Cultivation strategy to improve chemical profile and anti-oxidant activity of *Sideritis perfoliata* L. subsp. *perfoliata*

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**ABSTRACT**

Due to the remarkable medicinal properties of *Sideritis* spp., the commercial demand for the plant is continuously on the rise both in the European and in the global market. Cultivation of *Sideritis* species have been occurring to successfully meet the need for mass production of high-quality plant material. The present study was undertaken in order to investigate the impact of different cultivation practices (conventional versus organic cultivation; full versus deficit irrigation) in the yield and phytochemical profile of *S. perfoliata* L. subsp. *perfoliata* from Cyprus, under two harvestings for biomass production. Deficit irrigation decreased plant growth, but increased dry matter content. The content of chlorophylls and the nitrogen and potassium content decreased in organically grown plants. Both organic cultivation and/or deficit irrigation increased total phenolics, flavonoids, vitamin C and antioxidants. Essential oil yield increased under deficit irrigation at the 2nd harvest, while essential oil composition fluctuated among the treatments. Infusions of each plant material were prepared according to the European Medicines Agency (EMA) monograph. Based on their NMR spectra, the deficit-irrigated plants from conventional cultivation were the most rich in secondary metabolites and chosen for further chemical analysis. Six iridoids such as three flavonoids, two phenylethanoid glucosides and one phenolic acid have been isolated indicating new knowledge on the effects of cultivation practices on plant secondary metabolisms with putative industrial applications and interest.

1. Introduction

The widely and important use of *Sideritis* species in the folk medicine has been recorded in several ethnopharmacological studies. The genus *Sideritis* L. (Lamiaceae) comprises more than 150 species, located mainly in the Mediterranean, the Balkans and the Iberian Peninsula (González-Burgos et al., 2011). Among them, *S. perfoliata* L. is a perennial herb native to Greece, Turkey, Cyprus and Syria; it belongs to the section Embedoclea (Rafin) Bentham (Barber et al., 2002). In Cyprus and in Greece, the infusion of *S. perfoliata* L. by using mainly dried leaves and flowers, has been widely used in traditional medicine for the relief of dyspepsia, stomach disorders, as diuretic, aphrodisiac and calmative, as well as for the treatment of anemia, influenza, bronchitis, common cold and cough (Karousou and Deirmetzoglou, 2011).

The European Medicines Agency (EMA) has recognized *Sideritis* spp. as a traditional medicine and included herba *Sideritis* (S. scardica, *S. clandestina*, *S. raeseri*, *S. syriaca*) as a treatment against the common cold, for cough relief, and also for the relief of mild gastrointestinal disorders. A plethora of studies mention significant biological activities, such as anti-inflammatory, anti-oxidant and against gastrointestinal ulcer (González-Burgos et al., 2011; EMA, 2015). Furthermore, the use of *Sideritis* as a remedy against Alzheimer’s disease and memory disorders has been recently studied (Hofrichter et al., 2016). Loizzo et al. (2007) reported the cytotoxic activity of *S. perfoliata* essential oils and their ability to inhibit human tumor cell growth. The significant pharmacological activities and the high nutritional value of *Sideritis* spp. are attributed to their rich phytochemical profile. The herb is rich in minerals and various secondary metabolites, such as essential oils, terpenes, polyphenolic and phenolic derivatives (González-Burgos et al., 2011; EMA, 2015). As a result, during the last decade, different cultivation methods of *Sideritis* species have been occurred and focused on the optimization of the cultivation conditions aiming to maximize the...
yield of the bioactive secondary metabolites and to increase the pharmacological activities of the plant.

Nowadays, the consumption and preference of organic against non-organic (namely conventional) food has been increased compared to the past, as consumers demand healthier lifestyle, minimizing phytochemicals and protecting environment and living organisms (Lohr, 2001; Chrysargyris et al., 2017a). Organic agriculture is an integrated system based on ecological principles of professionalism. Especially for Medicinal and Aromatic Plants (MAP), there is a huge trend for organic cultivation (Bhattacharya et al., 2017) due to the lower inputs/demands (water, fertilizers, phytochemicals, cultivation practices), decreased growth rates and photosynthetic capacity and their often higher bioactivity on secondary metabolites as compared to conventionally grown MAPs (Bhattacharya et al., 2017). Quality and safety of MAP-derived products (fresh and dry plant tissue, essential oils, extracts and isolated components) is required and consists of major concern issues in health, cosmetics, and pharmaceuticals sector (Ekor, 2014).

Several environmental/abiotic (salinity, drought, minerals, temperature, wind etc.) and biotic (pathogens, living organisms etc.) factors are responsible for the crop’s productivity and adaptation to stress conditions. Considering the lack of fresh water for irrigation needs in Mediterranean basin and worldwide as this is influenced by the effects of Climate Change, farmers often have to deal with water shortage during the cultivation period. This affects yield and availability of field area for cultivation (Osakabe et al., 2014; Chrysargyris et al., 2016a).

