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Original Article

Chemical profile, antioxidant, antimicrobial, and cytotoxic activity of *Urtica dioica* L. under different cropping and irrigation practices

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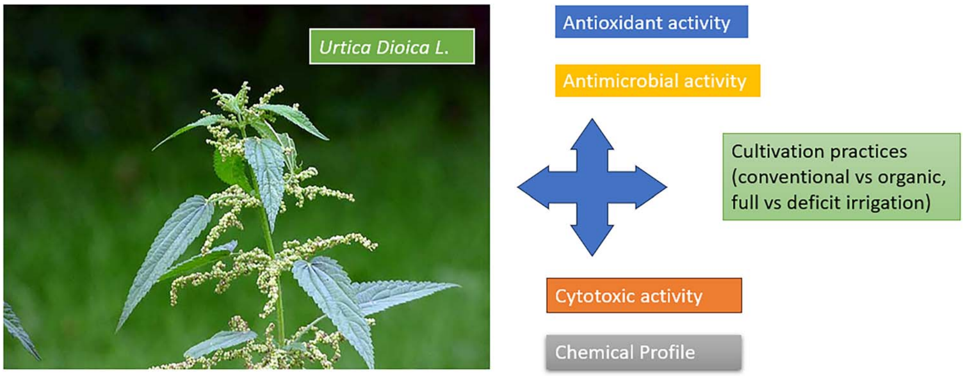
Abstract

Organic agriculture and regulated deficit irrigation (DI) are popular strategies for sustainable agricultural production. The present study investigated organically and conventionally cultivated *Urtica dioica* (L.) under different irrigation regimes (full and deficit irrigation) in the yield, physiology, phytochemical profile, antimicrobial, and cytotoxic properties under two harvestings periods. Deficit irrigation reduced plant development and yield, but raised dry matter content, especially in plants in organic cultivation. Plants adapted to DI with stomatal closure, while organically grown plants had decreased minerals content, with more profound effects at first harvesting. Plants grown in organic cultivation stimulated hydroxytyrosol and suppressed Rutin levels, compared to conventionally full irrigated plants at the first harvesting. At the second harvesting, Fraxin levels were stimulated, while hydroxytyrosol, chlorogenic acid, and citric acid were decreased with the DI treatment. The DI increased total phenolics, flavonoids, and antioxidants, especially at the first harvest. Plants grown under conventional cultivation had stronger antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*. However, plants grown under organic cultivation had stronger cytotoxic properties against two human colorectal cancer cell lines, with more profound effects with the DI applications. Both organic agriculture and DI stimulated biocidal properties with new insights on cultivation management for *U. dioica*.

Keywords: antioxidant activity, deficit irrigation, minerals, organic cultivation, stringing nettle

Graphical abstract



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Introduction

Stinging or common nettle (*Urtica dioica* L.) is a perennial, nitrophilous plant (Bacci et al., 2009). It is regarded as a weed in intensive agriculture, as it spreads rapidly within the main crop (Di Virgilio et al., 2015), while it has been utilised as a wild vegetable (Bhusal et al., 2022). There are economic and ecological reasons to promote the cultivation of stinging nettle, as it has several uses in traditional medicine and is a greatly nutritious food for humans (Mohammadian et al., 2024). Furthermore, the increased interest for common nettle is ages agon, as Hippocrates (460–377 B.C.) recorded 61 stinging nettle-based treatments (Upton, 2013). Numerous physiologically active chemicals are thought to be abundant in this medicinal plant, including polyphenols, flavonoids, tannins, vitamins, fatty acids, and minerals (Bhusal et al., 2022; Dujmović et al., 2025; Joshi et al., 2014), render the plant products valuable sources to reduce free radical generation and oxidative stress conditions.

Aqueous and ethanolic extracts of *U. dioica* plants, have been used for long period against anaemia (Taheri et al., 2022), gout, and eczema (Orcic et al., 2014), and commonly used in the treatment of prostatic cancer (Asgarpanah & Mohajerani, 2012), on improving the comfort of breastfeeding and boosting milk production (Bhusal et al., 2022). Additionally, stinging nettle has antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and antiulcer activities (Bhusal et al., 2022; Di Virgilio et al., 2015; Dujmović et al., 2025; Orcic et al., 2014). Beneficial effects on inflammation, hypoglycaemia, hypotension, cardiovascular diseases, arthritis, and allergic rhinitis have been reported (Di Virgilio et al., 2015; Taheri et al., 2022; Upton, 2013). Chlorophyll, a green pigmented component (E140) used in foods and medications, is obtained from the *U. dioica* plants for commercial utilisation (Jan et al., 2017). Extracts from *U. dioica* have been used on weed management on lentil crops (Rhioui et al., 2023), for soil phytoremediation of heavy metals (Jeannin et al., 2020), for animal feeding (Zhang et al., 2023), and in cosmetics and pharmaceutical sector (Upton, 2013).

Despite rising yields, chemical fertilisers represent substantial health risks to people, particularly to young children's, pregnant women, and nursing mothers (Tagkas et al., 2024). As people seek healthier lifestyles that minimise phytochemicals and safeguard ecosystems and organisms, they are increasingly choosing and consuming organic food over non-organic (i.e., conventional) food (Chrysargyris et al., 2017; Hajisolomou et al., 2024). Organic agriculture relies on healthy living systems, taking advantage of biodiversity and recycling, whereas conventional agriculture uses synthetic fertiliser and pesticides (Le Campion et al., 2020). Health issues related to inorganic cultivation have been eliminated as a result of the organic cultivation practices, which use natural fertilisers and pesticides, to prevent such issues. Additionally, organic farming can be explored more successful to medicinal and aromatic plants (MAPs) and herbs as they are commonly less demanding crops, with lower inputs and demands (water, minerals, pesticides, and cultivation practices), slower rate of growth and photosynthetic capacity, and frequently greater biological activity on secondary metabolites, if compared to vegetables and floriculture cropping systems (Bhattacharya et al., 2017; Kazimierczak et al., 2015).

