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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 occurred 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

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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 CO_2 availability and consumption of reduction equivalents ($NADPH + H^+$) for CO_2-fixation *via*Calvin cycle), resulting in a reduction status of the photosynthetic apparatus (Bloem 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 m^2, and the soil had 3.01% organic matter; available $CaCO_3$ 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-Porometer AP4 (Delta-T Devices-Cambridge, UK). Leaf chlorophyll fluorescence (chlorophyll fluoremeter, 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 $a = 0.0127 \times A_{663} - 0.00269 \times A_{645}$; Chl $b = 0.0229 \times A_{645} - 0.00468 \times A_{663}$; and t-Chl $= 0.0202 \times A_{645} + 0.00802 \times A_{663}$. 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 Calın-Sanchez et al. (2013) and preliminary tests. Then dry plant material was chopped and hydrodistilled for 3 h, using Clevenger

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 plant extracts was determined by using the assays of 2,2-diphenyl-1-picrylhydrazyl (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 Wojdyło et al. (2007). Results were expressed as Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent (mg trolox/g of fresh weight). Total phenolic content was measured using the Folin-Ciocalteu method, and results expressed as gallic acid equivalents (μmol GAE/g of fresh weight) as described previously by Tzortzakis et al. (2011). Total flavonoid content was determined according to aluminium chloride colorimetric method (Meyers et al., 2003) and expressed as Rutin equivalents (mg Rutin/g of fresh weight). Ascorbic acid (AA) content was quantified by titration with 2,6-dichlorophenol- indophenol (AOAC International, 2007) and results were expressed as mg of AA per g of fresh weight.

2.6. General experimental procedures of isolation and identification of the secondary metabolites

The structures of the isolated compounds were established by means of 1D & 2D NMR, *i.e.* (^{1}H-^{1}H-COSY (COrrelation SpectroscopY), ^{1}H-^{13}C-HSQC (Heteronuclear Single Quantum Correlation), HMBC (Heteronuclear Multiple Bond Correlation), NOESY (Nuclear Overhauser Effect SpectroscopY), ROESY (Rotating-frame Overhauser Effect SpectroscopY)]. ^{1}H, ^{13}C and 2D NMR spectra were recorded in Bruker DRX 400 and Bruker AC 200 (50.3 MHz for ^{13}C NMR) instruments at 295 K. Chemical shift are reported in ppm (δ) using the residual solvent signal (δ_H 3.31 in ^{1}H and δ_C 49.0 in ^{13}C, CD_3OD) 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 $[\alpha]_D$ 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 Neu's reagent for phenolics (Neu, 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 P_2O_5 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 ^{1}H-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 detail characterization and enabled us to choose the more abundant infusion. Therefore, the infusion of the conventional deficit-irrigated *S. perfoliata* subsp. *perfoliata* was chosen for further chemical analysis based on its ^{1}H-NMR spectrum. The infusion (1.0 g) was fractionated by CC over silica gel using as eluent mixtures of CH_2Cl_2:MeOH:H_2O 1:0:0-0:1:0 to yield finally 23 fractions (A–Y). Fractions J, O, R, U, W and X (eluted with CH_2Cl_2:MeOH:H_2O 8.3:1.7:0.1-6:4:0.4) 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 L (18.0 mg; eluted with CH_2Cl_2:MeOH:H_2O 8:2:0.2) was further purified by preparative Thin-Layer Chromatography (TLC) on cellulose using AcOH:H_2O 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 CH_2Cl_2:MeOH:H_2O 7.5:2.5:0.2) was subjected to CC over silica gel (EtOAc:MeOH:H_2O 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 CH_2Cl_2:MeOH:H_2O 7.0:3.0:0.3) was submitted to CC over silica gel (EtOAc:MeOH:H_2O 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 CH_2Cl_2:MeOH:H_2O 6.5:3.5:0.3) was further purified by CC over silica gel (EtOAc:MeOH:H_2O 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

Table 1
Effect of cultivation (conventional or organic) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Sideritis* plants growth, chlorophylls (Chl a, Chl b, total Chls) content and essential oils yields under two harvestings.

Compound	Height (cm)	Fresh weight (g)	Dry matter content (%)	Chl a (mg/g)	Chl b (mg/g)	Total Chls (mg/g)	EO yield (%)
	1st harvest						
Conventional FI	55.50 ± 1.18a	151.49 ± 17.12a	22.07 ± 0.61ab	1.001 ± 0.058a	0.254 ± 0.023a	1.255 ± 0.082a	0.456 ± 0.014a
Conventional DI	51.41 ± 4.34a	121.42 ± 6.26ab	26.57 ± 0.39a	1.026 ± 0.048a	0.272 ± 0.021a	1.297 ± 0.069a	0.617 ± 0.088a
Organic FI	64.00 ± 2.95a	135.48 ± 13.52a	20.73 ± 0.36b	0.779 ± 0.021b	0.189 ± 0.003b	0.968 ± 0.025b	0.450 ± 0.031a
Organic DI	55.85 ± 6.21a	92.33 ± 4.65b	27.04 ± 0.46a	0.815 ± 0.047b	0.194 ± 0.004b	1.009 ± 0.052b	0.488 ± 0.053a
	2nd harvest						
Conventional FI	30.66 ± 2.27b	135.31 ± 14.73ab	21.37 ± 2.36ab	0.960 ± 0.055a	0.244 ± 0.024a	1.204 ± 0.078a	0.565 ± 0.048c
Conventional DI	39.16 ± 2.24a	93.95 ± 7.69b	24.53 ± 1.25a	0.985 ± 0.046a	0.260 ± 0.020a	1.249 ± 0.068a	0.750 ± 0.024ab
Organic FI	36.50 ± 0.84a	142.57 ± 14.01a	18.71 ± 0.44b	0.748 ± 0.021b	0.182 ± 0.003b	0.932 ± 0.022b	0.660 ± 0.012bc
Organic DI	29.50 ± 1.34b	107.44 ± 13.66b	23.79 ± 0.73a	0.782 ± 0.042b	0.187 ± 0.003b	0.965 ± 0.045b	0.835 ± 0.044a
Three-way Anova	Height	Fresh weight	Dry matter content	Chl a	Chl b	Total Chls	EO yield
Cultivation (C)	ns	ns	ns	***	***	***	ns
Irrigarion (I)	*	***	***	ns	ns	ns	***
Harvesting (H)	***	ns	*	ns	ns	ns	***
C x I	ns	ns	ns	ns	ns	ns	ns
C x H	ns	*	ns	ns	ns	ns	*
I x H	ns	ns	ns	ns	ns	ns	ns
C x I x H	ns	ns	ns	ns	ns	ns	ns

Values (n = 6 for plant growth; n = 4 for chlorophylls and oil yileds) in column for each harvest followed by the same letter are not significantly different, $P \leq 0.05$. ns, *, **, and *** indicate non-significant or significant differences at $P \leq$ 5%, 1% and 0.1%, respectively, following three-way ANOVA.