Generally speaking, water deficiency is affecting plant growth negatively (decreasing leaf water potential and opening of stomata, decreasing CO2 availability and consumption of reduction equivalents (NADPH+H+) for CO2-fixation viaCalvin cycle), resulting in a reduction status of the photosynthetic apparatus (Bloom et al., 2014; Al-Gabbiesh et al., 2015). In consequence, this reduction and the excess/non consumed NADPH+H+ is favoring the synthesis and might reflect some positive impacts on secondary metabolites, including the increase in antioxidants and higher insecticidal activities (Laribi et al., 2009; Bettaieb et al., 2012; Chrysargyris et al., 2016a). Cultivation strategies of using deficit irrigation, alternative sources (treated-waste water, saline water etc.) of water for irrigation, and less water demanding crops, such as most of the MAP, are attracting the interest for exploitation and research.

Although organic cultivation practice is often a recommended choice for growing MAPs (Malik et al., 2011) few efforts have been devoted to the study of the organic cultivation conditions (Carrubba, 2014; Bhattacharya et al., 2017). Taking into consideration that the cultivation conditions may affect the biosynthesis of active ingredients in the plants and the high demand of Sideritis spp., the present study was undertaken in order to investigate the effect of different cultivation practices (conventional versus organic cultivation and full versus deficit irrigation) in the yield, total antioxidant activities, and phytochemical profile of S. perfoliata subsp. perfoliata from cultivated populations collected at different time intervals (six weeks) of harvestings.

2. Materials and methods

2.1. Plant material and experimental conditions

S. perfoliata subsp. perfoliata seedlings were purchased from the Cypriot National Centre of Aromatic Plants in trays, at the growth stage of 3–4 leaves and 5–6 cm height. Seedlings were established under field conditions, during spring-summer of 2018 in a commercial organic farm, Limassol, Cyprus (34°38′N, 32°56′E, 7 m). The experimental farmland occupied approximately 350 m², and the soil had 3.01% organic matter; available CaCO₃ 21.23%; pH 8.42; EC 0.78 mS/cm. The climate of the region is dry with the average midday temperature and air humidity during the summer months were ca.34.2°C and 59%, respectively.

2.2. Cultivation practices

Seedlings were transplanted in soil and arranged in triple rows (rows were 0.2 m apart and plants were separated by 0.33 m) at a plant density of 51,950 plants/ha. Seedlings were grown for c.a. four months. The experimental farm was divided into four treatments: i) Conventional with full irrigation (Conv.FI), ii) Conventional with deficit irrigation (Conv.DI), iii) Organic with full irrigation (Org.FI), and iv) Organic with deficit irrigation (Org.DI). Each treatment had three plots (replicates) and each plot had 30 plants. A total of 360 plants used in the current study. Registered organic or conventional fertilizers and pesticides were used accordingly.

The amount of irrigation applied was programmed according to the soil volumetric water content of the irrigation treatment measured by field-scout TDR300, equipped with 20 cm rods (Spectrum Technologies Inc, Aurora, IL, USA). Irrigation water was supplied approximately every 5–7 days. Soil water content measurements took place at 5–7 days intervals (Fig. 1S). Plants were grown for two months under full irrigation. Then, deficit irrigation (ca.50% of the full irrigation treatment) was applied for three weeks before the 1st harvest (May 2018) of the crops at the early flower stage of the plants, and then for three weeks before the 2nd harvest (June 2018). Between the two harvests (three weeks period), crop was irrigated normally, according to the plant water needs, in order to recover the biomass production.

2.3. Plant growth, physiology, and minerals

During the three weeks of water stress, before the 1st and 2nd harvest, physiological records took place, such as leaf stomatal conductance and chlorophyll fluorescence. Stomatal conductance measurements were carried out using a ΔT-Portometer AP4 (Delta-T Devices-Cambridge, UK). Leaf chlorophyll fluorescence (chlorophyll fluores- meter, opti-sciences OS-30p, UK) was measured on three fully expanded, sun-exposed leaves per plant (Chrysargyris et al., 2017b). Plant height was recorded in six plants/treatment before harvesting. Plants were harvested at 3 cm above soil in order to recover the biomass production, upper fresh weight was weighed (g), dried and total dry matter content (%) was then calculated.

Chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (t-Chl) content was determined as described by Richardson et al. (2002). Briefly, leaf disk (0.1 g consisted of a pool of two plants tissue) incubated in heat bath at 65°C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO, Sigma Aldrich, Germany) for chlorophyll extraction. Photosynthetic leaf pigments (Chl a, Chl b and t-Chl) content were calculated using the following equations: Chl a = 0.0127 × A₆6₃/0.0269 × A₆₄₅; Chl b = 0.0229 × A₆₄₅/0.00468 × A₆₆₅; t-Chl = 0.0202 × A₆₄₅ + 0.00802 × A₆₆₅. Results were expressed as mg of chlorophylls per g of fresh weight.