Crop production and stress tolerance are affected by several biotic (pathogens, organisms, etc.) and environmental/abiotic (salinity, drought, minerals, temperature, wind, etc.) variables. Farmers frequently struggle with water scarcity throughout the growing season due to limited freshwater availability in the Mediterranean basin and globally, a situation exacerbated by the impacts of climate change. This has an impact on crop productivity and available fertile soil, whereas soil erosion and desertification are taken place (Chrysargyris et al., 2016; Osakabe et al., 2014). Plants may cope with water stress and saving energy by reducing their photosynthetic rates, closing leaf stomata, decreasing CO_2 availability. Therefore, as a consequence of the water shortages, plant decrease growth and yield, with changes on the quality of the final product (Bloem et al., 2014; Chrysargyris et al., 2019). However, these changes in plant metabolism might favoured the synthesis of several secondary metabolic components, and give a boost in antioxidants and bioactive compounds (Chrysargyris et al., 2019). Research interest and exploitation are being drawn to cultivation systems that use deficit irrigation, alternate irrigation water sources (such as salty water, treated wastewater, etc.), and less water-demanding crops like most MAPs.

Despite the lack of information on water requirements and usage efficiency in the literature, stinging nettle is nevertheless regarded as a water-demanding crop (Di Virgilio et al., 2015). The best scenario is for the plant to receive water consistently throughout the growing season, which means that in certain situations, irrigation support may be needed throughout the establishment year (Vogl & Hartl, 2003). *Urtica dioica* plants could sustain without watering when there was 53 mm of summer precipitation (Bacci et al., 2009), whereas in several Mediterranean countries, including Cyprus, the summer precipitation is almost zero.

Few attempts have been made to research the organic culture conditions, despite the fact that organic cultivation practices are frequently advised for growing MAPs (Bhattacharya et al., 2017; Malik et al., 2011). *Urtica dioica* plants are an example of a nitrate-accumulating species, and while few studies evaluated successful agronomic practices (Opačić et al., 2024), they can be a good candidate in organic farming. This is particularly relevant because soil fertility in organic farming is typically lower than in conventional farming, where synthetic fertilisers are commonly over-applied. The present study was conducted to examine the effects of various farming methods (conventional versus organic cultivation and full versus deficit irrigation) on the yield, physiology, total antioxidant capacity, mineral accumulation, antimicrobial and cytotoxic activities, and phytochemicals profile of *U. dioica* from commercial cultivated crops under two harvesting periods. This was done considering the substantial demand of *U. dioica* and the possibility that cultivation conditions, especially under water shortage in deficit irrigation and/or minerals lack under organic farming, might impact the metabolism of active ingredients in the plants and possible enhance plants extracts biocidal properties.

Materials and methods

Experimental study set up

Urtica dioica seedlings were produced from the Ministry of Agriculture, Nicosia, Cyprus. Young plants had 3–4 leaves and height of 4–5 cm when transferred to a commercial organic farm (Limassol, 34°38′ N, 32°56′ E, 7 m) and transplanted in soil during Spring–Summer period. The experimental field was approximately 365 m^2, and the soil had 2.98% organic matter; 21.18% available $CaCO_3$; pH 8.29; EC 0.81 mS/cm. The microclimate of the area was dry with a mean midday temperature of 35.1 °C and air humidity of 61% during the summer period.

Seedlings were placed in soil with an arrangement in triple rows (rows were separated by 0.2 m, while plants were 0.33 m apart) at a plant density of 51,950 plants/ha. Stringing nettle plants were grown for approximately 17 weeks. Four treatments were used to split the experimental farm as follows: (a) Conventional with full irrigation (ConFI), (b) Conventional with deficit irrigation (ConDI), (c) Organic with full irrigation (OrgFI), and (d) Organic with deficit irrigation (OrgDI). Each treatment had three replicate plots, and each plot had 33 plants, resulting in 396 plants in total. Common registered organic or conventional fertilisers and pesticides were used accordingly.

Irrigation was applied every 5–6 days based on measurements that took place (every 3 days) with a field-scout TDR300 (0.2 m rods, Spectrum Technologies Inc., Aurora, IL, USA) on soil volumetric water content. Plants were fully irrigated for the first 8 weeks. The plants were subjected to deficit irrigation (ca. 50% of the full irrigation treatment) for 3 weeks before the first harvest (May 2021), and then for another three weeks before the second harvest (June 2021). Normal irrigation was applied between the two harvests (3 weeks period), according to the plant water needs for biomass production recovery.

Plant growth, physiology, and nutrient uptake

Before the first and second harvest, when the plants were subjected to deficit irrigation several plant growth and physiology measures took place. A ΔT-Porometer AP4 (Delta-T Devices, Cambridge, UK) was used to measure the leaf stomatal conductance, while leaf chlorophyll fluorescence was estimated with a chlorophyll fluorometer (opti-sciences OS-30p, UK) (Chrysargyris et al., 2017). Prior to each harvest, the height of six plants/treatment was recorded. For the recovery of the biomass production, plants were harvested (at 3 cm above soil), where the upper fresh weight was recorded (g), followed by the calculation of the dried and total dry matter content (%).

The content of chlorophylls (chlorophyll a—Chl a, chlorophyll b—Chl b, and total chlorophyll—total Chl) was estimated as reported by Richardson et al. (2002), and results were expressed as mg of chlorophylls per g of fresh weight. The Chl a:Chl b ratio was also computed.

Leaf's mineral content was determined on four replications/treatment. Dried samples were burned to ash at 480 °C for 5.5 hr and then acid-digested (2 N HCl). Mineral assessment for potassium-K, sodium-Na, phosphorus-P, and nitrogen-N was performed with the procedures mentioned by Chrysargyris et al. (2023), while magnesium-Mg, calcium-Ca, iron-Fe, copper-Cu, and zinc-Zn were determined by employing an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). The content of macronutrients and micronutrients was presented as g/kg and mg/kg of dry weight, respectively.

Polyphenols, flavonoids, and antioxidant activity

Methanolic plants extracts ($n = 4$) were used to determine total phenols, total flavonoids and antioxidant activity by employing three assays [2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)], as previously described (Chrysargyris et al., 2019). Total phenols content was estimated following the Folin–Ciocalteu method, and results presented as equivalents of gallic acid (μmol GAE/g of fresh weight) (Tzortzakis et al., 2022). Total flavonoid content was measured with the aluminium chloride colorimetric method and expressed as Rutin equivalents (mg Rutin/g of fresh weight), as previously described (Chrysargyris et al., 2019). Results from antioxidant activities were expressed as Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent (mg Trolox/g of fresh weight).

