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Agronomic and phytochemical evaluation of lavandin and lavender cultivars cultivated in the Tyrrhenian area of Tuscany (Italy)

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ABSTRACT

This study aimed to evaluate the possibility to organically cultivate lavender (Lavandula angustifolia Miller) and lavandin (Lavandula hybrida Reverchon), under the pedo-climatic conditions of central Italy. So, the growth and productive parameters as well as the essential oil content and composition of one lavender cultivar (Mailette) and three lavandin cultivars (Sumiens, Super A and Grosso) were assessed through 2-year field experiment (2014 and 2015 growing season). The results showed that the cold sensitivity of both lavender and lavandin plants decreased with ageing. Along the two years of cultivation, stem and inflorescences yields remained stable, and the lavandin cultivar Super A showed always the higher yields in comparison with the other varieties. A slight increase of essential oil (EO) yield was observed for the three cultivars of lavandin during the second year of experiment (2015), while the EO yield of lavender showed a slight decrease. The composition of these essential oils highlighted an important variation which affected all the classes of compounds, except for oxygenated monoterpenes (OM). The antioxidant capacity of EOs was also evaluated and the obtained results pointed out as the growing season was an important factor in influencing the antioxidant capacity of EOs of these aromatic plants.

1. Introduction

Lavandula genus belongs to the Lamiaceae (Labiatae) family and includes 39 known species (Hassiotis et al., 2014), mostly distributed in the Mediterranean area (Kara and Baydar, 2013) but also in Asia, Middle East and Northern Africa. *Lavandula angustifolia* Miller is one of the most famous aromatic and medicinal plants and its use as a therapeutic agent goes back to the ancient Romans and Greeks (Cavanagh and Wilkinson, 2002).

Lavender essential oil (EO) has a popular fragrance and is easily recognisable with several applications in cosmetics, perfumery, household cleaning products and air fresheners. Small bags of dried lavender are often used in the folk Italian tradition to scent cupboards and to repel unwanted moths. *Lavandula* extracts are recommended in aromatherapy to treat a wide range of ailments including stress, anxiety, depression, fatigue, motion sickness and hypertension (Ju et al., 2013; Kenner and Requena., 1996; Peirce, 1999; Chu and Kemper, 2001). Often administered with massage in Europe (Jäger et al., 1992), the EO is used to aid in relaxation, treat colic and stimulate the appetite (Duke et al., 2002). The combination with peppermint EO is also recommended to relieve tension headaches (Chu and Kemper, 2001).

Lavender EOs have antifungal properties (Adam et al., 1998; Pattnaik et al., 1997) and it is also effective for burns and insect bites (Cavanagh and Wilkinson, 2002; Gattefossé, 1937). Many of these properties depend on the EO composition (Bakkali et al., 2008; Mejri et al., 2010) and, despite the technological progress, many factors are far from the human control, such as climatic conditions and edaphic factors (Figueiredo et al., 2008; Pereira et al., 2000).

Several authors consider the physiochemical characteristics as determinant factors in the secondary metabolite biosynthesis of plants, as well as the pedo-climatic conditions (Lopez-Carbonell et al., 1996; Lappin et al., 1987) and cultivation techniques. These factors explained the differences found in the EO analysis of the same species and cultivar grown in the same conditions (Menghini et al., 2013). The intraspecific variations are particularly frequent in the Lamiaceae family; in fact different chemotypes or genetically based types have been reported in *Mentha citrata* (Murray and Lincoln, 1970), *Mentha spicata* (Kokkini and Vokou, 1989), *Origanum vulgare* (Vokou et al., 1993), *Rosmarinus officinalis* (Lakušić et al., 2013), *Salvia fruticosa* (Karousou et al., 1998) and in the *Thymus* genus (Adzet et al., 1977; Thomson et al., 2003; Stahl-Biskup and Sáez, 2002; Thompson, 2002) as well as in *Lavandula* genus.

Most of the lavender production is concentrated in France and

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Bulgaria, but many other European countries, Italy included, have a significant production. Despite of the commercial importance of *Lavandula* EO in several industries, it is not yet clear how environmental and genetic factors influence the *Lavandula* EO production and quality. The aim of this work was to evaluate the introduction of lavender (*Lavandula angustifolia* Miller. cv Mailette) and lavandin (*Lavandula hybrida* cv. Sumiens, Super A and Grosso) in organic cultivation in the central Italy environment and assess the effect of crop age on EO yield and quality. Through two years of field experiments, carried out in an organic farm located in the Tyrrhenian coast (Tuscany, Italy), the main biological and biometric characteristics, as well as crop yields and essential oil content and composition were assessed. The obtained EOs were also evaluated for their antioxidant capacity.

