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, an- xiety, 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 re- commended 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 de- terminant 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* (Lakušié 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 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 environ- mental 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

2.2 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 realized, 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 pro- gram 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.

137 *2.3.3. Rate of variation*

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

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

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143 Where V_i = 2014 value and V_f = 2015 value

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145 *2.4. Radical scavenging activity*

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147 The antioxidant capacity of EOs was determined by DPPH (2,2 di- phenyl-1-picrylhydrazyl) free
148 radical method according to Brand-Williams et al. (1995), with some modifications (Tavarini et al.,
149 2015). For the analysis, 200 µl of the methanolic solution of the essential oils at five different
150 concentrations (30, 50, 80, 100 and 200 mg mL^{-1}) were used. Radical scavenging activity was
151 calculated as the inhibition of the free radical by the sample using the formula% inhibition (%I) =
152 [(A0 − At)/A0] × 100, where A0 is the absorbance of the control DPPH solution at 0 min and At is
153 the absorbance in the presence of the extract after t = 60 min. The amount of sample necessary to
154 decrease the absorbance of DPPH by 50% (IC50) was graphically calculated, plotting the inhibition
155 percentage against the sample concentration in the reaction system. Trolox was used as a positive
156 control.

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158 *2.4. Statistical analysis*

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160 'Past 3 software package' was used for statistical analysis. The hierarchical cluster analysis HCA was
161 performed using Ward's method, with squared Euclidian distances as a measure of similarity.
162 Agronomic data were subjected to one-way ANOVA analysis in order to evaluate the effect of
163 species/variety on the main biometric and productive parameters, using the statistical software
164 COSTAT Cohort V6.201 (2002). Means were separated on the basis of Least Significance Difference

(LSD) test only when the ANOVA F-test per treatment was significant at the 0.05 probability level (Gomez and Gomez, 1984).

3. Results

3.1. Growing season conditions

Monthly meteorological conditions of the two growing seasons were showed in Fig. 1. Total rainfall during growing season, from March (vegetative regrowth) to June (time of harvest) was comparable be- tween the two years (229.4 mm in 2014, and 227.2 mm in 2015). On the other hand, the months before vegetative re-growth (January and February) were characterized by strong differences in total precipita- tion (320.8 *vs* 138.8 mm, in 2014 and 2015, respectively). Average mean temperatures from March to June were 16.0 and 16.4 °C in 2014 and 2015, respectively, even if during May and June 2015, an increase in maximum air temperature was registered in comparison with the previous year (+8.0% and +3.5%, in May and June, respectively).

3.2. Biological, biometric and productive characteristics

Depending on the species and cultivar, large variations in the main biological, biometric and productive traits were observed (Tables 1 and 2). The lavender cultivar Mailette was the earliest to flower, 5–18 days earlier than the lavandin cultivars. This behavior has been previously confirmed by Renaud et al. (2001). In both years of cultivation, the two lavandin cultivars Sumiens and Super A, produced the most vigorous and homogeneous plants, while, in contrast, Mailette and Grosso showed plants with the lowest vigor and homogeneity (Table 1). All the cultivars presented, as early as the first year, a flowering percentage higher than 90%, exception given for Grosso in the 1st year of pro- duction (89.5%). In the lavander cultivar Mailette, the inflorescence stalk was typically unbranched with a compact spike, while in the lavandin cultivar Sumiens, the inflorescence stalk was branched. The other two lavandin cultivars showed a variable pattern. Moreover, the morphology of the

inflorescence stalk changed over the season. Since the plant survival at the end of the winter increased from the 1st (95.3%) to the 2nd year (100%), it could be hypothesized that, in lavender and lavandin, the cold sensitivity of the plants decreased with ageing. At the same time, Sumiens was characterized by the highest plant height and plant width, while Mailette presented the lowest stem length with flowered cymes (Table 1). Regarding productive para- meters, they were significantly influenced by species/cultivar, while no effect of the year of cultivation and cultivar x year interaction has been assessed, exception given for fresh stemless yield which was affected also by the reciprocal interaction (Table 2). Super A was characterized by higher fresh and dry stem and inflorescences yields in comparison with the other varieties. On the other hand, and as expected, Mailette showed always the lowest yields (Table 2). Generally, along the second and third year after planting, the production remained stable, even if a slight decrease in fresh inflorescence yield was observed for all varieties, exception given for Mailette. To the best of our knowledge, very few studies have been conducted about lavender and lavandin cultivation and biomass yield evaluation in Italy. It is known that lavandin cultivars have a more vigorous growth habit, with higher biomass yield, in comparison with lavender varieties. Kara and Baydar (2013), comparing lavender and lavandin cultivars in a two-year field experiment, found that Super A, and Dutch lavandin were the high-yielding cultivars, producing the highest fresh and dry stemless flower yields. These findings confirm our results, which emphasized the greater performances of Super A. Previous studies (Ceylan et al., 1988, 1996; Arabaci and Bayram, 2005) reported that, for lavender, it was possible to obtain dry stemless flower yields ranging from 1.02 to 4.43 t ha^{-1}, depending on environmental conditions, cultivation practices, crop age, etc. In our study, under the environmental conditions of Tyrrhenian coast of Tuscany (central Italy), comparable dry stemless flower yields were achieved, varying between 1.07 t ha^{-1} (for lavender Mailette) to 2.00 t ha^{-1} (for Super A). In such conditions, 1 kg of dry inflorescences was obtained by drying 5.0 and 4.5 kg fresh stem flower of lavender and lavandin, respectively. These findings were in agreement to those reported by Kara and Baydar (2013), under the agro-ecological conditions of Isparta Province, Turkey.