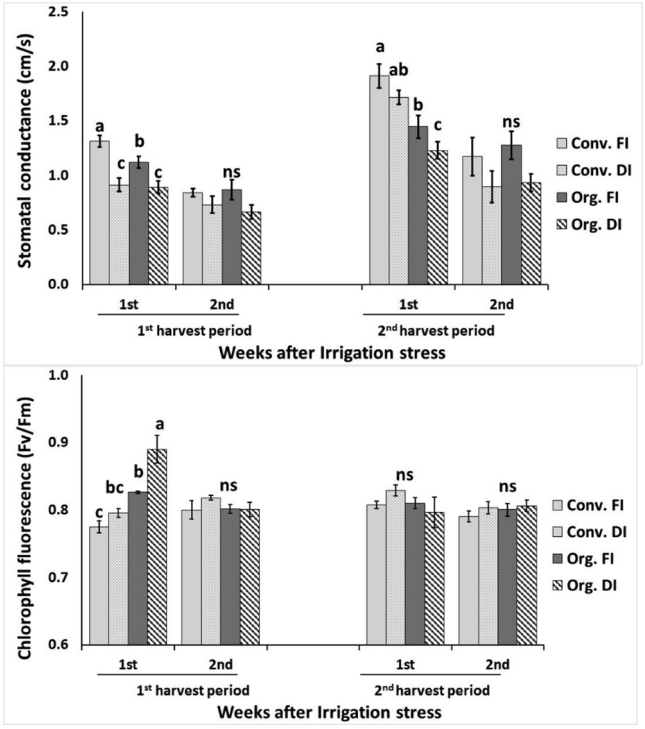

Fig. 1. Effect of cultivation (conventional or organic) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Sideritis* plants physiological parameters under two harvestings. Values represent mean (± SE) of measurements made on 6 independent replications per treatment. Mean values followed by the same letter do not differ significantly at P ≥ 0.05 according to Duncan's MRT. ns: no significance.

practice*irrigation*harvesting did not significantly ($P \geq 0.05$) affect plant growth and chlorophylls content.

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 146.78 mg/kg, Zn content averaged in 57.32 mg/kg and Cu content averaged in 148.59 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 ($P < 0.01$) by cultivation*irrigation (Table 3). The content of Zn was 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
Effect of cultivation (conventional or organic) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Sideritis* plants minerals content under two harvestings.

Compound	N (g/kg)	K (g/kg)	P (g/kg)	Ca (g/kg)	Mg (g/kg)	Na (g/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
	1st harvest								
Conventional FI	24.24 ± 0.12a	29.58 ± 0.59a	2.38 ± 0.25bc	5.46 ± 0.11b	3.97 ± 0.02b	0.220 ± 0.010b	151.62 ± 1.54a	59.08 ± 1.51a	150.37 ± 14.32a
Conventional DI	20.23 ± 0.21c	29.84 ± 0.14a	3.10 ± 0.32ab	6.24 ± 0.05b	4.37 ± 0.10a	0.223 ± 0.003b	149.11 ± 1.98a	57.78 ± 1.83a	143.92 ± 18.45a
Organic FI	21.96 ± 0.39b	23.76 ± 0.25b	3.31 ± 0.19a	6.18 ± 0.47b	4.26 ± 0.04a	0.273 ± 0.013a	144.61 ± 5.29a	58.85 ± 4.41a	136.26 ± 8.85a
Organic DI	21.67 ± 0.48b	20.65 ± 0.26c	2.08 ± 0.07c	8.51 ± 0.67a	4.37 ± 0.12a	0.300 ± 0.010a	141.75 ± 5.19a	53.55 ± 3.70a	163.80 ± 17.93a
	2nd harvest								
Conventional FI	28.43 ± 1.26a	36.23 ± 0.47a	3.66 ± 0.52a	10.19 ± 1.38a	4.80 ± 0.09a	0.380 ± 0.045c	138.25 ± 0.79a	65.25 ± 3.44a	171.80 ± 14.82a
Conventional DI	24.89 ± 0.19b	26.18 ± 0.28c	2.83 ± 0.09b	11.91 ± 1.15a	4.21 ± 0.05b	0.690 ± 0.020a	135.47 ± 0.77ab	58.56 ± 0.94a	170.77 ± 2.65a
Organic FI	22.41 ± 0.41c	28.82 ± 0.47b	2.88 ± 0.06b	9.02 ± 0.22a	3.91 ± 0.06c	0.520 ± 0.011b	129.95 ± 4.16bc	61.11 ± 1.79a	99.30 ± 20.99b
Organic DI	21.76 ± 0.28c	25.73 ± 0.24c	2.79 ± 0.11b	10.29 ± 0.66a	3.60 ± 0.11d	0.510 ± 0.010b	122.82 ± 1.95c	65.25 ± 2.73a	137.49 ± 5.11ab
Three-way Anova	N	K	P	Ca	Mg	Na	Fe	Zn	Cu
Cultivation (C)	ns	ns	ns	**	ns	ns	ns	**	ns
Irrigarion (I)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Harvesting (H)	ns	ns	ns	ns	**	ns	ns	ns	*
C x I	ns	ns	ns	ns	ns	ns	ns	ns	ns
C x H	ns	ns	ns	ns	ns	ns	ns	ns	ns
I x H	ns	ns	ns	**	ns	ns	ns	ns	*
C x I x H	ns	ns	ns	ns	ns	ns	ns	ns	**

Values (n = 4) in column for each harvest followed by the same letter are not significantly different, $P \leq 0.05$. ns, *, **, and *** indicate non-significant or significant differences at $P \leq$ 5%, 1% and 0.1%, respectively, following three-way ANOVA.

antioxidants increased in organically and full-irrigated plants compared to the relevant full-irrigated plants at conventional practice (Table 3). Indeed, at the second harvesting period, antioxidants increased in the deficit-treated plants, in both organic and conventional practices.

Considering the interaction among the examined factors, three way ANOVA analysis is presented in Table 3, revealed that cultivation practice affected the total phenolics, total flavonoids, ascorbic acid, antioxidant status assayed by DPPH and FRAP ($P < 0.001$) and by ABTS ($P < 0.05$). Irrigation affected total phenolics, total flavonoids, ascorbic acid, antioxidant status assayed by DPPH and FRAP ($P < 0.001$). Harvesting impacted total phenolics, total flavonoids, ascorbic acid, antioxidant status (DPPH, ABTS and FRAP) ($P < 0.001$). The interaction of cultivation*harvesting affected total phenolics, ascorbic acid, and DPPH ($P < 0.001$), FRAP ($P < 0.01$) and total flavonoids ($P < 0.05$). Indeed, irrigation*harvesting affected ($P < 0.05$) only total phenols. The interaction of cultivation*irrigation affected total flavonoids and antioxidant status (DPPH, ABTS and FRAP) ($P < 0.001$). The interaction of cultivation*irrigation*harvesting had impact on total flavonoids, ascorbic acid and antioxidant status (DPPH, ABTS and FRAP) ($P < 0.001$).

3.4. Essential oil yield and composition

Essential oils 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%), sesquiterpenes 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%), valeranone (7.72–12.94%), β-pinene (6.51–7.29%), sabinene (4.20–5.02%), the β-myrcene, 3-carene, terpinolene, β-caryophyllene, germacrene-D and cubenol-1-epi varied from 1 to 3%, while other compounds were identified in amounts lower than 1% of the total volatile components content (Table 4).