2. Materials and methods

2.1. Plant material and experimental conditions

Three cultivars of lavandin *Lavandula hybrida* Reverchon (sin. *L. x intermedia* Emeric ex Loisel.), Sumiens, Super A and Grosso, and one cultivar of lavender (*Lavandula angustifolia* Miller, cv Mailette) were cultivated with an organic agricultural system in a small farm located in the Tyrrhenian coast of Tuscany (Bibbona, Livorno, Italy; 43°27′N. 10°58′E. 60 m a.s.l), where these species had never been cultivated before. The physical and chemical soil characteristics were examined at the beginning of cultivation and soil samples were collected at 30 cm depth. Macro-Kjeldahl digestion procedure (Bremner and Mulvaney, 1982) was adopted for total nitrogen evaluation, while Olsen method (Olsen and Sommers, 1982) for available phosphorus determination. Soil organic carbon (SOC) was determined according to Nelson and Sommers (1982) and the amount of soil organic matter (SOM) was estimated by multiplying the SOC concentration by 1.724 (Nelson and Sommers, 1982). Soil pH was measured on a 1:2.5 soil:water suspension (McLean, 1982). The soil was sandy loam, characterized by 72.0% sand, 16.5% silt and 11.5% clay, with neutral reaction (pH 7.6). The soil presented low levels of both organic matter (1%) and total nitrogen (0.61 g N kg^{-1}) and medium level of available phosphorus (16.8 mg P kg^{-1}).

Changes in minimum, maximum and mean air temperatures and total rainfall were recorded by a weather station located nearby the farm.

Plants were purchased from a local nursery, specializing in the production of aromatic and medicinal plants with organic certification. They were manually transplanted in June 2013, when they were 15 cm in height with a well expanded root system. Soil tillage was done at the end of winter with medium ploughing (0.30 m), followed by superficial disk harrowing in order to prepare the transplanting bed. Planting was realised adopting a plant density of 1.4 plants m^2 with an inter-row spacing of 2.00 m and an intra-row spacing of 0.35 m. For each cultivar/species, 5 rows were realised, with 75–80 plants for row. Plants were grown on biodegradable plastic mulch with an irrigation drip automated system. Pelleted dry organic fertilizers were applied in pre-planting. From the second year from planting, mechanical weed control was performed among rows, while manual weeding was carried out on the row. No pests and diseases have been recorded.

In each year of growth, plant survival at the end of winter was measured.

2.2. Biological, biometric and productive characteristics

The plants were collected for two successive years (2014 and 2015 respectively in the 2nd and 3rd year after planting). The plants were harvested manually at the full flowering stage (which occurred on June, in both years), when volatile oil content was maximum. The date of full bloom was estimated when 75% of inflorescences were open. At each harvest, three randomized samplings on a minimal area of 10 m^2 for each cultivar/species were manually collected (excluding the plants on outer rows), and the main agronomic parameters were evaluated: plant vigour and uniformity; percentage of flowering plants; plant height (cm) and width (cm); length of flower stem (cm); fresh and dry yield of stem flower (i.e. inflorescence stalk) (t ha^{-1}); fresh and dry yields (t ha^{-1}) of stemless flowers; and essential oil content (%). To evaluate the plant vigour and the plant uniformity index, a value scale from 1 (low vigour/no uniformity) to 5 (high vigour/absolute uniformity) was adopted.

Dry weight measurements were carried out after drying samples in a ventilated oven at 40 °C until constant weight.

2.3. EO extraction and EO analysis

All the EOs were obtained by hydrodistillation from dried aerial parts of each plant samples using a Clevenger-type apparatus according to the Italian Pharmacopoeia (Helrich, 1990). The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate. The extracted EOs were kept in sealed air-tight glass vials and covered with aluminum foil at 4 °C until further analysis. The essential oil yield was determined as a percentage.

2.3.1. GC-FID analysis

Analysis with GC are performed by HP-5890 Series II instrument equipped with HP-Wax and HP5 capillary columns (30m × 0.25 µm film thickness), and with the following conditions: temperature program of 60 °C for 10 min with an increase of 5 °C/min–220 °C; injector and detector temperatures at 250 °C; carrier gas helium (2 ml/min); detector FID; split ratio 1:30; injection of 0.5 µl of a 10% hexane solution of the essential oil.

2.3.2. GC–MS analysis

They were performed with a Varian C*P*-3800 apparatus equipped with a DB-5 capillary column (30m × 0.25 mm i.d., film thickness 0.25 µm) and a Varian Saturn 2000 ion-trap mass detector. The oven temperature was programmed rising from 60 °C to 240 °C at 3 °C/min; injector temperature 220 °C; transfer-line temperature 240 °C; carrier gas He (1 ml/min).

The identification of the constituents was based on the comparison of their retention times (Rt) with those of pure reference samples and their linear retention indices (LRIs) determined relatively to a series of *n*-alkanes. The mass spectra were compared with those listed in the commercial libraries (NIST 2011 and ADAMS) and in a home-made mass-spectral library, built up from pure substances and with MS literature data.

2.3.3. Rate of variation

Also, called rate of evolution, allows us to calculate the variation between two values in percentage as the increase of percentage of the class of compounds in the EO between the two years of cultivation. To calculate it the following formula was used:

$$\text{variation (en \%)} = \frac{Vf - Vi}{Vi} \times 100$$

Where V_i = 2014 value and V_f = 2015 value

2.4. Radical scavenging activity

The antioxidant capacity of EOs was determined by DPPH (2,2 diphenyl-1-picrylhydrazyl) free radical method according to Brand-Williams et al. (1995), with some modifications (Tavarini et al., 2015). For the analysis, 200 µl of the methanolic solution of the essential oils at five different concentrations (30, 50, 80, 100 and 200 mg mL^{-1}) were used. Radical scavenging activity was calculated as the inhibition of the free radical by the sample using the formula% inhibition (%I) = [(A0 − At)/A0] × 100where A0 is the absorbance of the control DPPH