3.3. EO content and composition

The comparison among the EO yields obtained in the two years of trial, evidenced a slight increase in the three cultivars of lavandin, passing from the 1st to the 2nd year of cultivation, while the EO content of lavender decreased in 2015 in comparison with 2014. As expected, and in agreement with previous findings (Erbaș and Bayader, 2008), the yield of lavandin EO was higher than that obtained from lavender, even though lavender EO was characterized by a higher quality, due to the lower content of camphor (Lis-Balchin, 2002; Shellie et al., 2000). Super A showed to be the most productive in terms of EO, with a yield of about 9% (8.81% in 2014 vs 9.35% in 2015), followed by Grosso (8.25% in 2014 vs 8.50% in 2015). In the literature, it is generally reported that EO content of lavandin varied between 1.0–1.5% in fresh stem flowers and between 5.0–6.0% in dry stemless flowers (Kara and Baydar, 2013). Our data showed higher values in comparison with previous reports. This can be due to both the specific pedo-climatic conditions and the use of only flowery parts that were manually selected for successive distillation. The chemical compositions of lavandin/lavender EOs are reported in Table 3. Fifty-three compounds were identified with a total percentage of identification ranged between 99.7 and 100%. The chemical composition of lavender EO evidenced a loss of non-terpene derivatives with a rate of decrease of 41.7% passing from 3.6% in 2014–2.1% in 2015 (Fig. 2). The other classes of constituents showed more or less the same trend during the two years, such as oxygenated monoterpenes (88.5 in 2014 vs. 88.8% in 2015), sesquiterpene hydrocarbons (1.6 in 2014 vs 1.8% in 2015) and oxygenated sesquiterpenes (0.4 in 2014 vs 0.5% in 2015). On the contrary, monoterpene hydrocarbons reflected a slight increase of order of 10.2 (5.9 in 2014 vs 6.5% in 2015). Linalool (compound N° 21) was the most abundant oxygenated monoterpenes despite its percentage ranged from 48.4% in 2014 to 45.5% in 2015 followed by linalyl acetate (compounds N° 38), which exhibited the same amount (26 in 2014 vs 26.2% in 2015) in the two years of harvesting. These two compounds (linalool and linalyl acetate) were the main chemical constituents in the EO of *L. angustifolia* cultivar Mailette, accounting for more than 70% of the total.

Our results didn't agree with those reported in the literature (Chu and Kemper, 2001, 1994; Jäger et al., 1992), where these two constituents showed higher percentage (up to 90%). In all lavandin cultivars, the amounts of oxygenated monoterpenes increased from 2014 to 2015. The percentage of increase was of 5% > 4% > 1% respectively in Grosso > Sumiens > Super A cultivars. On the contrary, the percentage of monoterpene hydrocarbons showed a significant decrease especially in the Sumiens cultivar. Linalool was the main constituent of lavandin EOs providing a percentage ranging from 33.5% in Super A in 2014–42.9% in Grosso in 2015, followed, for both these cultivars, by linalyl acetate which presented a rate of decrease of 26.1 in Super A (21.1 in 2014 vs 15.6% in 2015) against 11.9 in Grosso (16.7 in 2014 vs 14.7% in 2015) (Fig. 2). Sumiens sample showed 1.8-cineole (compound N.12) as the second most abundant compound (12.7% in 2014 vs 11.7% in 2015, see Table 3) compounds in the class of oxygenated monoterpenes. Different papers reported that linalyl acetate and borneol accounted for over than 70% in Lavandula hybrida (Buckle, 1993; Marotti et al., 1989; Peracino et al., 1994). However, in the present study, the total amount of these compounds did not exceed 25% in all the lavandin cultivars. The essential oil composition of *Lavandula angustifolia* cv. Mailette was codified by the 'French Association for Standardization' (AFNOR) and by the 'European Pharmacopoeia' (EPh). The quality of the EO from Lavender obtained in this study was quite similar in the two years of harvesting and agreed with the levels accepted by EPh (Table 4). Regarding to the composition of all lavandin EOs, only linalool respected the limit assigned by EPh (Table 5). Camphor and linalyl acetate showed very low amount, and 1,8-cineol exhibited higher percentage in comparison with EPh standard. According to this data, the lavandin EO produced in this environment could not be used for pharmaceutical purposes. Hierarchical Cluster Analysis HCA (Fig. 3) showed the presence of two main groups: the first group included only lavender cultivar (Mailette), and the second one the three lavandin cultivars (Grosso, Super A and Sumiens). This latter can be divided into two subgroups: one with Sumiens for both years of cultivation, and the other one with Super A and Grosso cultivars, with Super A_15 that showed a composition slightly different from the others. This classification was confirmed by the Principal Component Analysis (PCA) (Fig.