Mineral content in leaves was determined on three replications/treatment (three pooled plants/replication). Samples were dried to constant weight (at 65°C for 4 d) and milled at <0.42 mm. Sub samples (‘0.5 g) were burned to ash in a furnace (Carbolite, AAF 1100, GERO, Germany) at 450°C for 5 h and then were digested with acid (2 N HCl). Mineral assessment for K, Na, P and N was performed according to Chrysargyris et al. (2019) and Mg, Ca, Fe, Cu, and Zn by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). Data were expressed in g/kg and mg/kg of dry weight, for macronutrients and micronutrients, respectively.

2.4. Essential oil extraction and analysis

Sideritis plants at the early flowering stage (leaves and flowers) were harvested and three biological replicates (pooled of three individual plants/replicate) from each treatment were air-dried at 42°C in oven according to Calin-Sánchez et al. (2013) and preliminary tests. Then dry plant material was chopped and hydrodistilled for 3 h, using Cleveger
apparatus for essential oil (EO) extraction. The EO yield was calculated (%) and oils were analyzed by Gas Chromatography-Mass Spectrometry (GC/MS- Shimadzu GC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010plus mass spectrometer) and constituents were determined (Chrysargyris et al., 2016b).

2.5. Polyphenols, flavonoids, ascorbic acid and antioxidant activity

Plants (0.5 g) at the early flowering stage (leaves and flowers) of four replicates (pooled by two individual plants/replicate) for each treatment were milled with 10 mL methanol (50%) and extraction was assisted with ultrasound. The antioxidant activity of the methanol extract was determined by using the assays of 2,2-diphenyl-1-pi- crylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), as previously described by Chrysargyris et al. (2016b) as well as the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay according to the methodology described by Wojdylo et al. (2007). Results were expressed as Trolox (1D&2DNMR, Overhauser Effect SpectroscopY), ROESY (Rotating-frame Overhauser Effect Spectroscopy), HSQC (Heteronuclear Single Quantum Correlation), HMBC (Heteronuclear Multiple Bond Correlation), NOESY (Nuclear Overhauser Effect Spectroscopy), ROESY (Rotating-frame Overhauser Effect Spectroscopy). 1H, 13C and 2D NMR spectra were recorded in Bruker DRX 400 and Bruker AC 200 (50.3 MHz for 13C NMR) instruments at 295 K. Chemical shift are reported in ppm (δ) using the residual solvent signal (δH 3.31 in 1H and δC 49.0 in 13C, CD3OD) as reference. COSY, HSQC, HMBC, NOESY were performed using standard Bruker microprograms. UV–vis spectra were recorded using a Shimadzu UV-160A spectrophotometer, according to standard procedures (Mabry et al., 1970). Optical rotation was recorded on a Perkin Elmer 341 polarimeter. The [α]D values were obtained in methanol at 20°C. Column chromatography (CC): silica gel (Merck, Art. 9385), gradient elution with the solvent mixtures indicated in each case. Preparative TLC plates pre coated with cellulose (Merck, Art. 5716). Fractionation was always monitored by TLC silica gel 60 F-254, (Merck, Art. 5554) and cellulose (Merck, Art. 5552) with visualization under UV (254 and 365 nm) and spraying with vanillin-sulfuric acid reagent (vanillin Merck, Art. No. S26047 841) and with Neuf’s reagent for phenolics (Neuf, 1957), respectively. All obtained extracts, fractions, and isolated compounds were evaporated to dryness in vacuum under low temperature and then were put in activated desiccators with P2O5 until their weights had stabilized. This procedure allows the elimination of the moisture from the samples that might influence pre-saturation performance and then lead to an intense water signal in the 1H-NMR spectra, making it difficult to observe near signals.

