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Phenophase effects on sage (*Salvia officinalis* L.) yield and composition of essential oil

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ABSTRACT

The main goal of the current study was to assess the phenological stage influences on yield and quality of essential oil of *Salvia officinalis* originating from two different regions and cultivated under the same experimental conditions. Results revealed the highest essential oil yielding (1.45, 1.49%) at the flowering phase. Analysis of the essential oils by GC/MS permitted the identification of sixty nine components and showed that the main components were 1,8-cineole (17.64–20.44%), α-thujone (15.66–25.23%), β-thujone (5.28–7.10%), camphor (6.00–24.36%) and viridiflorol (3.10–16.32%), but the percentages of these compounds varied depending on the phenological period. The flowering period was characterized by the highest production of 1,8-cineole (20.44, 19.28%). Camphor and viridiflorol percentages had a contrasting evolution in the course of the growth cycle, with the maximal levels detected respectively, at fruiting and vegetative phases. Additionally, substantial proportions of thujones (30.61, 27.96%) marked the fruiting stage. The accumulation of oxygenated monoterpene progressed during the phenological cycle of *S. officinalis*. Results from PCA revealed a clear discrimination between the three studied phenological stages. *S. officinalis* essential oil yields and volatile compounds percentages were significantly ($p < 0.05$) affected by the harvesting time.

1. Introduction

Sage (*Salvia officinalis* L.) is a culinary, aromatic and medicinal plant of economic importance since it has been used in prolonging the shelf life of food (Altindal and Altindal, 2016), as flavoring agent in food products, perfumes and cosmetics and it has been credited with a long list of medicinal uses such as antihydrotic, spasmolytic, antiseptic, anti-inflammatory and the treatment of mental, nervous conditions (Baricevic and Bartol, 2000), Alzheimer's disease (Perry et al., 2003) and cancer (Ho et al., 2000; Altindal and Altindal, 2016). Many useful secondary metabolites including terpenes and phenolics and their derivatives are produced by *S. officinalis* plants and have been in the center of pharmacopoeias of many countries (Tepe et al., 2006). *S. officinalis* is one of the most important aromatic plants cultivated worldwide as a source of essential oils. Many phytochemical studies so far investigated the chemical composition of *S. officinalis* essential oils (Avato et al., 2005; Hayouni et al., 2008; Santos-Gomes and Fernandes-Ferreira, 2001). As known, the biosynthesis of the volatile compounds is influenced by various environmental factors namely the soil mineral fertilization (Piccaglia and Marotti, 1993), the light intensity (Li et al., 1996), the climate conditions (Maksimović et al., 2007; Russo et al., 2013), the culture site (Ben Farhat et al., 2009; Perry et al., 1999), the developmental stages (Mirjalili et al., 2006) and the genetic baggage (Skoula et al., 1999; Li et al., 2015). Plants produce a high diversity of volatile terpenes playing different ecological functions in relation with their interactions with the environment. Terpenoids may be produced in defense against herbivores, but may also serve as secondary functions attracting the natural enemies of these herbivores or may provide a competitive advantage to several angiosperm species as allelopathic agents (Croteau et al., 2000).

The main goal of the present study was to assess the essential oil yield and composition of *S. officinalis* obtained from two different origins and cultivated in an experimental field, at different plant phenological stages (vegetative, flowering, fruiting). The current investigation will permit the achievement of two important

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scientific and practical key points namely, the determination of optimal harvesting time with the desired aromatic and/or therapeutic qualities and the most productive plant origin, which may help increasing economical feasibility of the essential oil production and the obtention of new scientific data on the changes of *S. officinalis* volatile secondary metabolites in the course of its phenological cycle.

2. Materials and methods

2.1. Plant material

The field experiment was carried out in the IMIDA (Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario) research field in the region of Torreblanca (Murcia, Spain). The experimental area (37°47′N, 0°54′W, 30 m above the sea) was characterized by an annual average temperature of 18.2 °C and rainfall of 308.3 mm in a semi-arid bioclimatic stage.

Seeds of *Salvia officinalis* were collected in June 2007 from two different regions in the north of Tunisia. The collection site Bou Arada (36°21′ N, 9°37′ W, 252 m above the sea) was characterized by a semi-arid moderate climate with an annual average temperature of 17.8 °C and rainfall of 450 mm and the coastal locality Soliman (36°41′N, 10°29′W, 16 m above the sea) belonged to a higher semi-arid climate with an annual average temperature of 19.2 °C and rainfall of 500 mm. The seeds were germinated and the plantlets were grown under greenhouse conditions for three months, then, the plants were transplanted in the experimental field. Analysis of the essential oil composition of cultivated *S. officinalis* plants was performed during vegetative, flowering and fruiting phenological stages in 2009. Five individual plants were considered for the analysis of each origin and phenological stage.