4) where the first axis (PC1) explained for 68.9% and PC2 for 23.6%, which resumed for 92.5% of the total variability (Fig. 4). PCA evidenced that the lavender group was distinguished by the presence of geranyl acetate, (E)-β-ocimene and 3-octanone, relative the other lavandin cultivars. The Sumiens subgroup (Sumiens_14 and Sumiens_15) was characterized by borneol, trans and cis linalool oxide, and (Z)-β-ocimene and was situated in the low right quadrant of the plot. In the second subgroup camphor, lavandulyl acetate and 1,8- cineol were the typical compounds of Grosso_14 and Grosso_15 together with Super A_14. Super A_15 confirmed its position slightly different from the others, since it was situated in the high left quadrant due to the higher content of α-terpineol.

3.4. Radical scavenging activity

The in-vitro radical scavenging activity (RSA) of the essential oils showed significant differences depending on cultivar/species and year of cultivation, pointing out as both species/cultivar and growing season are important factors to influence the antioxidant capacity of EOs of these plants (Table 6). Interestingly, the methanolic solutions of EO of L. angustifolia cv. Mailette, was always characterized by a strong AA, as proved by its lowest IC50 concentration. In addition, the AA of Mailette EO strongly increased in the second year of experiment. The higher maximum air temperatures registered in May and June 2015, in comparison with the previous year may be one of the important factor effect the chemical composition of EO and consequently on its AA activity (Fig. 1). A similar behavior was also observed for the lavandin cultivar Grosso. It is widely reported that several environmental factors, such as temperature, photoperiod, light intensity, water availability, can directly affect the biosynthesis of secondary metabolites and, consequently, their related AA (Carvalho et al., 2010; Tavarini et al., 2015). Biological and antioxidant properties of lavender essential oil have been assessed by several studies (Cavanagh and Wilkinson, 2002; Peana et al., 2002; Silva et al., 2015). However, discordant results about AA of *Lavandula* spp. EOs, have been reported (Hohmann et al., 1999; Dapkevicius et al., 1998; Miliauskas et al., 2004; Baptista et al., 2015; Silva et al., 2015), due to the different assays used for their evaluation, but also to other

294 important pre- (genotype, pedo-climatic conditions, geographic origin, harvest time) and post-harvest
295 (drying, storage) factors (Figueiredo et al., 2008; Topal et al., 2008; Baptista et al., 2015). Our study
296 showed that the lavender and lavandin essential oils presented an interesting AA and this ability was
297 concentration-dependent. However, it has been found that the AA of lavender EO was less potent
298 than that recorded for the EOs obtained from other Lamiaceae members (Baptista et al., 2015;
299 Martucci et al., 2015). At this regard, Martucci et al. (2015) observed that oregano EO was
300 characterized by a ten-fold higher AA than that lavender one. These authors (Martucci et al., 2015)
301 related this behavior to the presence, in the oregano EO, of specific compounds such as phenols,
302 carvacrol and thymol, able to act as electron donors (Burt, 2004).

303 **4. Conclusion**

304 This study evidenced that organically-grown lavender and lavandin cultivars can be successfully
305 cultivated in Tyrrhenian coast of Tuscany (central Italy), with good stemless flower and EO yields.
306 The introduction of these species into organic cultivation systems could contribute to obtain high
307 quality raw material, as well as to enhance the diversification of crop rotation, which is of pivotal
308 importance in the management of organic farms. The EOs obtained were of good quality and with
309 satisfied yields to be used for industrial purposes, as cosmetics application.

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Figure captions

Figure 1. Meteorological data (monthly rainfall and monthly mean temperatures) for 2014 and 2015 growing seasons.

Figure 2. Chemical classes of compounds present in the EO of the four *Lavandula* cultivars (%) and graphical representation of their variation (%) in 2015 with respect to 2014.

Figure 3. Dendrogram of the Hierarchical Cluster Analysis (HCA) volatile constituents from different cultivars of *Lavandula* EOs.