2.7. Preparation and isolation

Infusions of all samples were prepared based on the monograph of EMA, namely 4.0 g of air-dried aerial parts (early flowering stage, including leaves and flowers) of each cultivation were dropped into 150 mL boiled distilled water for 5 min, then were filtrated and concentrated to dryness. All residues were monitored and traced down using an NMR metabolomics strategy, which permitted their detailed characterization and enabled us to choose the most abundant infusion. Therefore, the infusion of the conventional deficit-irrigated S. perfoliata subsp. perfoliata was chosen for further chemical analysis based on its 1H-NMR spectrum. The infusion (1.0 g) was fractionated by CC over silica gel using as eluent mixtures of CH3Cl2:MeOH:H2O 1:0-0:1:0 to yield finally 23 fractions (A–Y). Fractions J, O, R, W and X (eluted with CH3Cl2:MeOH:H2O 8.3:1:7.0:6:1:4.0) were identified as compounds 4 (0.9 mg), 3 (0.4 mg), 7 (0.8 mg), 12 (7.5 mg), 11 (23.8 mg) and 6 (0.4 mg), respectively. Fraction 1 (18.0 mg; eluted with CH3Cl2:MeOH:H2O 8:2:0:2) was further purified by preparative Thin-Layer Chromatography (TLC) on cellulose using AcOH:H2O 3:7 as eluent and afforded compounds 3 (1.6 mg), 5 (4.5 mg) and 9 (8.2 mg). Fraction S (52.8 mg; eluted with CH3Cl2:MeOH:H2O 7:5:2:5:0:2) was subjected to CC over silica gel (EtOAc:MeOH:H2O 9:1:5:1:5:5:0) and yielded compounds 7 (3.9 mg) and 10 (1.0 mg). Fraction T (120.0 mg; eluted with CH3Cl2:MeOH:H2O 7.0:3:0:0:3:0:0) was submitted to CC over silica gel (EtOAc:MeOH:H2O 9:1:5:1:5:5:0) and afforded compounds 1 (0.9 mg) and 8 (2.2 mg). Fraction V (100.0 mg; eluted with CH3Cl2:MeOH:H2O 6.5:3:5:5:0:3) was further purified by CC over silica gel (EtOAc:MeOH:H2O 9:1:5:1:5:5:0) and yielded compounds 2 (5.0 mg), 6 (0.4 mg), 11 (5.3 mg) and 12 (7.0 mg).

2.8. Statistical methods

Statistical analysis was performed using IBM SPSS version 22 where the effects of cultivation practice, irrigation and harvesting period as well as their interactions on the plant growth, physiological, biochemical and mineral levels of samples were assessed with three way ANOVA. Data means were also compared with one-way analysis of variance (ANOVA) and Duncan’s multiple range tests for comparisons of treatment means at $P < 0.05$. Measurements were done in three to six biological replications/treatment (each replication consisted of a poll of three individual measures/samples).

3. Results

3.1. Plant growth

Plants were cultivated in an open field and the aerial parts were used in the study, after two harvesting periods. Plant height increased in DI-treated plants compared to FI-treated plants in conventional treatment, as well as in FI-treated plants compared to DI-treated plants in organic treatment at the 2nd harvest (Table 1). However, no differences on plant height (averaged in 56.7 cm) were found at the 1st harvest. In organic cultivation, deficit irrigation decreased plant biomass production and increased dry matter content.

Plants subjected to deficit irrigation, especially in organic cultivation, decreased the leaf stomatal conductance at the first week of the applied water stress in order to maintain water storage in the leaves (Fig. 1). The oppose results were observed for the chlorophyll fluorescence at the 1st harvest, while no differences on chlorophyll fluorescence could be observed thereafter. Chlorophylls content decreased in organic cultivation compared to the conventional one, while deficit irrigation did not cause any effects (Table 1).

Three way ANOVA analysis shown in Table 1 revealed that cultivation practice (conventional versus organic) affected the content of chlorophylls (Chl a, Chl b and total Chls) ($P < 0.001$). The irrigation (full versus deficit) affected plant height ($P < 0.05$), fresh weight and dry matter content ($P < 0.001$). Harvesting period significantly affected plant height ($P < 0.001$) and dry matter content ($P < 0.05$). Fresh weight was significantly ($P < 0.05$) impacted by the interaction of cultivation practice*harvesting period. The interactions of irrigation*harvesting period, cultivation practice*irrigation, and cultivation
3.2. Mineral content

The mineral content varied among treatments and harvesting period as presented in Table 2. Overviewing, different mineral accumulation was found among the 1st and the 2nd harvest. At the 1st harvest, deficit irrigation decreased N but increased Mg content in conventional cultivation while DI decreased K and P but increased Ca content in organic cultivation (Table 2). *Sideritis* grown in organic versus conventional cultivation revealed decreased N and K, but increased P, Mg, and Na content. No differences were found on microelements as Fe content averaged in 148.59 mg/kg, Zn content averaged in 146.78 mg/kg, and Cu content averaged in 57.32 mg/kg. At the 2nd harvest, DI decreased N, K, P and Mg but increased Na content in conventional cultivation while DI decreased K and Mg content in organic cultivation (Table 2). Plants grown in organic versus conventional cultivation revealed decreased N, K, P, Mg, Na, Fe and Cu content. No differences were found on Ca content averaged in 9.71 g/kg and Fe content averaged in 131.63 mg/kg.

Multi-variance analysis revealed that Ca content affected \( P < 0.01 \) by cultivation and irrigation*harvesting period while Mg content affected \( P < 0.05 \) by cultivation while the Cu content was affected by harvesting and irrigation*harvesting \( P < 0.05 \) and by cultivation*irrigation*harvesting \( P < 0.01 \).