UHPLC-QToF-MS extraction and identification

Phenolic compounds were extracted using a solid–liquid extraction method (Vajić et al., 2015). Briefly, 1 g of nettle powder was mixed with 20 ml of 50:50 methanol/water (vol/vol) (HPLC grade) and vortexed for 1 min. The mixture was then shaken (table shaker) for 15 min in the dark. Following this, the extract was placed in an ultrasonic bath and sonicated for 30 min at 40 °C. Afterwards, the extract was centrifuged at 4,000 rpm for 10 min. A 0.22 μm membrane filter was used for filtering the supernatant, which was then stored at −20 °C until analysis. Ultra-high-performance liquid chromatography-triple/time-of-flight mass spectrometry (UHPLC-QToF-MS) analysis was conducted, according to the procedure reported by Koulis et al. (2022), using the same instrumentation, chromatographic conditions, and mass spectrometry settings. The LC gradient elution and flow rate programme are provided in Supplementary Table 1.

Antimicrobial and cytotoxic properties of ethanolic extracts

Preparation of ethanolic extracts

Appropriate amount of dried plant tissue's powder was macerated for 72 hr with absolute ethanol in a 1:2.5 vol/vol ratio under continuous shaking at 160 rpm.(Lall et al., 2013) Afterwards, the material was filtered (Whatman No. 1) and ethanol was removed to complete dryness with a rotary evaporator (Laborota 4011 digital, Heidolph Instruments, Schwabach, Germany).

Antimicrobial activity

Bacteria and cultivation media

The following bacteria were tested: *Staphylococcus aureus* (ATCC 11632; Gram positive) and *Escherichia coli* (ATCC 25922; Gram negative). Bacterial cultures were prepared by overnight incubation (16–18 hr) at 37 °C in Brain Heart Infusion broth (BHI, Himedia, India) to reach a concentration of 1.0×10^8 colony forming units per ml (CFU/ml). As for the control, streptomycin (Sigma-Aldrich, Germany) antibiotic was used.

Microdilution method

The microtiter plate assay was used to determine the antibacterial activity of the prepared ethanolic extracts, where the inhibitory concentration for 50% reduction (IC_{50}) and minimum inhibitory concentration (MIC) were calculated as described by Lall et al. (2013). Ethanolic plant extracts dissolved in 5% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) (diluted with sterile ddH_2O) to obtain stock solutions (75 mg/ml) that were then two-fold diluted reaching concentrations ranging from 1.17 to 75 mg/ml. Briefly, a 96-well round-bottom microtiter plate, was supplemented with 50 μl of BHI and 45 μl of each plant extract dilution. To that, 5 μl of the bacterial culture (1.0×10^8 CFU/ml) were added to each well (1.0×10^6 CFU/ml per well) (final well volume: 100 μl). A standard antibiotic; streptomycin (1.56–100 μg/ml) was employed as a positive control. The absorbance was recorded every 30 min at a wavelength of 630 nm at 37 °C for 20 hr with an ELx800TM Absorbance Microplate Reader (BioTek Instruments Inc., Vermont, USA). The MIC and the concentration inhibited the growth of the bacterial population by 50% (IC_{50}) were computed and presented as mg/ml.

Cytotoxic properties

Cell culture

Two human colorectal cancer cell lines were used as follow: HT-29 (less invasive colorectal cancer cells) and HCT-116 (highly invasive colorectal cancer cells). McCoy's 5A medium (supplemented with 10% foetal bovine serum [FBS] and 1% penicillin/streptomycin) was used for culturing both cancer cell lines, while the cell lines were sub-cultured in 0.05% trypsin solution and incubated in a humidified atmosphere of 5% CO_2 at 37 °C. All reagents for cell culture were purchased from Invitrogen Life Technologies (Invitrogen, CA, USA).

MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) assay was used to examine the cytotoxic activity of the plant extracts (Liu & Nair, 2010). Ethanolic extracts were dissolved in 0.1% DMSO to obtain stock solutions (1,000 μg/ml) that were serially diluted (in phosphate-buffered saline solution) achieving concentrations ranging from 100 to 1,000 μg/ml. HT29 and HCT116 cells were seeded in triplicates in 96-well plates at a density of 10^4 cells/well and incubated at 37 °C in 95% humidity and 5% CO_2 for 24 hr. Afterwards, the culture medium was removed from the wells and 100 μl of each plant extract sample were added to each well. The plate was further incubated for 72 hr. Then, 10 μl of MTT (Biotium, USA) were then added, and the plate was incubated for another 4 hr. Formazan crystals were dissolved with the addition of 200 μl of DMSO (PAN-Biotech, Germany). The absorbance was then measured at a wavelength of 570 nm by using ELx800TM Absorbance Microplate Reader (BioTek Instruments Inc., Vermont, USA). The percentage of cytotoxic activity compared to that of the untreated cells was determined as follows:

$$\text{Cell viability } (\%) = \frac{(\text{OD sample} - \text{OD blank})}{(\text{OD control} - \text{OD blank})} \times 100\%$$

and IC_{50} was then calculated as the concentration to inhibit the 50% of the cells.

Statistical methods

The experiment was set up based on the split-split-plot design with three factors (cultivation practice, irrigation, and harvesting period), while each treatment consisted of three replicates-plots. All data was processed with a one-way analysis of variance (ANOVA) ($p < .05$) in IBM SPSS v.22 (IBM Corp., Armonk, NY, USA). A Duncan's multiple range test was applied to separate the treatments' means. Measurements were made in four to six biological replications/treatment (each replication consisted of a pool of individual measures/samples). The R programme was used for the calculation of pairwise metabolites effects correlations by the Pearson's correlation test.

Results and discussion

Plant growth and physiology

The impacts of cultivation practices—as conventional instead of organic-, and irrigation regimes—as full instead of deficit irrigation—on stringing nettle plant growth and physiology are shown in Tables 1 and 2 and Figure 1. Plant height was decreased in DI-treated plants in comparison to conventional and FI-treated plants at the first harvest (Table 1). Plant fresh weight was increased (up to 37.2%) at ConFI treated plants compared with the other treatments. The dry matter content was increased in plants grown in organic system and DI application, at the first harvest being in accordance with previous reports on *Sideritis perfoliata* under similar experimental studies (Chrysargyris et al., 2019). At the second harvest, significantly higher fresh biomass was obtained in conventional and FI-treated plants (indicated the enriched minerals and water availability for plant development), but the same plants had the least dry matter content. Plant height at the second harvest was unchanged and averaged in 25.02 cm (being almost 30% decreased than the averaged plant height of 36.1 cm, at the first harvest) (Table 1). As a result, the use of well watering in plants on conventional farms that increase the utilisation of minerals, especially on nitrogen fertilisers, as well as the yield increases can mirror the increased water content in the plant cells, which results the low dry matter content in conventional plants (Kazimierczak et al., 2015). Previous studies indicated that nettle DM content was affected by the different sampling times (Hakala et al., 2009) with increased yield to be evidenced at the first harvesting time (Opačić et al., 2024), being in accordance with the presence results. It has been documented that the maximum plant height in stringing nettle was found in plant with density of 4 plants/m^2 followed by density of 5 plants/m^2, while the least one was found in density of 10 plants/m^2 (Kakabouki et al., 2020). In the present study, the plant density was 5 plants/m^2, indicating that plants had an appropriate spacing to grow under the examined cultivation practices. Prior reports indicated that organic medicinal plants had significantly more dry matter content and less water content than the conventionally-grown plants (Kazimierczak et al., 2015), being in line with the observation of the present research.