Figure 4. Principal Compound Analysis (PCA) plot of volatile constituents from different cultivars of *Lavandula* EOs.

Table 1. Main biometric and morphological characteristics of the four cultivars of *L. hybrida* and *L. angustifolia* at harvest.

Parameters	2014				2015			
	Mailette	Grosso	Sumiens	Super A	Mailette	Grosso	Sumiens	Super A
Plants survival (%)	98.6 a	91.7 c	94.0 b	96.9 b	100.0 a	100.0 a	100.0 a	100.0 a
Flowering plants (%)	92.7 a	89.5 a	91.5 a	91.0 a	92.5 a	90.0 a	91.5 a	90.2 a
Vigour [a] (1 - 5)	4.04 b	4.29 b	4.91 a	4.64 a	4.05 b	4.17 b	4.95 a	4.65 a
Plant height (cm)	48.7 c	50.4 c	74.1 a	67.7 b	52.4 c	62.7 c	81.7 a	76.70 b
Length of stem flower (cm)	31.49 c	41.36 b	46.79 a	44.89 b	25.40 c	35.68 b	45.47 a	37.38 b
Plant width (cm)	52.0 c	67.0 b	96.0 a	97.0 a	68.9 d	90.0 c	129.4 a	126.10 b
Date of harvest	June 21	June 26	June 26	June 26	June 10	June 28	June 15	June 28

For each year, means of each parameter in the columns followed by the same letter are not significantly different for 0.05 probability level (LSD test), according to the one-way ANOVA test, with cultivar as variability factor.

[a]Vigour scale from 1 to 5 (1= low vigour; 2 = slow growth rate; 3 = moderate vigour; 4 = vigorous; 5 = high vigour)

Table 2. Fresh and dry yields (t ha^{-1}) of stem flowers and inflorescences of the four cultivars of *L. hybrida* and *L. angustifolia* at harvest in the 2 consecutive growing seasons.

		2014	2015	Mean Cultivar
Fresh stem flower yield (t ha^{-1})	cv. Mailette	5.01 ± 0.17	5.31 ± 0.35	5.16 C
	cv. Grosso	5.48 ± 0.32	5.14 ± 0.41	5.31 C
	cv. Sumiens	5.88 ± 0.26	5.60 ± 0.36	5.74 B
	cv. Super A	8.99 ± 0.42	8.14 ± 0.35	8.57 A
	Significance	*Cv = ***; Y = n.s.; CvxY = n.s.*		
Dry stem flower yield (t ha^{-1})	cv. Mailette	1.79 ± 0.06	1.90 ± 0.10	1.85 C
	cv. Grosso	2.37 ± 0.05	2.21 ± 0.10	2.29 B
	cv. Sumiens	2.24 ± 0.02	2.13 ± 0.13	2.19 B
	cv. Super A	3.74 ± 0.02	3.58 ± 0.18	3.66 A
	Significance	*Cv = ***; Y = n.s.; CvxY = n.s.*		
Fresh stemless flower yield (t ha^{-1})	cv. Mailette	2.39 ± 0.12 e	2.53 ± 0.17 de	2.45 C
	cv. Grosso	3.55 ± 0.08 b	2.45 ± 0.21 de	3.00 B
	cv. Sumiens	2.64 ± 0.03 d	3.26 ± 0.08 c	2.95 B
	cv. Super A	4.22± 0.02 a	3.58 ± 0.10 b	3.90 A
	Significance	*Cv = ***; Y = n.s.; CvxY = ******		
Dry stemless flower yield (t ha^{-1})	cv. Mailette	1.07 ± 0.06	1.14 ± 0.04	1.11 C
	cv. Grosso	1.31 ± 0.03	1.17 ± 0.05	1.24 B
	cv. Sumiens	1.31 ± 0.04	1.30 ± 0.07	1.31 B
	cv. Super A	2.00 ± 0.05	1.95 ± 0.20	1.98 A
	Significance	*Cv = ***; Y = n.s.; CvxY = n.s.*		

Results are the means ± SD of three replicates. A two-way ANOVA was used to evaluate the effect of the interaction between cultivar (Cv) and year of growth (Y) (CvxY). Lower case letters indicate CvxY interaction, upper-case letters indicate effect of cultivar (Cv) and year of growth (Y). Significance was as follows: ns, not significant; ***, significant at $p < 0.001$ level.

478 **Table 3**. Chemical composition of EOs of three cultivars of *Lavandula hybrida* Reverchon (Grosso, Super A and Sumiens) and one cultivar of lavander (*Lavandula angustifolia*
479 Miller. cv Mailette) collected in two years of harvest (2014 and 2015).