### 3.3. Phenolics, flavonoids, ascorbic acid and antioxidant status

The content of polyphenols, flavonoids, ascorbic acid and antioxidant activities (as assayed by FRAP, DPPH, ABTS) were increased in conventional and deficit irrigation treatments as well as in organic (full and deficit irrigation) treatments for both harvesting periods (Table 3). Interestingly, organically-grown plants under deficit irrigation revealed up to 5.7 times higher total phenolics when compared to the control treatment (conventional and fully-irrigated plants) at the 1st harvest. Total flavonoids increased in both 1st and 2nd harvestings in plants subjected to organic practice and/or deficit irrigation with more pronounced effects on organically and deficit-irrigated *Sideritis*. The content of ascorbic acid increased in deficit-irrigated plants under conventional practice during the 1st harvest comparing to the full-irrigated plants but this increase did not persist during the 2nd harvest. In general, plants subjected to either mineral limitations (organic practice) and/or water shortage (deficit irrigation) stress had increased AA levels (Table 3).

The antioxidant capacity fluctuated among the harvesting periods as well as the applied cultivation practices. At the first harvesting period,
Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fe (mg/kg)</th>
<th>Cu (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Mg (mg/kg)</th>
<th>Ca (mg/kg)</th>
<th>Na (mg/kg)</th>
<th>P (mg/kg)</th>
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<tbody>
<tr>
<td>1st harvest</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Conventional FI</td>
<td>0.12 ± 0.01c</td>
<td>3.37 ± 0.22b</td>
<td>6.48 ± 0.57a</td>
<td>6.48 ± 0.47a</td>
<td>2.86 ± 0.12a</td>
<td>2.34 ± 0.07b</td>
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<td>2.34 ± 0.07b</td>
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<td>2nd harvest</td>
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Values (n = 4) in columns for each harvest followed by the same letter are not significantly different, P > 0.05, ns, *; **, *** indicate non-significant or significant differences at P < 0.05, 1% and 0.1%, respectively.

Essential oil yield did not differ at the 1st harvest but increased (up to 32%) with the deficit irrigation practice at the 2nd harvest (Table 1). The higher EO yield was found in organic cultivation when plants subjected to deficit irrigation, while the lowest yield was evidenced in conventional and full-irrigated plants. Three-way ANOVA analysis for essential oil yield is presented in Table 1. Essential oil yield was significantly affected by irrigation and harvesting (P < 0.001), as well as cultivation practice*harvesting period.

Essential oils composition is presented in Table 4, and analysis revealed the presence of thirty-seven (excluding cis-Ocimene) and thirty-four (excluding neral, carvone, geranial, and bourbonene b) individual compounds, representing ≥96.43% of the total oil profile, for the 1st and 2nd harvests respectively. It can be further noticed that monoterpenes hydrocarbon compounds were the most abundant class (72.03% to 84.39%), followed by oxygenated sesquiterpenes (8.73% to 15.22%), sesquiterpene hydrocarbons (3.54% to 5.89%) and oxygenated monoterpenes (0.52% to 2.42%) (Table 4). The major constituents of the examined *Sideritis* EO in decreasing order were *β*-phellandrene (26.54–31.72%), *α*-pinene (25.35–28.15%), valerenal (7.72–12.94%), *β*-pinene (6.51–7.29%), sabinene (4.20–5.02%), the *β*-phellandrene (26.54–31.72%), *α*-pinene (25.35–28.15%), valerenal (7.72–12.94%), *β*-pinene (6.51–7.29%), sabinene (4.20–5.02%), the **3.4. Essential oil yield and composition**

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The major component of the essential oil (*β*-phellandrene) increased in deficit-irrigated *Sideritis* in conventional (at 1st harvest) and organic (1st and 2nd harvest) cultivation. Sabinen had similar trend with *β*-phellandrene. The content of *α*-pinene increased (up to 10%) in FI-organically grown plants at the 1st harvest comparing with the FI-conventionally grown plants, while this was not evident at the 2nd harvest. Indeed, full irrigation increased the content of *β*-pinene in organic (1st and 2nd harvest) and conventional (at 2nd harvest) cultivation (Table 4). The highest content of valeranone was found in FI-conventionally grown plants at the 1st harvest but not similar outcomes were found at the 2nd harvest whereas valeranone content increased in FI-organically grown plants. In general, deficit irrigation increased the content of 3-carene, *β*-myrcene and terpinolene either at the 1st or at the 2nd harvest.