Leaf stomatal conductance was mainly affected by DI in both conventionally and organically grown plants and this mirrored the plant response to maintain water savings in leaves after water stress events (Figure 1A). The opposed results were evidenced for the leaf chlorophyll fluorescence and SPAD values, mainly at the first harvesting, as relevant increased values were obtained in DI-treated plants (Figure 1B and C). In line with the current study on the decreased leaf stomatal conductivity, plants under water stress coordinated shifts in ion transport, abscisic acid signalling, and leaf stomatal closure (Osakabe et al., 2014).

Table 2 presents the impacts of cultivation practices and irrigation regimes on stringing nettle plants, with relevant changes to be found at the first harvest than at the second harvest. Therefore, at the first harvesting, total chlorophylls content, as a result of the Chl a and Chl b contribution, was decreased in plants grown to organic cultivation and full-irrigated when compared to organically grown plants under DI and/or conventionally grown plants (independent of the irrigation practices). The Chl a:Chl b ratio was the greatest at ConFI applications and the least at the OrgDI applications. Harvesting of *U. dioica* is related to the final product, as it was suggested the first harvesting during spring for fodder, medical or other industrial uses, such as chlorophyll production; the second harvesting mid-summer for fibre production; and the third harvesting early autumn for using leaves (Vogl & Hartl, 2003). However, this can varied in different countries and microclimate conditions as well as the cultivation practices and plants vigour's (Di Virgilio et al., 2015). Chlorophyll levels were obtained in the present study were in accordance with prior reports, when nettle leaves were packed and stored for 14 days under chilled conditions (Dujmović et al., 2025). The increased chlorophyll levels and the response of nettle to N treatment was reported previously (Rutto et al., 2012), which agrees with the current findings of the increased chlorophylls levels and increased N accumulation in plants at the conventional cultivation system.

Phenolics, flavonoids, and antioxidant status

Three methods (as assayed by FRAP, DPPH, and ABTS) were employed to acquire the most accurate assessment of the

Table 1. Effect of cultivation (conventional-Con or organic-Org) and irrigation (full irrigation [FI] or deficit irrigation [DI]) practices on stringing nettle plants growth under two harvestings.

Treatment	Height (cm)	Fresh weight (g)	Dry matter content (%)	Yield dry weight (ton/ha)
First harvest				
ConFI	42.08 ± 1.18a	85.05 ± 3.77a	21.53 ± 0.52b	0.88 ± 0.04a
ConDI	32.75 ± 1.37b	63.75 ± 8.30b	22.36 ± 0.20b	0.60 ± 0.03b
OrgFI	37.00 ± 2.07ab	67.35 ± 3.72b	22.47 ± 0.18b	0.76 ± 0.13ab
OrgDI	32.67 ± 3.99b	53.40 ± 5.80b	23.51 ± 0.36a	0.53 ± 0.05b
Second harvest				
ConFI	26.00 ± 1.02	74.74 ± 5.35a	20.35 ± 0.26b	0.78 ± 0.06a
ConDI	24.08 ± 1.28	38.85 ± 4.91b	21.67 ± 0.28a	0.40 ± 0.05b
OrgFI	24.58 ± 1.12	35.44 ± 3.56b	21.50 ± 0.38a	0.37 ± 0.04b
OrgDI	25.42 ± 1.91	30.33 ± 3.31b	21.35 ± 0.28a	0.32 ± 0.04b

Note. Values ($n = 6$) ± *SE* in column for each harvest followed by the same letter are not significantly different.

Table 2. Effect of cultivation (conventional-Con or organic-Org) and irrigation (full irrigation [FI] or deficit irrigation [DI]) practices on stringing nettle plants content (mg/g Fw) for chlorophyll a, chlorophyll b, and total chlorophylls and Chl a:Chl b ratio under two harvestings.

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophylls	Chl a: Chlb
First harvest				
ConFI	1.23 ± 0.02a	0.32 ± 0.01a	1.55 ± 0.03a	3.80 ± 0.07a
ConDI	1.34 ± 0.10a	0.38 ± 0.03a	1.72 ± 0.13a	3.52 ± 0.07bc
OrgFI	0.88 ± 0.07b	0.24 ± 0.02b	1.12 ± 0.09b	3.61 ± 0.02b
OrgDI	1.27 ± 0.08a	0.38 ± 0.03a	1.64 ± 0.11a	3.39 ± 0.05c
Second harvest				
ConFI	0.37 ± 0.01a	0.34 ± 0.01	0.72 ± 0.01	1.08 ± 0.01b
ConDI	0.35 ± 0.02ab	0.31 ± 0.02	0.66 ± 0.03	1.10 ± 0.01a
OrgFI	0.36 ± 0.01ab	0.33 ± 0.01	0.69 ± 0.03	1.09 ± 0.01ab
OrgDI	0.33 ± 0.02b	0.30 ± 0.01	0.63 ± 0.03	1.08 ± 0.01b

Note. Values ($n = 6$) ± *SE* in column for each harvest followed by the same letter are not significantly different.

antioxidant properties of nettle leaves because of their diverse chemical profile, which includes compounds like fatty acids, vitamins, polyphenols, carotenoids, minerals, etc. (Đurović et al., 2020, 2024; Opačić et al., 2022). All assays indicated that plants grown in OrgDI had the greatest antioxidant capacity. Therefore, total phenols, flavonoids, and antioxidant activity of plant methanolic extracts were stimulated mainly in organic and deficit irrigation treatments for both harvesting periods (Table 3). In details, at the first harvesting, both OrgDI and ConFI grown plants revealed increased total phenols content compared to the other treatments (OrgFI and ConDI). Similarly, the greatest total flavonoids, ABTS, DPPH, and FRAP values were obtained in OrgDI treated plants when compared mainly with the ConDI grown plants.