				Relative abundance (%)							
	Component	**LRI[a]**	**Class**	**Mailette_14**	**Mailette_15**	**Sumiens_14**	**Sumiens_15**	**Super A_14**	**Super A_15**	**Grosso_14**	**Grosso_15**
1	tricyclene	938	MH				0.4		0.4		0.3
2	α-pinene	940	MH			0.5		0.5		0.5	0.1
3	Camphene	955	MH	0.3	0.3	0.5	0.4	0.4	0.3	0.4	0.4
4	sabinene	978	MH			0.2		0.2		0.1	
5	β-pinene	981	MH	0.2	0.1	0.6	0.4	0.6	0.4	0.6	0.4
6	1 octen-3-ol	982	NT						0.2		0.2
7	3-octanone	988	NT	1.1	0.7	1.0	0.7				
8	myrcene	993	MH	1.5	1.6	0.8	0.7	1.1	1.3	1.2	1.2
9	3-octanol	998	MH	0.3		0.2					
10	N-hexyl acetate	1013	NT	0.8	0.7			0.2	0.2	0.2	0.2
11	limonene	1032	MH	0.3	0.4	1.4		0.8	0.3	0.9	
12	1,8 cineole	1036	OM			12.7	11.2	8.1	5.7	8.4	7.8
13	(Z) β-ocimene	1042	MH	1.7	2.3	2.1	1.6	1.2	1.3	1.3	1.1
14	(E) β-ocimene	1053	MH	1.6	1.5	1.1	0.9	0.6	0.8	0.8	0.7
15	γ-terpinene	1062	MH					0.1	0.2	0.1	0.1
16	cis-sabinene hydrate	1070	OM			0.2	0.2	0.3	0.1	0.3	0.1
17	cis-linalool oxide (furanoid)	1079	OM		0.8		2.0		0.2		0.2
18	terpinolene	1090	MH		0.3				0.8		0.6

19	p-mentha-2,4(8)-diene	1094	OM	0.9		1.5		0.6		0.8	
20	trans-linalool oxide (furanoid)	1094	OM	0.7	0.7	1.4	2.0	0.3		0.4	
21	linalool	1102	OM	48.4	45.5	39.9	40.8	33.5	38.8	33.8	42.9
22	1-octen-3-yl acetate	1117	NT	1	0.5	0.2	0.1	0.6	0.6	0.4	0.4
23	3-octanol acetate	1129	NT	0.1	0.1						
24	camphor	1148	OM	0.4	0.5	8.9	5.3	7.8	5.4	8.5	7.7
25	hexyl isobutyrate	1153	NT	0.1		0.2		0.2	0.2	0.2	
26	borneol	1169	OM	1.1	1.5	7.4	13.1	3.1	4.3	3.1	4.5
27	lavandulol	1170	OM					0.1		0.2	
28	4-terpineol	1180	OM	0.1	0.2	0.1	0.2	2.7	3.9	3.1	4.1
29	cryptone	1187	NT			0.4	0.5				
30	hexyl butyrate	1191	NT	0.5		0.3		0.2		0.2	
31	α-terpineol	1192	OM	5.7	6.6	2.5	3.5	3.7	6.3	4.5	4.3
32	nerol	1228	OM	0.5	0.8	0.2	0.2	0.3	0.6	0.5	0.2
33	isobornyl formate	1230	OM			0.2	0.5				
34	hexyl-2-methylbutyrate	1234	NT		0.1		0.1		0.2		0.1
35	hexyl 3-methyl butanoate	1242	OM			0.2	0.1	0.2	0.3	0.2	
36	cuminaldehyde	1244	OM			0.1	0.3				0.2
37	carvone	1248	OM				0.2				
38	linalyl acetate	1260	OM	26.0	26.2	10.7	9.1	21.1	15.6	16.7	14.7
39	isobornyl acetate	1287	OM	0.1	0.2		0.1				
40	lavandulyl acetate	1289	OM	0.4	0.6	0.2	0.3	4.2	4.8	4.4	3.7

41	hexil tiglate	1333	NT			0.2	0.2	0.1	0.1	0.1	
42	neryl acetate	1368	OM	1.5	1.8	0.5	0.7	0.9	1.2	1.1	0.7
43	geranyl acetate	1386	OM	2.7	3.4	0.9	1.3	1.7	2.3	2.0	1.3
44	β-caryophyllene (= E-caryophyllene)	1418	SH	1.1	1.1	0.3	0.2	0.8	0.6	0.7	0.4
45	β-(E)-farnesene	1460	SH	0.5	0.7	0.1		0.5	0.4	0.5	0.3
46	germacrene D	1481	SH			0.1		0.3	0.2	0.4	0.2
47	lavandulyl isovalerate	1510	NT			0		0.3	0.2	0.3	0.1
48	trans-γ-cadinene	1513	SH			0.1					
49	caryophyllene oxide	1582	OS	0.4	0.5	0.4	0.7	0.1		0.2	
50	T-cadinol (=epi-α-cadinol)	1642	OS			1.0	1.0	1.0	0.6	1.1	0.3
51	α-muurolol (=torreyol)	1651	OS				0.3				
52	α-cadinol	1655	OS							0.1	
53	epi-α-bisabolool	1685	OS			0.5	0.6	1.6	1.0	1.7	0.5
	Total identified			100	99.7	99.8	99.9	100	99.8	100	100
	EO Yield (%w/w)			5.08	4.49	6.36	6.95	8.81	9.35	8.25	8.50

480 *.a) LRI: Linear retention index*

481 **Table 4**. Percentage of the main compounds in the lavender EO samples in comparison with the accepted percentage of
482 AFNOR and European Pharmacopoeia (EPh).