3.5. Phytochemical profile

Eight infusions were prepared in order to investigate the effect of different cultivation practices (conventional versus organic cultivation;
full versus deficit irrigation) in the yield and phytochemical profile of S. perfoliata subsp. perfoliata from Cyprus, harvested two times (in May 2018 and in June 2018). Based on the $^1$H-NMR spectra, we noticed that the infusions of all the cultivation practices harvested in May 2018 showed richer phytochemical profiles compared to the other infusions of the plant materials harvested in June 2018 (Fig. 2A and B). Moreover, the $^1$H-NMR spectra of conventional and organic cultivations of the deficit-irrigated plants proved abundant in metabolites and exhibited signals attributed to iridoids and polyphenolic derivatives (phenylethanoid glucosides, flavonoids and phenolic acids) (Fig. 2A). At a first step, the infusions of the conventional cultivation; deficit-irrigated plants was chosen due to the high antioxidant activity that exhibited over, the $^1$H-NMR spectra of conventional and organic cultivations of Sideritis plants was chosen due to the high antioxidant activity that exhibited consequently to their antioxidant activity. To the best of our knowledge, the present study deals with the first phytochemical analysis of conventional cultivation; deficit-irrigated of S. perfoliata subsp. perfoliata revealed the presence of phenolic compounds 1-6, which belong to the chemical groups of phenylethanoid glucosides, flavonoids and phenolic acids (Ezer et al., 1992; Charami et al., 2008; Petreska et al., 2011). It is noteworthy to point out that all six isolated iridoids (7-12) had not been previously detected in S. perfoliata subsp. perfoliata. Plants of the Lamiaceae family contain a wide range of iridoid derivatives. Although, Sideritis was considered as an iridoid poor genus (Charami et al., 2008; González-Burgos et al., 2011), during the last two decades, many phytochemical studies have revealed their presence in different Sideritis species, such as S. perfoliata subsp. perfoliata (Charami et al., 2008), S. clandestina subsp. peleponnesiaca (Vasilopoulou et al., 2013), S. lanata (Alipieva et al., 2009), S. libanotica Labill. subsp. linearis (Charami et al., 2008), S. lycia (Akcoş et al., 1998), S. montana (Koleva and Handjieva, 1997), S. montana subsp. montana (Venditti et al., 2016), S. romana L. (Venditti et al., 2016), S. scardica (Koleva and Handjieva, 1997) and S. syriaca (Koleva and Handjieva, 1997). This is an interesting point, since the biosynthetic pathways of phenolics and iridoids are totally different. It seems that the treatment during the deficit-irrigated conventional cultivation triggered the biosynthesis of non-volatile monoterpenes. Iridoids represent a large group of non-volatile monoterpenes. Iridoids represent a large group of non-volatile monoterpenes and are reported to play an important role in plant defense and communication (Villasenor, 2007). They are biosynthesized from isoprene units via the mevalonic acid pathway of the plants (Dewick, 2009). Moreover, some minerals (e.g. Mg, Ca, Zn) have been mentioned to increase the content of terpenes and to be cofactors for enzymes which participate in the mevalonic acid pathway (Supanjani et al., 2005; Fagan and Palfrey, 2010). Thus, the different mineral accumulation between the different treatments may influence the mevalonic acid pathway of the plants. We believe that there is a strong correlation between the fluctuation of mineral contents and the biosynthesis of iridoids. Further investigation and application of different cultivation methods could provide new insights into the biosynthesis of secondary metabolites, mainly in iridoids of this genus, as well as into the biological activities of cultivated Sideritis species, with putative industrial interest and uses (infusion, pharmaceuticals, cosmetics, insecticide etc) and preparation of nutraceutical diet supplements and herbal drugs.

Conventional farming is usually an intensive cultivation practice with the use of chemical fertilizers and phytochemicals. This might increase the crop yield of MAP, but not necessarily increase the quality and safety of the consumed/used product, as the mineral fertilizers lower the concentration of bioactive components and therefore the nutritional value of the plant tissue (Kazimierzczak et al., 2015).

### Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total phenols</th>
<th>Total flavonoids</th>
<th>ABTS</th>
<th>DPPH</th>
<th>FRAP</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st harvest</td>
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<tr>
<td>Conventional FI</td>
<td>17.83 ± 1.85b</td>
<td>0.89 ± 0.19b</td>
<td>3.81 ± 0.61b</td>
<td>4.34 ± 0.21c</td>
<td>9.93 ± 0.80c</td>
<td>0.025 ± 0.001c</td>
</tr>
<tr>
<td>Conventional DI</td>
<td>82.31 ± 7.98a</td>
<td>4.24 ± 0.28a</td>
<td>6.34 ± 0.34a</td>
<td>11.13 ± 0.57b</td>
<td>25.27 ± 2.44ab</td>
<td>0.062 ± 0.006a</td>
</tr>
<tr>
<td>Organic FI</td>
<td>97.49 ± 13.16a</td>
<td>4.50 ± 0.46a</td>
<td>6.87 ± 0.17a</td>
<td>13.25 ± 0.51a</td>
<td>27.31 ± 1.51a</td>
<td>0.050 ± 0.000b</td>
</tr>
<tr>
<td>Organic DI</td>
<td>120.78 ± 21.43a</td>
<td>3.77 ± 0.32a</td>
<td>5.31 ± 0.91ab</td>
<td>10.64 ± 0.66b</td>
<td>21.84 ± 0.57b</td>
<td>0.051 ± 0.001b</td>
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<tr>
<td>2nd harvest</td>
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<tr>
<td>Conventional FI</td>
<td>25.02 ± 0.06b</td>
<td>1.57 ± 0.06c</td>
<td>2.61 ± 0.07b</td>
<td>4.81 ± 0.21c</td>
<td>6.89 ± 0.19c</td>
<td>0.056 ± 0.002c</td>
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<tr>
<td>Conventional DI</td>
<td>34.41 ± 9.6a</td>
<td>2.29 ± 0.16b</td>
<td>3.14 ± 0.03a</td>
<td>6.38 ± 0.40ab</td>
<td>9.89 ± 0.34ab</td>
<td>0.054 ± 0.002c</td>
</tr>
<tr>
<td>Organic FI</td>
<td>32.17 ± 2.63a</td>
<td>2.09 ± 0.12ac</td>
<td>3.04 ± 0.14ab</td>
<td>5.73 ± 0.49bc</td>
<td>8.99 ± 0.75ab</td>
<td>0.068 ± 0.001b</td>
</tr>
<tr>
<td>Organic DI</td>
<td>38.09 ± 2.62a</td>
<td>3.04 ± 0.23a</td>
<td>3.48 ± 0.23a</td>
<td>7.18 ± 0.41a</td>
<td>11.55 ± 1.05a</td>
<td>0.092 ± 0.004a</td>
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<td>Irrigation (I)</td>
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<td>Harvesting (H)</td>
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<td>C x I x H</td>
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</table>

Values (n = 4) in column for each harvest followed by the same letter are not significantly different, P ≤ 0.05. ns, *, **, and *** indicate non-significant or significant differences at P ≤ 5%, 1% and 0.1%, respectively, following three-way ANOVA.
However, in the present study, full-irrigated Sideritis obtained same yield in both organic and conventional practices. Indeed, the lack of irrigation water was the main factor that influenced crop productivity and plant physiology (i.e. open leaf stomata). Plant subjected to water stress regulated changes in abscisic acid signaling, ion transport, and leaf stomatal closure (Osakabe et al., 2014), being in accordance with the results obtained same Sideritis plants grown in conventional or organic cultivation and subjected to full (FI) or deficit (DI) irrigation.

Values (n = 3) in rows for each harvest followed by the same letter are not significantly different, P ≤ 0.05.
the present finding related to the reduced leaf stomatal conductivity. Dry matter content did not differ in the present study, being in accordance with previous reports on organically versus conventionally-grown peppermint (*Mentha piperita* L.) and rosemary (*Rosmarinus officinalis* L.) (Kazimierczak et al., 2015).

The organic *Sideritis*, independently of the irrigation scheme applied, contained higher level of total phenolics than the conventional full-irrigated *Sideritis*. Similarly, the conventional deficit-irrigated *Sideritis* had higher content of phenolics comparing with the full-irrigated plants. These findings are in agreement with previous studies of Kazimierczak et al. (2015) who reported increased levels of total phenolics and individual phenolic acids in the organic than in the conventional rosemary (*R. officinalis* L.) and lemon balm (*Melissa officinalis* L.). In contrast, Lv et al. (2012) found no differences on total phenolics on peppermint (*M. piperita* L.) and cinnamon (*Cinnamomum verum* L.) grown under organic and conventional cultivation practices. However, the same authors detailed the changes on individual phenolic components, as for example the organically grown peppermint had greater level of insoluble bound and total caffeic acid, and soluble free p-coumaric acid compared with the conventional counterpart, whereas the conventional peppermints had increased amounts of total and insoluble syringic and ferulic acids (Lv et al., 2012).

The importance of *Sideritis* spp (*Sideritis montana* L.) for pharmaceutical use (anti-biocidal) to treat a variety of disorders is attributed to the presence of flavonoids and phenylethanoid glycosides, has been previously reported (Satter et al., 1993). In the present study, increased levels of flavonoids were observed in organic farming (FI, DI) as well as in deficit irrigation for the conventional farming, highlighting the

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**Fig. 3.** $^1$H-NMR spectrum of the infusion of the conventional cultivation; deficit-irrigated of *S. perfoliata* L.