At the second harvest total phenols increased up to 37.9% in plants grown in OrgDI treatment when compared to the other tested applications. Total flavonoids increased up to 28.3% in plants grown in OrgDI compared to plants grown in OrgFI and ConDI applications. Plant grown in OrgDI revealed increased DPPH, FRAP, and ABTS values, whereas the ABTS was also increased in ConFI and ConDI applications (Table 3).

Increased total phenols and flavonoids were observed in organically cultivated *Centella asiatica* at the first harvesting compared to the non-organically grown plants, and the samples obtained at the second and third harvesting (Bhattacharya et al., 2017). Harvesting time and season are affecting the yield and quality of the final product (Vogl & Hartl, 2003). *Centella asiatica* samples from the first harvesting were richer in phytochemicals and revealed the greatest memory enhancing activity, compared to the second and third harvesting, when plants were grown in organic cultivation compared to non-organic ones (Bhattacharya et al., 2017). Moreover, it has been reported that organic medicinal plants (rosemary, peppermint, lemon balm, and sage) contained much higher levels of phenolic acids and total flavonoids than the plants from conventional production (Kazimierczak et al., 2015), being in line with the observation of the present study. However, plant response to cultivation practices and tolerance to water stress might differ, as organically grown *S. perfoliata* contained higher levels of phenolics, independently of the irrigation regimes applied (full versus deficit irrigation) compared to the conventionally grown plants (Chrysargyris et al., 2019). In the same study, it was demonstrated that the biosynthesis of phenolics and iridoids is totally different as conventional cultivation triggered the biosynthesis of non-volatile monoterpenes (Chrysargyris et al., 2019). Several medicinal uses of stinging nettle and its relevant biological activity was attributed to flavonoids presence (Farag et al., 2013; Upton, 2013), phenylpropanoids (Farag et al., 2013), and carotenoids (Upton, 2013).

According to their structures, the production of vitamin C, flavonoids, and phenolics is connected to the carbon sources that are accessible. The increased fertility and fertilisers application in conventional culture, is reflecting the increased N sources available that stimulate plant development and N-containing metabolites, including free amino acids, proteins, and alkaloids (Elshafie et al., 2023). Conversely, when N is scarce, such as in organic agriculture, the available carbon gets reallocated and plants diversify and increase secondary metabolism, which includes defensive components, including phenolics, flavonoids, and vitamin C increases (Pagare et al., 2015; Zheng, 2009). In other words,

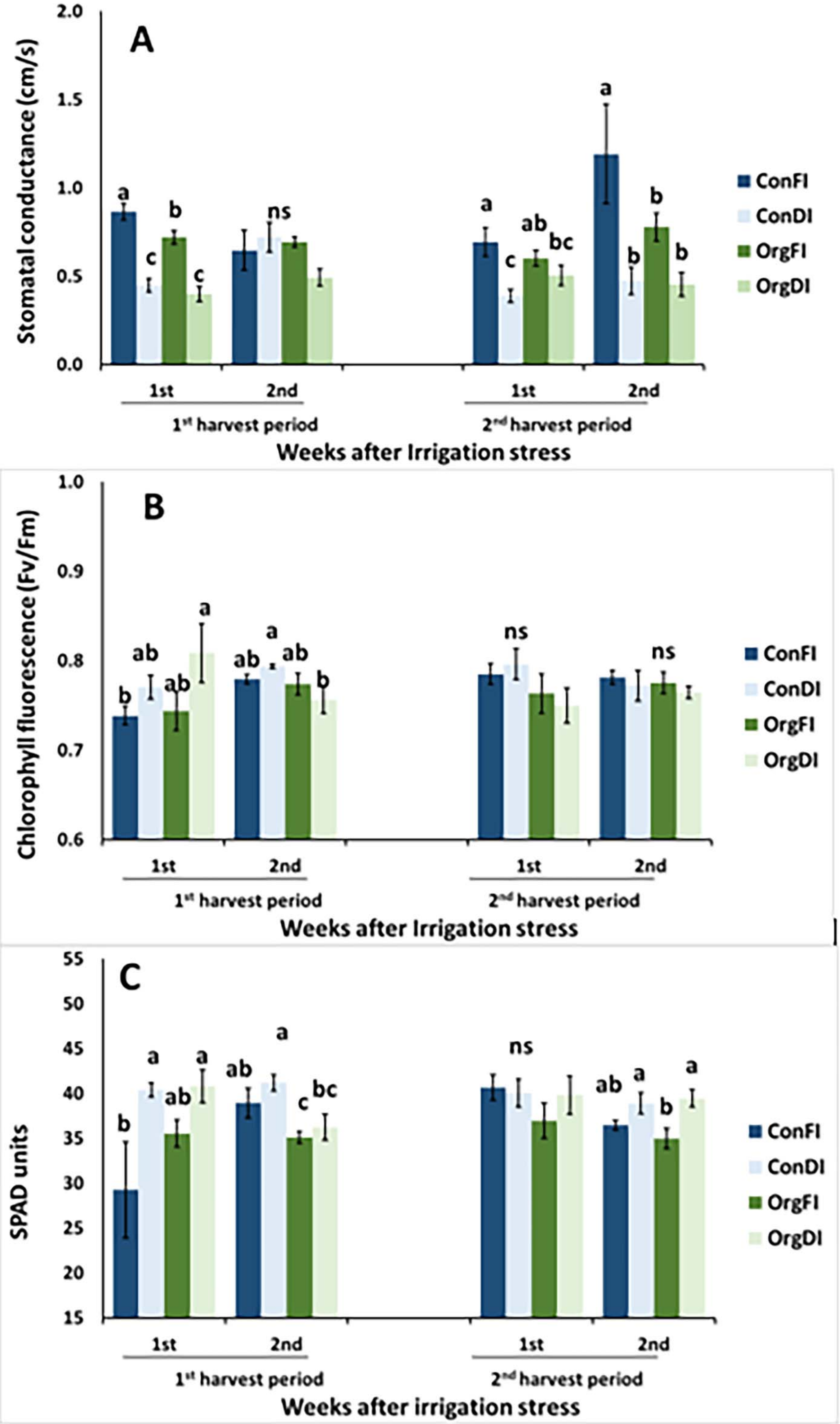

Figure 1. Effect of cultivation (conventional-Con or organic-Org) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on stringing nettle plants (A) stomatal conductance (cm/s), (B) chlorophyll fluorescence (Fv/Fm), and (C) SPAD values, under two harvestings. Values represent mean (±SE) of measurements made on six independent replications per treatment. Mean values followed by the same letter do not differ significantly at $p \geq .05$ according to Duncan's MRT. ns = no significance.

the higher amounts of phenolics, flavonoids, and antioxidants discovered in the current study are mirrored in the decreased N levels reported in plants grown organically and/or under deficit irrigation, as illustrated in Figure 2A.