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. Sabinene 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;

Table 3
Effect of cultivation (conventional or organic) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Sideritis* plants total phenolics (μmol GAE/g Fw), total flavonoids (mg Rutin/g Fw), antioxidant status (ABTS, DPPH, FRAP; mg Trolox/g Fw) and ascorbic acid content (mg AA/g Fw) under two harvestings.

Compound	Total phenols	Total flavonoids	ABTS	DPPH	FRAP	AA
	1st harvest					
Conventional FI	17.83 ± 1.85b	0.89 ± 0.19b	3.81 ± 0.61b	4.34 ± 0.21c	9.93 ± 0.80c	0.025 ± 0.001c
Conventional DI	82.31 ± 7.98a	4.24 ± 0.28a	6.34 ± 0.34a	11.13 ± 0.57b	25.27 ± 2.44ab	0.062 ± 0.006a
Organic FI	97.49 ± 13.16a	4.50 ± 0.46a	6.87 ± 0.17a	13.25 ± 0.51a	27.31 ± 1.51a	0.050 ± 0.000b
Organic DI	120.78 ± 21.43a	3.77 ± 0.32a	5.31 ± 0.91ab	10.64 ± 0.66b	21.84 ± 0.57b	0.051 ± 0.001b
	2nd harvest					
Conventional FI	25.02 ± 0.06b	1.57 ± 0.06c	2.61 ± 0.07b	4.81 ± 0.21c	6.89 ± 0.19c	0.056 ± 0.002c
Conventional DI	34.41 ± 0.96a	2.29 ± 0.16b	3.14 ± 0.03a	6.38 ± 0.40ab	9.89 ± 0.34ab	0.054 ± 0.002c
Organic FI	32.17 ± 2.63a	2.09 ± 0.12b	3.04 ± 0.14ab	5.73 ± 0.49bc	8.99 ± 0.75b	0.068 ± 0.001b
Organic DI	38.09 ± 2.62a	3.04 ± 0.23a	3.48 ± 0.23a	7.18 ± 0.41a	11.55 ± 1.05a	0.092 ± 0.004a
Three-way Anova	Total phenols	Total flavonoids	ABTS	DPPH	FRAP	AA
Cultivation (C)	***	***	*	***	***	***
Irrigarion (I)	***	***	ns	***	***	***
Harvesting (H)	***	***	***	***	***	***
C x I	ns	***	***	***	***	ns
C x H	***	*	ns	***	**	***
I x H	*	ns	ns	ns	ns	ns
C x I x H	ns	***	***	***	***	***

Values (n = 4) in column for each harvest followed by the same letter are not significantly different, $P \leq 0.05$. ns, *, **, and *** indicate non-significant or significant differences at $P \leq$ 5%, 1% and 0.1%, respectively, following three-way ANOVA.

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 and flavone glucosides) (Fig. 2A). At a first step, the infusion of the conventional cultivation; deficit-irrigated plants was chosen due to the high antioxidant activity that exhibited and their rich content in iridoids based on its ^{1}H-NMR spectrum (Fig. 3). Fig. 4 presents the ^{1}H NMR spectra of the isolated iridoids for comparison to the total extracts (Fig. 2A). Consequently, this infusion was further fractionated by several CC over silica gel and afforded in total twelve compounds **1-12**. It is worth mentioning that during the whole isolation course of the infusion, all fractions were continuously controlled and traced down by ^{1}H NMR, which permitted their detailed identification. The obtained pure compounds were identified as acteoside **(1)** (Li et al., 2005), lavandulifolioside **(2)** (Ackoş et al., 1999), isoscutellarein-7-*O*-[6‴-O-acetyl-*β*-D-allopyranosyl-(1→2)]-*β*-D-glucopyranoside **(3)** (Rodríguez-Lyon et al., 2000), 4′-methylisoscutellarein-7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1→2)]-*β*-D-glucopyranoside **(4)** (Halfon et al., 2013), 4′-methylhypolaetin-7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1→2)]-*β*-D-glucopyranoside **(5)** (Halfon et al., 2013), chlorogenic acid **(6)** (Tomou and Skaltsa, 2018), ajugol **(7)** (Nykmukanova et al., 2017), gardoside **(8)** (Inouye et al., 1974), 7-acetyl-8-*epi*-loganic acid **(9)** (Kotsos et al., 2001), 8-*epi*-loganic acid **(10)** (Venditti et al., 2015), melittoside **(11)** (Venditti et al., 2016) and monomelittoside **(12)** (Venditti et al., 2016) (Fig. 5).

4. Discussion

The results indicate that the different cultivation practices affect the plants' response to their biosynthesis of secondary metabolites and 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*. Previous studies of wild *S. perfoliata* L. 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. *peloponnesiaca* (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 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 Palfey, 2010). Thus, the different mineral accumulation between the different treatments may influence the mevalonic acid pathway. 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 (Kazimierczak et al., 2015).

Table 4
Chemical composition (%) of essential oils of *Sideritis* plants grown in conventional or organic cultivation and subjected to full (FI) or deficit (DI) irrigation.