2.2. Chemicals

Homologous series of C_6–C_{17} *n*-alkanes and high-purity standards were purchased from Sigma–Aldrich (Madrid, Spain). Anhydrous sodium sulfate was supplied from Scharlau Chemie S.A. (Sentmenat, Spain).

2.3. Essential oil extraction

Aerial parts of individual plants were dried in an oven at 35 °C until it reached a constant weight. The dried plant material of each sample was submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oil obtained was separated from water and dried over anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis (Jordán et al., 2009). Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

2.4. Gas chromatography–mass spectrometry analysis

Samples of 0.1 μL were subjected to analysis by gas chromatography–mass spectrometry (GC–MS). An Agilent HP 6890 gas chromatograph (GC), equipped with a 30 m × 0.25 mm HP-5 column with 0.25 μm film thickness, and a DB-Wax (30 m × 0.32 mm i.d.) and 1.0 μm film thickness was used. Both stationary phases were supplied by Agilent Technologies (Palo Alto, CA, USA). Helium was used as the carrier gas (constant pressure, β-ionone eluting at 27.6 min for HP-5MS column and 41.38 min for DB-Wax column) and the split ratio was set to 100:1. The GC was linked to an Agilent model 5972 inert mass spectrometry detector (Agilent, Palo Alto, CA). For both stationary phases, the initial oven temperature was set at 60 °C then increased at 2.5 °C/min to 155 °C and finally raised to 250 °C at a rate of 10 °C/min; the injection port and the transfer line to the mass selective detector were kept at 250 and 280 °C, respectively. The mass spectrometer was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m*/*z* 50 to 500 at 3.21 scan/s. The quadrupole temperature was 150 °C and the electron multiplier voltage was maintained at 1300 V.

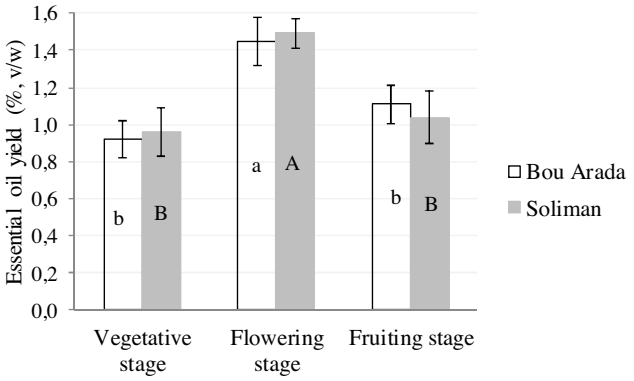

Fig. 1. *S. officinalis* essential oil yields at different growth stages. Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter, yield values represented the mean of five individual plants, bars sharing the same small letter did not share significant differences at $p < 0.05$ (Duncan test).

2.5. Compounds identification

The individual peaks were identified by retention times and retention indices (relative to C_6–C_{17} *n*-alkanes), compared with those of compounds published in bibliography or literature, and by comparison of mass spectra using the NBS75 K library (U.S. National Bureau of Standards, 2002) and spectra obtained from the standard. Percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current.

2.6. Statistical analysis

All data were reported as mean ± standard deviation of five experiments. Data were analysed by an analysis of variance ($p < 0.05$) and the means were separated by Duncan's multiple-range test (ANOVA procedure). Pearson's correlation coefficients were calculated between grouped chemical classes. A Principal Component Analysis (PCA) was performed to assess the variations between *S. officinalis* individual plants and growth stages based on their essential oil composition. Results were processed by computer programs Excel and STATISTICA software version 5.1.

3. Results and discussion

3.1. Effect of the phenological stage on S. officinalis essential oil yield

The variations of the essential oil yields of *S. officinalis* plants of two origins and three phenological stages are shown in Fig. 1. For both *S. officinalis* origins, the essential oil content rose significantly from a minimum of (0.92, 0.96%) collected at the vegetative phase to a maximum of (1.45, 1.49%) obtained at the flowering stage, then, decreased significantly (1.11, 1.04%) in the course of the fruiting. Flowering essential oil yields differs significantly ($p < 0.05$) from both vegetative and fruiting yields as calculated on the basis of dry matter weight. During the flowering phase, plants may produce substantial amounts of essential oils in order to attract more pollinators (Palá-Paúl et al., 2001), however, the low rate of biosynthesis of volatile compounds during the vegetative phase may be