COMPOUNDS	AFNOR	EPh	Maillette_14	Maillette_15
Linanool	25 – 37	25 - 45	48.4	45.5
Linalyl acetate	35 – 47	25 - 46	26	26.2
1.8-cineol	3 – 7	Max 2.5	TR	TR
Borneol	1.4 – 3		1.1	1.5
Camphor	3.5 – 6.5	Max 1.2	0.4	0.5

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485 **Table 5**. Percentage of the main compounds in the EO of lavandin cultivars in comparison with the accepted percentage
486 of European Pharmacopoeia (EPh).

COMPOUNDS	EPh	Sumiens_14	Sumiens_15	SuperA_14	SuperA_15	Grosso_14	Grosso_15
Linanool	25 - 45	39.9	40.8	33.5	38.8	33.8	42.9
Linalyl acetate	25 - 46	10.7	9.1	21.1	15.6	16.7	14.7
1.8-cineol	Max 2.5	12.7	11.2	8.1	5.7	8.4	7.8
Camphor	12 - 18	8.9	5.3	7.8	5.4	8.5	7.7

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Table 6. Radical scavenging activity, measured by DPPH assay, of lavender and lavandin EOs, obtained in the two years of cultivation. Trolox has been used as reference standard.

	IC_{50} (mg mL^{-1}) year 2014	IC_{50} (mg mL^{-1}) year 2015	Mean
Super A	50.35 d	58.26 e	54.31 B
Sumiens	43.48 c	62.49 f	52.99 B
Grosso	63.86 f	58.13 e	70.00 C
Mailette	28.57 b	3.96 a	16.27 A
Trolox	0.022		

Results are the means of three replicates. A two-way ANOVA was used to evaluate the effect of the interaction between cultivar (Cv) and year of growth (Y) (CvxY). Lower case letters indicate CvxY interaction, upper-case letters indicate effect of cultivar (Cv).