Mineral content

The mineral accumulation in stringing nettle plants was affected by the cultivation and irrigation practices as well as varied within the two harvesting events (Figure 2). At the first

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

Treatment	Total phenols	Total flavonoids	ABTS	DPPH	FRAP
First harvest					
ConFI	69.53 ± 5.42a	4.54 ± 0.36ab	7.35 ± 0.23ab	12.80 ± 0.74ab	23.49 ± 1.20b
ConDI	53.02 ± 5.03b	3.35 ± 0.44b	5.93 ± 0.28b	10.16 ± 0.73b	17.93 ± 1.04b
OrgFI	39.21 ± 3.95b	4.01 ± 0.41ab	6.34 ± 0.59ab	11.77 ± 0.78ab	22.62 ± 1.40b
OrgDI	68.09 ± 4.69a	5.33 ± 0.67a	7.51 ± 0.66a	14.31 ± 1.41a	29.11 ± 2.97a
Second harvest					
ConFI	98.89 ± 1.72b	11.70 ± 0.71ab	10.34 ± 0.38a	23.28 ± 1.52b	37.54 ± 1.52b
ConDI	95.43 ± 3.96b	10.74 ± 0.52b	10.37 ± 0.42a	26.50 ± 1.71b	36.51 ± 1.18b
OrgFI	89.76 ± 2.85b	10.30 ± 0.31b	8.85 ± 0.12b	25.59 ± 0.74b	32.62 ± 0.76c
OrgDI	123.80 ± 9.46a	13.21 ± 0.40a	10.67 ± 0.25a	37.51 ± 2.40a	44.72 ± 1.42a

Note. Values ($n = 4$) ± SE in column for each harvest followed by the same letter are not significantly different. ABTS = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power.

harvest, deficit irrigation decreased plant's P (Figure 2C), Ca (Figure 2D), Na (Figure 2F), Zn (Figure 2G), but increased Mg (Figure 2E) in conventional cultivation. Moreover, deficit irrigation decreased plant's K, Ca, and Mg (Figure 2B, D, and E) but increased N (Figure 2A), Na (Figure 2F), and Zn (Figure 2G) in organic cultivation compared with the full-irrigated plants in the relevant cultivation practices. No differences were found in Cu contented averaged in 331.4 mg/kg.

At the second harvest, deficit irrigation decreased plant's K (Figure 2B), and P (Figure 2C), but increased N (Figure 2A), Ca (Figure 2D), Mg (Figure 2E), Na (Figure 2F), Zn (Figure 2G), and Cu (Figure 2H), in conventional cultivation compared with the full-irrigated plants. Moreover, deficit irrigation decreased Mg (Figure 2E), and Zn (Figure 2G), but increased N (Figure 2A), P (Figure 2C), and Na (Figure 2F) in organic cultivation compared with the full-irrigated plants.

Not supprisely, *U. dioica* has been documented as an indicator of soil nitrogen and signals the soil good fertility status (Solomou et al., 2020). Water availability is of great importance to the plant metabolism as lack of water is suppressing the N metabolism in soil and thereafter its availability to plants (Carrubba, 2014). Previous studies indicated that nettle is a rich in nutrients in both its roots and biomass, especially to Ca, N, and K (Hakala et al., 2009). Previous reports indicated high N rates demands (160–300 kg N/ha/year) for nettle, which is not environmental friendly agricultural practice and negatively affected quality and phytochemicals of the plants (as reviewed by Di Virgilio et al. (2015)). Organic fertilisers efficiency in soil might be lower than the chemical's ones during the year of application and possible crops can utilise N from organic sources in relevant lower amounts compared to the chemical sources (Naguib, 2011), while the organic fertilisers effectiveness in increased minerals, biodiversity, and soil organisms abundance, can be obtained over longer periods of use (Carrubba, 2014). Additionally, the organic form of N provided by the organic cultivation system is not directly and totally available for the plant's needs, resulting in poorer plant growth (Chrysargyris et al., 2019).

The present study's findings of lower N and K levels in organically produced plants, particularly during the first harvest due to the lower N availability in soil (N in organic form that need to be mineralised to inorganic forms, such as ammonium and nitrate ions), are consistent with earlier reports on *S. perfoliata* (Chrysargyris et al., 2019), while Fe, Mg, and P levels are increased in several organically grown crops relative to those in conventional crops (Rembiałkowska, 2007). Previous studies on *U. dioica* indicated Ca, K, Mg, P, and N content of 7.04, 18.04, 1.14, 2.86, and 5.76 g/kg, respectively (Hakala et al., 2009), whereas in the present study the relevant content were as 22.92, 24.48, 0.57, 1.36, and 21.34 g/kg. The high levels of K and Ca (even higher than the N levels) reported on *U. dioica* leaves in the present study support further the use of stinging nettle for cosmetical purposes as both K and Ca, including iron, vitamins A, D, and C, choline, and amines, are some of the main components underling nettle cosmetic properties (Upton, 2013; Vogl & Hartl, 2003). Moreover, the high content of minerals (Ca, K, P, and Fe) and vitamins (A and C) renders *U. dioica* an important nutraceutical plants. It is benefit for those with high blood pressure, diabetes, and heart disease, as it is consumed as a vegetable in several countries like Nigeria (Bhusal et al., 2022).

UHPLC-QToF-MS analysis

Heat maps (Figure 3), based on the relative expression of phenolic compounds in stinging nettle plants after different cultivation and irrigation practices, reveal a clear differentiation in plant metabolic responses among treatments. In the first harvesting period, DI appeared to reduce Fraxin levels in conventionally grown plants, while in organically cultivated plants it stimulated Hydroxytyrosol and suppressed Rutin levels compared to the ConFI application (Figure 3, Supplementary Table 2). Moreover, organically grown plants under FI exhibited lower levels of citric acid and chlorogenic acid compared with the ConFI application (Figure 3A).