		1st harvest				2nd harvest			
Compound	RI	Conv. FI	Conv. DI	Org.FI	Org.DI	Conv. FI	Conv. DI	Org.FI	Org.DI
α-Thujene	926	0.62 ± 0.03b	0.74 ± 0.01a	0.71 ± 0.04a	0.74 ± 0.00a	0.76 ± 0.02ab	0.80 ± 0.00a	0.72 ± 0.00b	0.78 ± 0.01a
α-Pinene	933	25.35 ± 0.35b	26.55 ± 0.40b	27.98 ± 0.59a	26.74 ± 0.23ab	27.77 ± 0.82a	26.86 ± 0.2a	28.15 ± 0.19a	27.86 ± 0.11a
Camphene	948	0.07 ± 0.00b	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.07 ± 0.00b
Sabinene	973	4.20 ± 0.13b	4.72 ± 0.04a	4.60 ± 0.20ab	4.73 ± 0.14a	5.00 ± 0.09ab	5.02 ± 0.01a	4.81 ± 0.02b	4.93 ± 0.04ab
β-Pinene	977	6.51 ± 0.13b	6.90 ± 0.04ab	7.13 ± 0.26a	6.90 ± 0.10ab	7.29 ± 0.22a	7.08 ± 0.07ab	7.25 ± 0.00a	6.8 ± 0.09b
β-Myrcene	989	2.31 ± 0.11c	2.80 ± 0.01a	2.46 ± 0.12bc	2.65 ± 0.03ab	3.32 ± 0.04b	3.46 ± 0.01a	2.90 ± 0.04c	3.59 ± 0.05a
3-octanol	1003	0.03 ± 0.01a	0.05 ± 0.00a	0.02 ± 0.01a	0.06 ± 0.00a	0.00 ± 0.00b	0.03 ± 0.00a	0.01 ± 0.01a	0.02 ± 0.00a
α-Phellandrene	1005	0.82 ± 0.05b	0.91 ± 0.00a	0.88 ± 0.03ab	0.93 ± 0.01ab	1.08 ± 0.05a	1.16 ± 0.07a	0.93 ± 0.02a	1.11 ± 0.02a
3-carene	1013	2.23 ± 0.05b	2.43 ± 0.00a	2.29 ± 0.01b	2.46 ± 0.02a	2.66 ± 0.05b	2.78 ± 0.06a	2.46 ± 0.05c	2.77 ± 0.04a
α-Terpinene	1017	0.18 ± 0.00b	0.20 ± 0.00a	0.18 ± 0.00b	0.19 ± 0.00ab	0.21 ± 0.00ab	0.23 ± 0.00a	0.16 ± 0.00c	0.20 ± 0.00b
o-Cymene	1024	0.16 ± 0.00b	0.15 ± 0.00b	0.16 ± 0.00b	0.19 ± 0.01a	0.13 ± 0.00b	0.12 ± 0.00bc	0.15 ± 0.00a	0.12 ± 0.00c
β-Phellandrene	1029	26.54 ± 0.22b	28.71 ± 0.08a	26.65 ± 0.64b	28.53 ± 0.07a	29.88 ± 0.49b	29.93 ± 0.23b	28.05 ± 0.43c	31.72 ± 0.12a
cis-Ocimene	1036	–	–	–	–	0.02 ± 0.01ab	0.05 ± 0.00a	0.01 ± 0.00b	0.03 ± 0.00ab
Benzene acetaldehyde	1041	0.05 ± 0.00a	0.02 ± 0.01b	0.06 ± 0.00a	0.04 ± 0.00ab	0.01 ± 0.00a	0.02 ± 0.01a	0.00 ± 0.00a	0.01 ± 0.00a
trans-Ocimene	1046	0.03 ± 0.01a	0.07 ± 0.00a	0.03 ± 0.02a	0.06 ± 0.00a	0.09 ± 0.00b	0.10 ± 0.00a	0.07 ± 0.00c	0.10 ± 0.00b
γ-Terpinene	1058	0.40 ± 0.01ab	0.41 ± 0.00a	0.39 ± 0.00b	0.39 ± 0.00ab	0.44 ± 0.01a	0.45 ± 0.00a	0.37 ± 0.00b	0.45 ± 0.00a
cis-Sabinenehydrate	1067	0.04 ± 0.02b	0.10 ± 0.00a	0.08 ± 0.00ab	0.10 ± 0.00a	0.07 ± 0.00b	0.11 ± 0.00a	0.07 ± 0.01b	0.08 ± 0.00b
Terpinolene	1089	2.58 ± 0.10bc	2.86 ± 0.00a	2.48 ± 0.03c	2.74 ± 0.04ab	3.57 ± 0.03b	3.57 ± 0.03b	2.89 ± 0.06c	3.76 ± 0.00a
trans-Sabinenehydrate	1100	0.38 ± 0.04b	0.46 ± 0.01ab	0.47 ± 0.01ab	0.51 ± 0.00a	0.26 ± 0.00ab	0.30 ± 0.00a	0.21 ± 0.02b	0.21 ± 0.02b
Terpinen-4-ol	1178	0.21 ± 0.04b	0.29 ± 0.01a	0.22 ± 0.02ab	0.28 ± 0.00ab	0.14 ± 0.00a	0.17 ± 0.00a	0.13 ± 0.01a	0.12 ± 0.03a
α-Terpineol	1191	0.09 ± 0.01b	0.13 ± 0.00a	0.10 ± 0.00b	0.13 ± 0.00a	0.02 ± 0.01a	0.05 ± 0.00a	0.02 ± 0.01a	0.03 ± 0.00a
Decanal	1204	0.16 ± 0.01a	0.16 ± 0.01a	0.17 ± 0.00a	0.17 ± 0.01a	0.08 ± 0.00bc	0.12 ± 0.00a	0.09 ± 0.00b	0.07 ± 0.00c
Neral	1242	0.16 ± 0.01a	0.03 ± 0.01b	0.03 ± 0.02b	0.00 ± 0.00b	–	–	–	–
Carvone	1244	0.94 ± 0.39a	0.38 ± 0.13a	0.37 ± 0.13a	0.49 ± 0.08a	–	–	–	–
Geranial	1271	0.25 ± 0.04a	0.04 ± 0.02b	0.06 ± 0.03b	0.00 ± 0.00b	–	–	–	–
β-Bourbonene	1386	0.17 ± 0.01a	0.16 ± 0.00a	0.17 ± 0.00a	0.20 ± 0.01a	–	–	–	–
β-Caryophyllene	1425	2.35 ± 0.06b	2.45 ± 0.17b	2.90 ± 0.12a	2.27 ± 0.01b	1.64 ± 0.06a	1.67 ± 0.02a	1.73 ± 0.00a	1.25 ± 0.01b
α-Caryophyllene	1462	0.06 ± 0.00c	0.07 ± 0.00b	0.09 ± 0.00a	0.07 ± 000bc	0.04 ± 0.00a	0.05 ± 0.00a	0.05 ± 0.00a	0.01 ± 0.00b
Caryophyllene-9-epi	1479	0.85 ± 0.02bc	0.79 ± 0.02c	0.97 ± 0.07ab	1.00 ± 0.00a	0.53 ± 0.02c	0.63 ± 0.03b	0.93 ± 0.00a	0.67 ± 0.03b
Germacrene D	1495	1.84 ± 0.04a	1.77 ± 0.01a	1.76 ± 0.09a	1.54 ± 0.06b	1.68 ± 0.14b	1.62 ± 0.0.04b	2.18 ± 0.02a	1.59 ± 0.00b
Caryophyllene oxide	1587	0.09 ± 0.01b	0.08 ± 0.00b	0.13 ± 0.00a	0.12 ± 0.00a	0.01 ± 0.01b	0.01 ± 0.00b	0.05 ± 0.00a	0.01 ± 0.00b
Viridiflorol	1592	0.23 ± 0.00a	0.17 ± 0.00b	0.20 ± 0.02ab	0.18 ± 0.00b	0.06 ± 0.03b	0.09 ± 0.01ab	0.14 ± 0.00a	0.06 ± 0.00b
Cubenol-1-epi	1617	1.95 ± 0.02a	1.68 ± 0.03b	1.83 ± 0.11ab	1.83 ± 0.02ab	1.13 ± 0.10ab	1.25 ± 0.05a	1.32 ± 0.02a	0.93 ± 0.05b
Valeranone	1673	12.94 ± 0.15a	10.15 ± 0.14b	11.24 ± 0.84b	11.08 ± 0.15b	8.06 ± 0.60b	8.47 ± 0.26ab	9.38 ± 0.17a	7.72 ± 0.20b
δ-Dodecalactone	1704	0.67 ± 0.05a	0.60 ± 0.09ab	0.40 ± 0.00bc	0.37 ± 0.00c	0.55 ± 0.03b	0.48 ± 0.00bc	0.68 ± 0.02a	0.46 ± 0.02c
Mint sulfide	1737	0.00 ± 0.00d	0.06 ± 0.00c	0.09 ± 0.00b	0.13 ± 0.00a	0.02 ± 0.01b	0.02 ± 0.01b	0.07 ± 0.01a	0.00 ± 0.00b
Isokaurene	1990	0.32 ± 0.02a	0.32 ± 0.04a	0.29 ± 0.04a	0.27 ± 0.02a	0.50 ± 0.11a	0.57 ± 0.00a	0.69 ± 0.07a	0.57 ± 0.02a
Sclareol	2135	0.40 ± 0.05a	0.31 ± 0.02a	0.30 ± 0.08a	0.25 ± 0.02a	1.14 ± 0.27a	0.87 ± 0.05a	1.08 ± 0.13a	0.70 ± 0.00a
Total Identified		96.43 ± 0.13	97.95 ± 0.17	98.04 ± 0.29	98.55 ± 0.06	98.40 ± 0.29	98.34 ± 0.13	97.98 ± 0.17	98.97 ± 0.00
Monoterpenes hydrocarbons		72.03 ± 0.27b	77.62 ± 0.27a	76.04 ± 1.99a	77.43 ± 0.31a	82.36 ± 1.71a	81.73 ± 0.66ab	79.07 ± 0.46b	84.39 ± 0.34a
Sesquiterpenes hydrocarbons		5.27 ± 0.15ab	5.26 ± 0.15ab	5.89 ± 0.29a	5.09 ± 0.06b	3.91 ± 0.23b	3.98 ± 0.11b	4.89 ± 0.02a	3.54 ± 0.02b
Oxygenated monoterpenes		2.42 ± 0.44a	1.59 ± 0.12b	1.52 ± 0.23b	1.69 ± 0.06b	0.59 ± 0.00b	0.76 ± 0.02a	0.53 ± 0.05b	0.52 ± 0.07b
Oxygenated sesquiterpenes		15.22 ± 0.03a	12.10 ± 0.19b	13.41 ± 0.98b	13.20 ± 0.18b	9.28 ± 0.75b	9.84 ± 0.32ab	10.90 ± 0.13a	8.73 ± 0.24b
Others		1.48 ± 0.15a	1.37 ± 0.18a	1.17 ± 0.18a	1.14 ± 0.04a	2.24 ± 0.43a	2.01 ± 0.05a	2.55 ± 0.23a	1.77 ± 0.00a

