatography–mass spectrometry) and a GC-FID (gas chromatography-flame ionization detector). GC-FID system (6890N Network GC system Agilent Technologies, Palo Alto, CA, USA) was equipped with a DB-5 narrow column (10 m × 0.1 mm × 0.1 μm). Helium was used as carrier gas. Front inlet was kept at 260 °C with a split ratio of 500:1. The volume of sample injection was 0.2 μL. The temperature program was, 60 °C for 1 min; 60–85 °C at a rate of 8 °C/min; 85–280 °C at a rate of 15 °C/min; 280–300 °C at 30 °C/min, and held at 300 °C for 5 min.

The GC–MS analysis was carried out with a GC–MS HP 6890 coupled with a HP 5972 MSD (Hewlett-Packard, Palo Alto, CA, USA). The GC was equipped with a DB-5ms capillary column (30 m × 0.25 mm × 0.25 μm film thickness). The carrier gas was Helium (2 mL/min). GC oven temperature program was: 60 °C for 4 min, rising to 100 °C at 5 °C/min, and then from 100 °C to 280 °C at 9 °C/min. The volatile compounds were identified by comparison of the mass spectra of every compound with a mass spectra library of volatile compounds and confirmed by comparison of the retention indices from literature (Adams, 2007). Some of the compounds were additionally checked by reference compounds. All the compounds are expressed in peak area percentage.

2.4. Statistical analysis

The statistical analyses of the data were done with SPSS program version 15.0 (SPSS, 2006). One factor ANOVA was done for population and year of harvest as sources of variation and a Duncan test was performed to analyze the differences among harvesting years for each species. In order to assess the patterns of variation, PCA was done by simultaneously considering the main compounds (choosing the compounds with an average higher than 1%, deleting those which were not significant, neither for population nor for year in the previous ANOVA). For this analysis the characters were initially scaled to make their variances equal. In the multivariate space that they defined, a new set of axes was then chosen so that the variance on each axis was as large as possible but at right angles to the preceding ones. The coefficient of each data point on each new axis was a weighted sum of its coefficients on the original axes.

3. Results

3.1. T. mastichina *(L.) L. variability*

In *T. mastichina*, a total of 63 compounds were identified (Table 2). The compounds were mainly oxygenated monoterpenes (82.40 ± 1.09%). Ten compounds showed averages higher than 1%: α-pinene, sabinene, β-pinene, myrcene, 1,8-cineol, limonene, β-*trans*-ocimene, linalool, α-terpineol and α-terpinyl acetate, being limonene, β-*trans*-ocimene, linalool and α-terpinyl acetate, whose showed the higher variability. The main compound was 1,8-cineol in all samples (58.52–68.82%), so all the studied populations belong to 1,8-cineol chemotype.

In relation to the population variation, a total of 39 compounds out of the 63 identified were statistically significant (Table 2). In