In the second harvesting period, Fraxin levels were generally enhanced under DI treatment regardless of cultivation method, while hydroxytyrosol, chlorogenic acid, and citric acid were suppressed somehow, with the DI treatment in both conventionally- and organically grown plants (Figure 3B).

The phenolic compounds observed in this study, such as Chlorogenic acid, Hydroxytyrosol, Rutin, and Fraxin, are interconnected through the phenylpropanoid pathway, responsible for the biosynthesis of many plants' secondary metabolites. For instance, Rutin and Chlorogenic acid are products of this pathway, and their modulation under stress suggests a metabolic redirection towards defence-related compounds. The increased Hydroxytyrosol levels under deficit irrigation in organically treated plants likely reflect a reconfiguration of plant metabolism to cope with stress, while Rutin levels were generally suppressed under similar conditions, suggesting a metabolic diversion towards stress-related metabolites. Fraxin appears to play a role in the plant's defence mechanisms, with its increased levels under water-limited conditions supporting its involvement in oxidative stress mitigation (Cheynier et al., 2013).

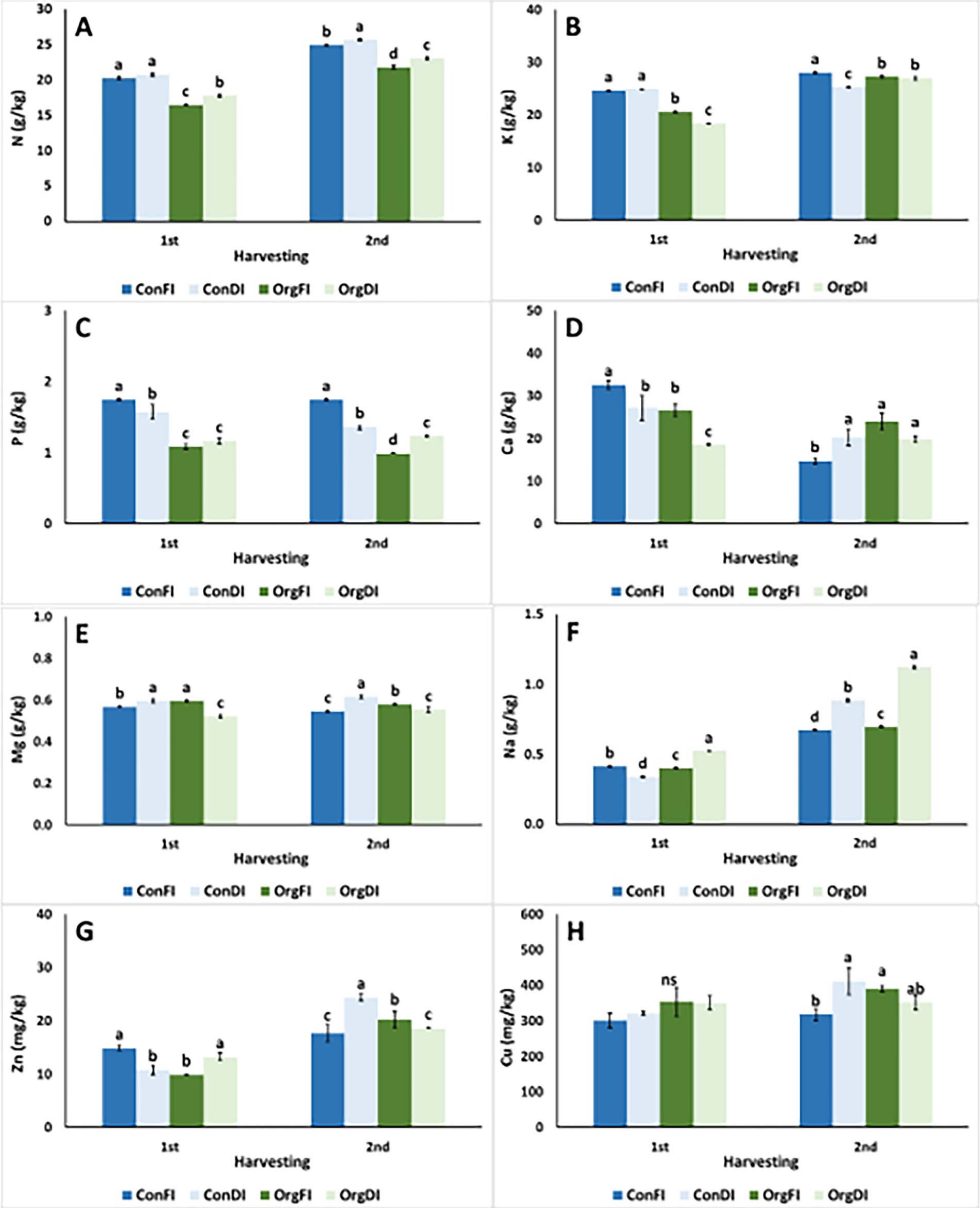

Figure 2. Effect of cultivation (conventional-Con or organic-Org) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on stringing nettle plants mineral composition under two harvestings. (A) NitrogenN, (B) potassium-K, (C) phosphorus-P, (D) calcium-Ca, (E) magnesium-Mg, (F) sodium-Na, (G) zinc-Zn, and (H) copper-Cu. Values represent mean (±SE) measurements made on four independent replications per treatment. Mean values followed by the same letter do not differ significantly at $p \geq .05$ according to Duncan's MRT. ns = no significance.

Many of the phenolic compounds identified in stinging nettle are well-known for their bioactive properties, including antioxidant, antimicrobial, and cytotoxic effects. Hydroxytyrosol has been shown to exhibit strong antioxidants and antimicrobial activity, which could help the plant mitigate oxidative stress and prevent microbial damage under deficit irrigation conditions. However, the antimicrobial efficacy of Hydroxytyrosol is subject to ongoing debate, with some studies suggesting limited activity depending on concentration and environmental factors (Medina-Martínez et al., 2016). Chlorogenic acid also demonstrates antioxidant and cytotoxic properties, with studies supporting its potential in cancer treatment. Similarly, Rutin has been