Values (n = 3) in rows for each harvest followed by the same letter are not significantly different, $P \leq 0.05$.

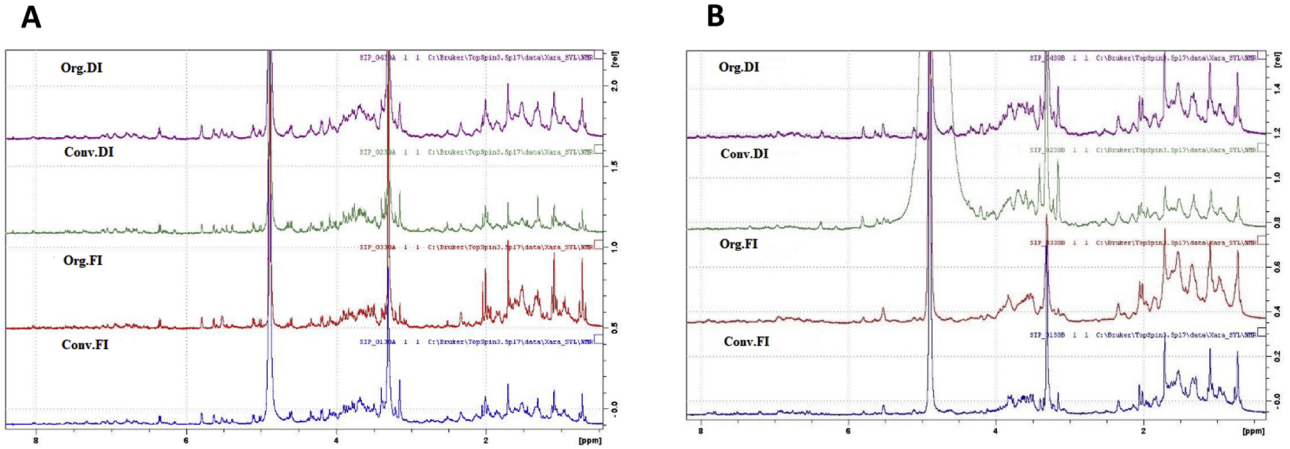

Fig. 2. ^{1}H-NMR spectra of the infusions of the plant harvested in (A) May 2018 and (B) June 2018.

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

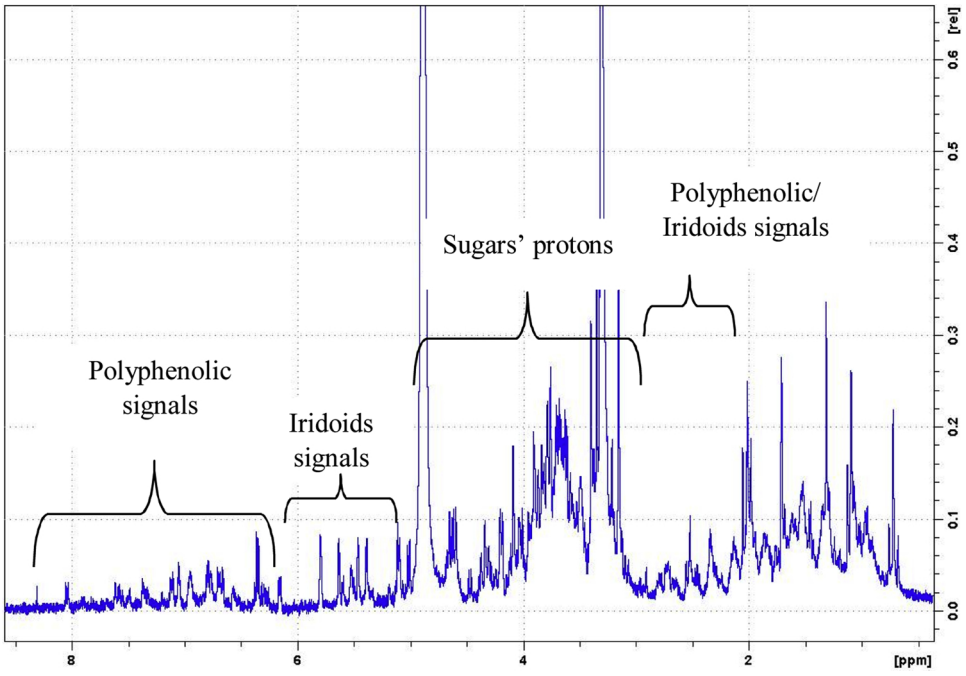

Fig. 3. [1]H-NMR spectrum of the infusion of the conventional cultivation; deficit-irrigated of *S. perfoliata* L.