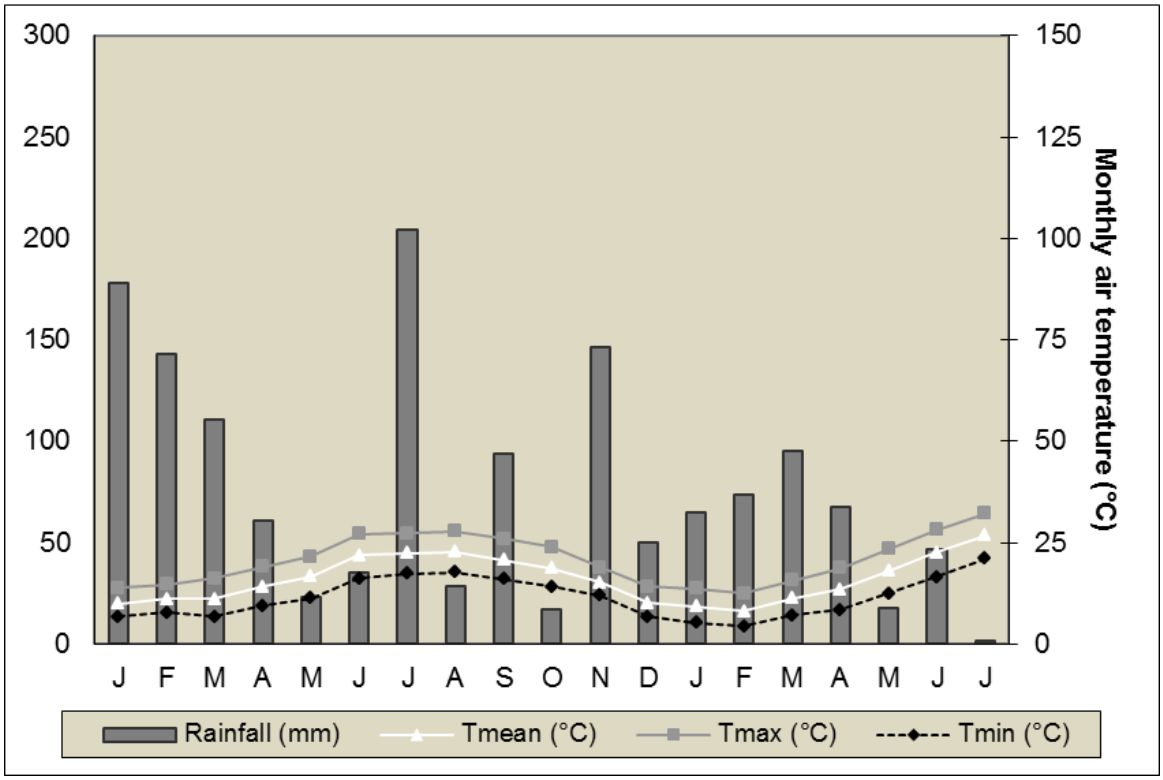
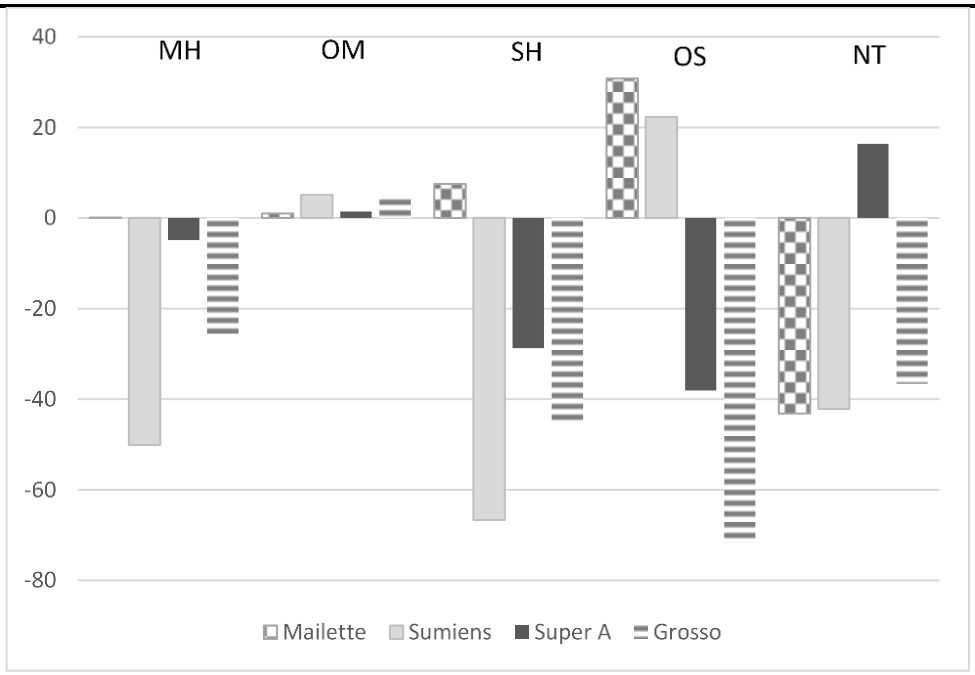


Fig. 1. Meteorological data (monthly rainfall and monthly mean temperatures) for 2014 and 2015 growing seasons.



class of compounds	Maillette_14	Maillette_15	Sumiens_14	Sumiens_15	SuperA_14	SuperA_15	Grosso_14	Grosso_15
Monoterpene Hydrocarbons (MH)	5.9	6.5	7.4	4.4	5.5	5.8	5.9	4.9
Oxygenated Monoterpenes OM)	88.5	88.8	87.6	91.1	88.6	89.5	88	92.4
Oxygenated Sesquiterpenes (OS)	0.4	0.5	1.9	2.6	2.7	1.6	3.1	0.8
Sesquiterpene Hydrocarbons (SH)	1.6	1.8	0.6	0.2	1.6	1.2	1.6	0.9
Non-Terpenes (NT)	3.6	2.1	2.3	1.6	1.6	1.7	1.4	1.0

Fig. 2. Chemical classes of compounds present in the EO of the four *Lavandula* cultivars (%) and graphical representation of their variation (%) in 2015 with respect to 2014.