**Fig. 4.** $^1$H-NMR spectra of the iridoids compounds 7-12.
importance of the cultivation practices applied in each farm. Similarly, the organic medicinal plants such as rosemary, peppermint, lemon balm and sage, had on average greater levels of total flavonoids compared to the conventional ones (Kazimierczak et al., 2015). Regarding the antioxidant status of the harvested *Sideritis*, both organic and deficit irrigation were the main factors influencing the antioxidant levels at the first and at the second harvest, respectively, being in agreement with previous reports (Chrysargyris et al., 2016a). The phenolics, flavonoids and vitamin C biosynthesis, based on their structure, is related to the available carbon sources. Increased levels of N fertilizers promote plant growth and nitrogen-containing metabolites, i.e. free amino acids, proteins and alkaloids and as such, N increase causes declines in phenolics, flavonoids and vitamin C content (Kazimierczak et al., 2015). The opposite is evident under nitrogen shortage; this drives plants to diversification, increasing secondary metabolism including defense components (Pagare et al., 2015). In other words, the decreased N levels found in organically-grown plants and/or deficit-irrigated plants is reflected the increased levels of bio-compounds in the organically grown medicinal plants (Kazimierczak et al., 2015). Organic farmers commonly use less mineral levels with the organic fertilizers/sources when compared to the conventional ones, and as a consequence, conventional fertilizers will support more the plant growth needs with minerals. However, ion uptake and mineral accumulation in plants is related to several factors, including the water content in soil (mainly through irrigation practices) which affect the cations and anions absorptions by the plant.

Plants subjected to water stress revealed increased EO yield at the 2nd harvest and this was evident in previous studies on sage (Chrysargyris et al., 2016a) as stressed plants have a tendency to produce terpenes (Turtola et al., 2003). Essential oil’s monoterpenes (C10) are synthesized through the plastid-derived geranyl diphosphate (GDP) pathway (Schilmiller et al., 2009). In this study, it can be concluded that the synthesis of monoterpenes as α-pinene, β-pinene, sabinene, β-phellandrene and myrcene seems to be accelerated when plants are cultivated under water stress conditions. The synthesis, in this case, favors α-phellandrene and α-pinene, two monoterpenes that derive from two different routes of the same pathway (Dewick, 2002). This is evident in the first sampling and there is a tendency for the same result...
at the second sampling period. Stress condition, as water deficiency, is known to improve quality of the essential oil, in medicinal plants (Chrysargyris et al., 2016a). In contrast, the cytoplasmic formation of sesquiterpenes (C15) that are synthesized from farnesyl diposphate (FDP) appears to be delayed when deficit irrigation is applied to the plants. Valeranone, carphophilene and germacrane D appear in smaller percentages when plants are under the regime of deficit irrigation and organic cultivation. All C15 terpenes contribute less in the profile of the oils after the second sampling, revealing the late formation of the components.

The importance of organic derived products is well appreciated as they have higher levels of several chemicals compounds. Lu et al. (2014) even suggested a flow-injection mass spectrometric (FIMS) fingerprinting techniques for the rapidly (1 min) differentiation of organic versus conventional-derived sweet basil. Additionally, the high-resolution melting-curve (HRM) assay developed for the rapid and straightforward identification and discrimination of various Sideritis species, highlighting the importance of species identification due to their specific properties and oriented future trends in research (Kalivas et al., 2014).

5. Conclusion

The current study investigated the effect of water deficit and cultivation practice on the growth, biochemical attributes, both volatile and non-volatile composition on Sideritis plants. Deficit irrigation was mainly negative affected the plant growth-related parameters but increased the dry matter content and the yield of essential oils at the 2nd harvest. Cultivation practice (i.e. organic versus conventional) decreased the chlorophyll content and the nitrogen and potassium accumulation in Sideritis. Plants subjected to either organic cultivation and/or deficit irrigation had increased levels on secondary metabolites including total phenolics, flavonoids, vitamin C and antioxidants, and this compound synthesis is related to a possible induced stress caused by the examined cultivation practices. Following NMR spectra, the deficit-irrigated plants from conventional cultivation were the richest in secondary metabolites and were chosen for further chemical analysis. Six iridoids, namely three flavonoids, two phelylenethanol glycosides and one phenolic acid have been isolated, providing new inputs on plant metabolism related to cultivation practices, with possible industrial applications and interest. Medicinal and Aromatic Plant species are low demanding crops that can be cultivated with low inputs and/or various abiotic stress conditions, revealing changes in plant secondary metabolites and possible identification/isolation of components of industrial interest. Future research is obviously needed to that direction.

Author contributions

R.V. carried out the crop cultivation/maintenance and antioxidant analyses; A.C. carried out the crop cultivation, essential oil and mineral analysis; C.K. carried out the chemical analyses; E.-M.T contributed to the chemical analyses; H.S. supervised the chemical analyses; N.T. supervised the agronomical/biochemical analyses. C.K. carried out the chemical analyses; E.-M.T contributed to the chemical analyses; N.T. supervised the agronomical/biochemical analyses. Additional, the high resolution melting-curve (HRM) assay developed for the rapid and straightforward identification and discrimination of various Sideritis species, highlighting the importance of species identification due to their specific properties and oriented future trends in research (Kalivas et al., 2014).

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2019.111694.

References


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