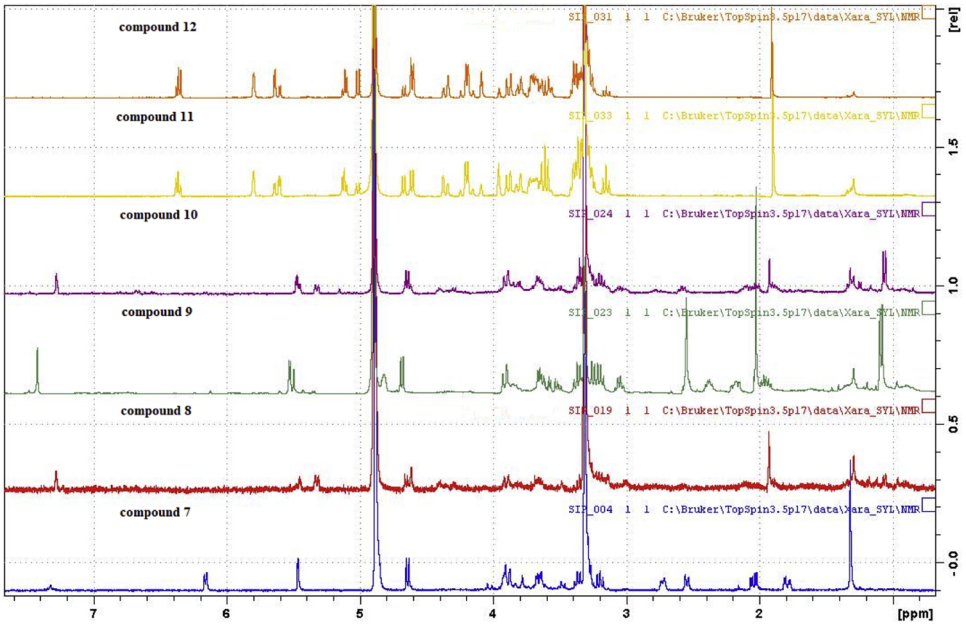

Fig. 4. [1]H-NMR spectra of the iridoids compounds **7-12**.

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

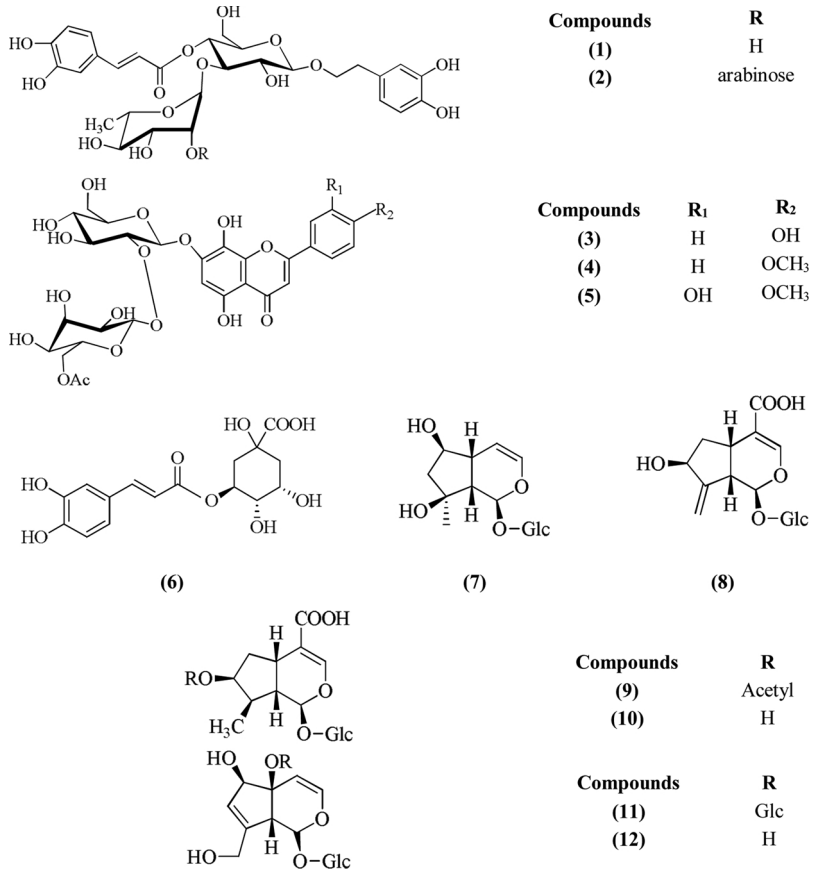

Fig. 5. Structures of isolated compounds **1-12**.

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 phenolics, flavonoids and antioxidants found in the present study, through the reallocation of carbon, as plant growth is reduced. Commonly, organic farmers use less nitrogen sources than the conventional ones, and thus less mineral content in soil or minerals available to plants (*i.e.*part of N is used for organic matter decomposition in soil) would affect the content of mineral in plants. The large amounts of polyphenols and antioxidants have an important role in adsorbing and neutralizing free radicals and reactive oxygen species (ROS). The increased antioxidant capacity of plant species and their products is closely related to the lower occurrence and lower mortality rates of various human diseases (Anderson et al., 2001). Fortunately, Mediterranean flora is composed by a large number of MAP species with well define protective and curative properties, posing anti-inflammatory, antioxidant, antirheumatic, antiulcer, digestive and antimicrobial activities (Armata et al., 2008; Charami et al., 2008; Chrysargyris et al., 2017b).

Mineral availability and accumulation in medicinal plants is of great importance and research interest focuses on the evaluation of appropriate mineral supplement during plant growth (Chrysargyris et al., 2016b, 2017b). The decreased N and K and the increased Mg levels in organically grown plants found in the present study, especially during the 1st harvest, are in agreement with previous reports (Rembiałkowska, 2007). The N provided with the organic cultivation system is mainly organic and not totally available for the plants and this could justify poor plant growth, decrease in chlorophyll content and low photosynthetic activity (Ouzounidou et al., 2008). The decreased levels of N 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 monoterepenes 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 diphosphate (FDP) appears to be delayed when deficit irrigation is applied to the plants. Valeranone, caryophyllene and germacrene 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 phenylethanoid glucosides 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. N.T. and H.S. conceived and designed the experiments. A.C.; C.K.; E.-M.T.; H.S. and N.T. contributed to the writing of the paper.

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

Ackoş, Y., Ezer, N., Çalis, R., Demirdamar, R., Tel, B.C., 1999. Polyphenolic compounds of *Sideritis lycia* and their anti-inflammatory activity. Pharm. Biol. 37, 118–122. https://doi.org/10.1076/phbi.37.2.118.6081.

Akcoş, Y., Ezer, N., Özçelik, B., Abbasoglu, U., 1998. Iridoid glucosides from *Sideritis lycia* Boiss. & Heldr. and its antimicrobial activities. FABAD J. Pharm. Sci. 23, 99–103.

Alipieva, K.I., Kostadinova, E.P., Evstatieva, L.N., Stefova, M., Bankova, V.S., 2009. An iridoid and a flavonoid from *Sideritis lanata* L. Fitoterapia 80, 51–53. https://doi.org/10.1016/j.fitote.2008.09.011.

Al-Gabbiesh, A., Kleinwächter, M., Selmar, D., 2015. Influencing the contents of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. Jordan J. Biol. Sci. 8 (1), 1–10.