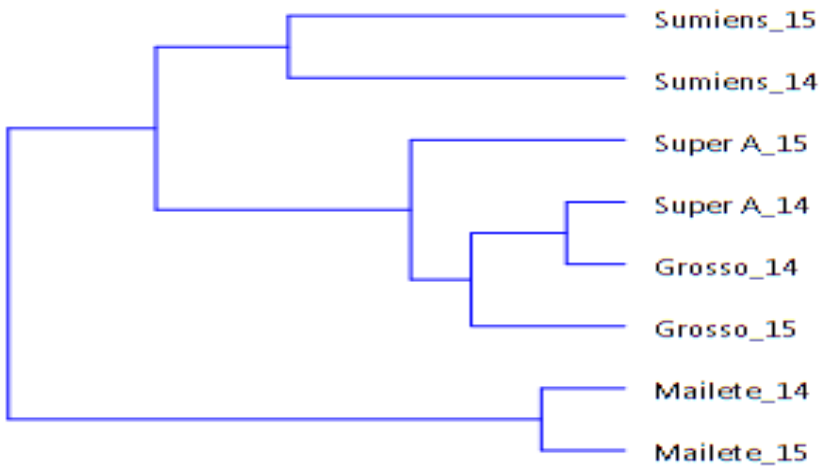
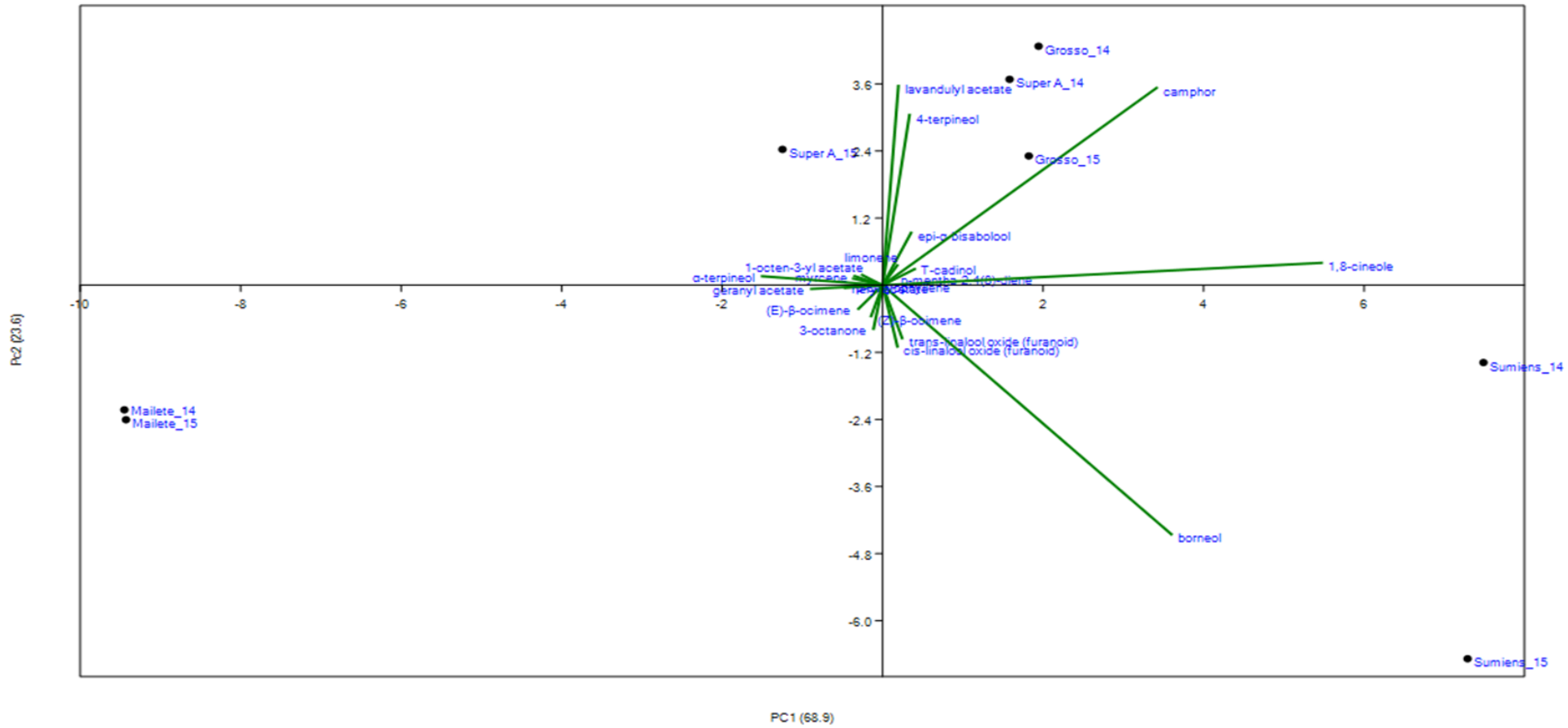


Fig. 3. Dendrogram of the Hierarchical Cluster Analysis (HCA) volatile constituents from different cultivars of *Lavandula* EOs.



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507 **Fig. 4.** Principal Compound Analysis (PCA) plot of volatile constituents from different cultivars of *Lavandula* EOs**.**

Highlights

- Lavender and lavandin are successfully and organically grown in Tuscany.
- Good and stable inflorescences yields are achieved in a 2-year field trial.
- EOs were analysed for composition and antioxidant properties.
- EO composition varies among species/cultivars, except for oxygenated monoterpenes.
- EO antioxidant activity is strongly affected by growing season.

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