Anderson, K.J., Teuber, S.S., Gobeille, A., Cremin, P., Waterhouse, A.L., Steinberg, F.M., 2001. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. J. Nutr. 131, 2837–2842.

International, A.O.A.C., 2007. Official Methods of Analysis, 18th ed. Gaithersburg.

Armata, M., Gabrieli, C., Termentzi, A., Zervou, M., Kokkalou, E., 2008. Constituents of *Sideritis syriaca* ssp. *syriaca* (Lamiaceae) and their antioxidant activity. Food Chem. 111, 179–186.

Barber, J.C., Francisco-Ortega, J., Santos-Guerra, A., Turner, K.G., Jansen, R.K., 2002. Origin of Macaronesian *Sideritis* L. (Lamioideae: lamiaceae) inferred from nuclear and chloroplast sequence datasets. Mol. Phylogenet. Evol. 23, 293–306. https://doi.org/10.1016/S1055-7903(02)00018-0.

Bettaieb, I.R., Jabri-Karoui, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., Marzouk, B., 2012. Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Ind. Crops Prod. 36, 238–245.

Bhattacharya, R.D., Parmar, K.M., Itankar, P.R., Prasad, S.K., 2017. Phytochemical and pharmacological evaluation of organic and non-organic cultivated nutritional *Centella asiatica* collected after different time intervals of harvesting. S. Afr. J. Bot. 112, 237–245.

Bloem, E., Haneklaus, S., Kleinwächter, M., Paulsen, J., Schnug, E., Selmar, D., 2014. Stress-induced changes of bioactive compounds in *Tropaeolum majus* L. Ind. Crops Prod. 60, 349–359.

Calın-Sanchez, A., Figiel, A., Lech, K., Szumny, A., Carbonell-Barrachina, A.A., 2013. Effects of drying methods on the composition of thyme (*Thymus vulgaris* L.) essential oil. Dry Technol. 31 (2), 224–235.

Carrubba, A., 2014. Organic and chemical N fertilization on coriander (*Coriandrum sativum* L.) in a Mediterranean environment. Ind. Crops Prod. 57, 174–187.

Charami, M.-T., Lazari, D., Karioti, A., Skaltsa, H., Hadjipavlou-Litina, D., Souleles, C., 2008. Antioxidant and antiinflammatory activities of *Sideritis perfoliata* subsp. *perfoliata* (Lamiaceae). Phytother. Res. 22, 450–454.

Chrysargyris, A., Laoutari, S., Litskas, V., Stravrinides, M., Tzortzakis, N., 2016a. Effects of water stress on lavender and sage biomass production, essential oil composition and biocidal properties against *Tetranychus urticae* (Koch). Sci. Hort. 213, 96–103.

Chrysargyris, A., Panayiotou, C., Tzortzakis, N., 2016b. Nitrogen and phosphorus levels affected plant growth, essential oil composition and antioxidant status of lavender plant (*Lavandula angustifolia* Mill.). Ind. Crops Prod. 83, 577–586.

Chrysargyris, A., Papakyriakou, E., Petropoulos, S.A., Tzortzakis, N., 2019. The combined and single effect of salinity and copper stress on growth and quality of *Mentha spicata* plants. J. Hazard. Mater. 368, 584–593.

Chrysargyris, A., Xylia, P., Botsaris, G., Tzortzakis, N., 2017b. Antioxidant and antibacterial activities, mineral and essential oil composition of spearmint (*Mentha spicata* L.) affected by the potassium levels. Ind. Crops Prod. 103, 202–212.

Chrysargyris, A., Xylia, P., Kontos, Y., Ntoulaptsi, M., Tzortzakis, N., 2017a. Consumer behavior and knowledge on organic vegetables in Cyprus. Food Res. 1, 57–65.

Dewick, P.M., 2002. The biosynthesis of C5-C25 terpenoid compounds. Nat. Prod. Rep. 19 (2), 181–222.

Dewick, P.M., 2009. Medicinal Natural Products: A Biosynthetic Approach, 3rd edition. John Wiley & Sons, Chichester, UK, pp. 188–192.

Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse challenges in monitoring safety. Front. Pharmacol. 4, 1–10.

EMA/HMPC/39455/2015. Assessment report on Sideritis scardica Griseb.; Sideritis clandestina (Bory & Chaub.) Hayek; Sideritis raeseri Boiss. & Heldr.; Sideritis syriaca L., herba. pp. 30.

Ezer, N., Sakar, M.K., Rodríguez, B., de la Torre, M.C., 1992. Flavonoid glycosides and a phenylpropanoid glycoside from Sideritis perfoliata. Int. J. Pharmacogn. 30, 61–65. https://doi.org/10.3109/13880209209054633.

Fagan, R.L., Palfey, B.A., 2010. Flavin-dependent enzymes. In: In: Begley, T.P. (Ed.), Comprehensive Natural Products Chemistry II 7. Cofactor Biosynthesis and Enzymology, pp. 37–113.

González-Burgos, E., Carretero, M.E., Gómez-Serranillos, M.P., 2011. *Sideritis* spp.: uses, chemical composition and pharmacological activities-A review. J. Ethnopharmacol. 135, 209–225. https://doi.org/10.1016/j.jep.2011.03.014.

Halfon, B., Çiftçi, E., Topçu, G., 2013. Flavonoid constituents of Sideritis caesarea. Turk. J. Chem. 37, 464–472. https://doi.org/10.3906/kim-1206-45.

Hofrichter, J., Krohn, M., Schumacher, T., Lange, C., Feistel, B., Walbroel, B., Pahnke, J., 2016. Sideritis spp. extracts enhance memory and learning in Alzheimer's -Amyloidosis mouse models and aged C57Bl/6 mice. J. Alzheimers Dis. 53, 967–980.

Inouye, H., Takeda, Y., Nishimura, H., 1974. Two new iridoid glucosides from *Gardenia jasminoides* fruits. Phytochemistry 13, 2219–2224. https://doi.org/10.1016/0031-9422(74)85031-4.

Kalivas, A., Ganopoulos, I., Xanthopoulou, A., Chatzopoulou, P., Tsaftaris, A., Madesis, P., 2014. DNA barcode ITS2 coupled with high resolution melting (HRM) analysis for taxonomic identification of *Sideritis* species growing in Greece. Mol. Biol. Rep. 41, 5147–5155.

Karousou, R., Deirmetzoglou, S., 2011. The herbal market of Cyprus: traditional links and cultural exchanges. J. Ethnopharmacol. 133, 191–203. https://doi.org/10.1016/j.jep.2010.09.034.

Kazimierczak, R., Hallmann, E., Rembiałkowska, E., 2015. Effects of organic and conventional production systems on the content of bioactive substances in four species of medicinal plants. Biol. Agric. Hortic. 31 (2), 118–127.

Koleva, I.I., Handjieva, N.V., 1997. Study of the iridoid glycosides in *Sideritis* (Lamiaceae). Nauchni Tr – Vissh Inst Khranit Vkusova Prom-st, Plovdiv 42, 75–79.

Kotsos, M., Aligiannis, N., Mitaku, S., Skaltsounis, A.L., Charvala, K., 2001. Chemistry of plants from Crete: stachyspinoside, a new flavonoid glycoside and iridoids from *Stachys spinosa*. Nat. Prod. Lett. 15 (6), 377–386. https://doi.org/10.1080/10575630108041307.

Laribi, B., Bettaieb, I., Kouki, K., Mougou, A., Marzouk, B., 2009. Water deficit effects on caraway (*Carum carvi* L.) growth, essential oil and fatty acid composition. Ind. Crops Prod. 30, 372–379.

Li, L., Tsao, R., Liu, Z., Liu, S., Yang, R., Young, J.-C., Zhu, H., Deng, Z., Xie, M., Fu, Z., 2005. Isolation and purification of acteoside and isoacteoside from *Plantago psyllium* L. by high-speed counter-current chromatography. J. Chromatogr. A 1036, 161–169.

Lohr, L., 2001. Factors affecting international demand and trade in organic food products. In: Regmi, A. (Ed.), Changing Structure of Global Food Consumption and Trade. USDA/Economic Research Service, Washington, DC, pp. 67–79.

Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., Statti, G.A., Menichini, F., 2007. Cytotoxic activity of essential oils from Labiatae and Lauraceae families against *in vitro* human tumor models. Anticancer Res. 27, 3293–3300.

Lu, Y., Gao, B., Chen, P., Charles, D., Yu, L., 2014. Characterisation of organic and conventional sweet basil leaves using chromatographic and flow-injection mass spectrometric (FIMS) fingerprints combined with principal component analysis. Food Chem. 154, 262–268.

Lv, J., Huang, H., Yu, L., Whent, M., Niu, Y., Shi, H., Wang, T.T.Y., Luthria, D., Charles, D., Yu, L., 2012. Phenolic composition and nutraceutical properties of organic and conventional cinnamon and peppermint. Food Chem. 132, 1442–1450.

Mabry, T.G., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer, New York, USA, pp. 354.

Malik, A.A., Suryapani, S., Ahmad, J., 2011. Chemical vs organic cultivation of medicinal and aromatic plants: the choice is clear. Int. J. Med. Arom. Plants 1 (1), 5–13.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H., 2003. Antioxidant and antiproliferative activities of strawberries. J. Agric. Food Chem. 53, 6887–6892.

Neu, R., 1957. Chelate von Diarylborsäuren mit aliphatischen Oxyalkylaminen als Reagenzien für den Nachweis von Oxyphenyl-benzo-γ-pyronen. Naturwissenschaften. 44, 181–183. https://doi.org/10.1007/BF00599857.

Nykmukanova, M.M., Eskalieva, B.K., Burasheva, G.Sh., Choudhary, M.I., Adhikari, A., Amadou, D., 2017. Iridoids from Verbascum marschallianum. Chem. Nat. Compd. 53 (3), 580–581. https://doi.org/10.1007/s10600-017-2056-6.

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.-S.P., 2014. Response of plants to water stress. Front. Plant Sci. 5, 1–8.

Ouzounidou, G., Asfi, M., Sotirakis, N., Papadopoulou, P., Gaitis, F., 2008. Olive mill wastewater triggered changes in physiology and nutritional quality of tomato (*Lycopersicon esculentum* Mill.) depending on growth substrate. J. Hazard. Mater. 158, 523–530.

Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., Bansal, Y.K., 2015. Secondary metabolites of plants and their role: overview. Curr. Trends Biotechnol. Pharm. 9 (3), 293–304.

Petreska, J., Stefkov, G., Kulevanova, S., Alipieva, K., Bankova, V., Stefovα, M., 2011. Phenolic compounds of mountain tea from the Balkans: LC/DAD/ESI/MS^n profile and content. Nat. Prod. Commun. 6, 21–30. https://doi.org/10.1177/1934578X1100600107.

Rembiałkowska, E., 2007. Review: quality of plant products from organic agriculture. J. Sci. Food Agric. 87, 2757–2762.

Richardson, A.D., Duigan, S.P., Berlyn, G.P., Richardson, A.D., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol. 153, 185–194.

Rodríguez-Lyon, M., Díaz-Lanza, A.M., Bernabé, M., Villaescusa-Castillo, L., 2000. Flavone glycosides containing acetylated sugars from Sideritis hyssopifolia. Magn. Reson. Chem. 38, 684–687. https://doi.org/10.1002/1097-458X(200008)38:83.0.CO;2-G.

Satter, A.A., Bankova, V., Sappov, S., Duddeck, H., Abdel-Sattar, A., 1993. Flavonoid glycosides from *Sideritis* species. Fitoterapia 64, 278–279.

Schilmiller, A.L., Schauvinhold, I., Larson, M., Xu, R., Charbonneau, A.L., Schmidt, A., Wilkerson, C., Last, R.L., Pichersky, E., 2009. Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. Proc. Natl. Acad. Sci. 106 (26), 10865–10870.

Supanjani Tawaha, A.R.M., Yang, M.S., Lee, K.D., 2005. Calcium effects on yield, mineral uptake and terpene components of hydroponic *Chrysanthemum coronarium* L. Int. J. Bot. 1, 196–200.

Tomou, E.-M., Skaltsa, H., 2018. Phytochemical investigation of the fern *Asplenium ceterach* L. Aspleniaceae. Nat. Prod. Commun. 13, 849–850.

Turtola, S., Manninen, A.-M., Rikala, R., Kainulainen, P., 2003. Drought stress alters the concentration of wood terpenoids in Scots pine and Norway spruce seedlings. J. Chem. Ecol. 29, 1981–1995.

Tzortzakis, N.G., Tzanakaki, K., Economakis, C., 2011. Effect of origanum oil and vinegar on the maintenance of postharvest quality of tomato. Food Nutr. Sci. 2, 974–982.

Vasilopoulou, C.G., Kontogianni, V.G., Linardaki, Z.I., Iatrou, G., Lamari, F.N., Nerantzaki, A.A., Gerothanassis, I.P., Tzakos, A.G., Margarity, M., 2013. Phytochemical composition of "mountain tea" from*Sideritis clandestina* subsp. *clandestina* and evaluation of its behavioral and oxidant/antioxidant effects on adult mice. Eur. J. Nutr. 52, 107–116.

Venditti, A., Frezza, C., Guarcini, L., Maggi, F., Bianco, A., Serafini, M., 2016. Reassessment of *Melittis melissophyllum* L. subsp. *melissophyllum* iridoidic fraction. Nat. Prod. Res. 30 (2), 218–222. https://doi.org/10.1080/14786419.2015.1040792.

Venditti, A., Frezza, C., Serafini, M., Biaco, A., 2015. Iridoids and phenylethanoid from *Pedicularis kerneri* Dalla Torre growing in Dolomites. Italy. Nat. Prod. Res. 30 (3), 327–331. https://doi.org/10.1080/14786419.2015.1060230.

Villasenor, I.M., 2007. Bioactivities of iridoids. Antiinflamm. Antiallergy Agents Med. Chem. 6, 307–314.

Wojdyło, A., Oszmiański, J., Czemerys, R., 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 105, 940–949. https://doi.org/10.1016/j.foodchem.2007.04.038.