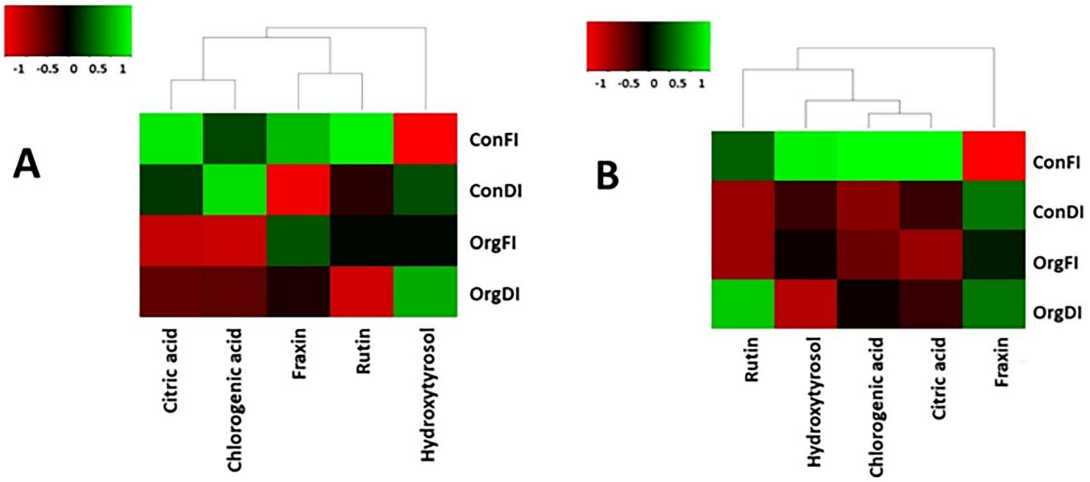

Figure 3. Metabolite changes in stringing nettle plants after different cultivation (conventional-Con or organic-Org) and irrigation (full irrigation-FI or deficit irrigation-DI) practices. Heat map representing relative expression of phenolics compounds traits elicited in plant tissue under two harvestings (A) first harvesting and (B) second harvesting as compared to the conventional and full-irrigated plants.

recognised for its anti-inflammatory and antimicrobial effects, while Fraxin is noted for its cytotoxic and anti-inflammatory properties, further contributing to the plant's therapeutic potential. The interaction between cultivation practices and irrigation strategies significantly influences the biosynthesis of bioactive phenolic compounds in stinging nettle.

Antimicrobial activity

The results from antimicrobial activity of ethanolic extracts are presented in Table 4, showing the MIC and IC_{50} values of each cultivation practice (conventional versus organic), irrigation (full versus deficit) in two different harvests against *E. coli* and *S. aureus*. These two bacteria are among the most prevalent causes of urinary tract infection, which is one of the most serious and frequent infections that can happen to anyone at any age (Molaabaszadeh et al., 2013). Due to its long-term therapeutic benefits, *U. dioica* is a well-known medicinal plant around the world (Mirtaghi et al., 2016). In the present research, comparing the effects of the different ethanolic extracts of *U. dioica* on both bacteria, the MIC and IC_{50} values after conventional full and deficit practices were almost the half of those from the organic practices, especially in the first harvest. The MIC values on *E. coli* after conventional treatments either full or deficit were 4.68 mg/ml, compared to the organic treatments that the MIC values were 9.37 mg/ml. On *S. aureus*, the MIC values after conventional and organic practices were 1.17and 2.34 mg/ml, respectively. In line with MIC values, the IC_{50} values on *E. coli* and *S. aureus* were significantly different in the first harvest compared to the second harvest after conventional treatments compared to the organic, indicating that the period of harvest might be important on the effect of the growth for the two investigated bacteria. These results indicated that the conventional treatment of the plant revealed stronger inhibiting effects on the growth of both bacteria. *Staphylococcus aureus*, however, proved to be the bacterium most vulnerable to the extract's activity.

The study's findings are quite consistent with research that has been published on the subject in recent years. Kiaei et al. (2010) described the antimicrobial activity of *U. dioica* ethanolic extracts against *E. coli* and several staphylococci species. Their findings showed that the growth of *E. coli* was not affected after plant extract treatment, contrast to the complete inhibition observed for *Staphylococcus epidermidis*, *S. aureus*, and *Staphylococcus saprophyticus*. According to a comprehensive research, it was showed that the ethanolic extract from *U. dioica* revealed higher antibacterial activity against Gram positive bacteria, such as *Streptococcus pyogenes*, *S. aureus*, and *S. epidermidis*, compared to other bacteria (especially Gram negative) such as *Citrobacter freundii*, *Serratia marcescens*, *Erwinia* sp., and *Proteus* sp. (Kőszegi et al., 2017).

Cytotoxic properties

The cytotoxic effects of *U. dioica* ethanolic extracts (with four different cultivation practices, as described above) were examined using the MTT assay was performed in order to analyse the cytotoxic effects of ethanolic extracts of the plant *U. dioica* with four different cultivation practices as described before. *Urtica dioica* is a traditional MAP with numerous pharmacological properties that is widely used as a therapeutic agent against cancer (Esposito et al., 2019). In this study, the two colon cancer cell lines investigated (HT-29 and HCT-116) were selected due to their different metastatic potential (low and high invasiveness, respectively) (Pape et al., 2019). Both cell lines were treated with the different ethanolic extracts of *U. dioica* for 72 hr. Table 5 presents the effects of cultivation practice (conventional versus organic), irrigation (full versus deficit), and the IC_{50} on the two colon cancer cell lines. Overall, the effects of extracts from the different treatments of *U. dioica* were more cytotoxic for the highly invasive HCT-116 cell line compared to the low invasive HT-29 cell line. As seen in Table 5, the IC_{50} values of all the treatments of the extracts were more than 1,000 μg/ml for HT-29 cells. However, the different cultivation practices exhibited different effects on the HCT-116 cells. More importantly, the extract from organic/deficit demonstrated low IC_{50} value which was 657.70 μg/ml at the first harvest, and it reduced to half at the second harvest. This indicates that organic cultivation with low water acts resulted in plants that gave extracts with stronger activity against the highly invasive cells (HCT-116) compared to the low invasiveness colon cancer cells (HT-29). This suggests that these extracts have immense and targeted anti-cancer potential, eliminating highly aggressive and metastatic cells. Previous studies showed that products obtained from organic cultivation practices present higher quality (i.e., nutritional value and biological properties) as opposed to the ones from conventional cultivation practices (Barbieri et al., 2017;

Table 4. Effect of cultivation (conventional-Con or organic-Org) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on stringing nettle plants antimicrobial properties. MIC and IC50 values of *Urtica dioica* extract and its groups on Gram (−) bacteria *E. coli* and Gram (+) bacteria: *S. aureus*.

	E. coli		***S. aureus***	
Treatment	**MIC (mg/ml)**	**IC_{50} (mg/ml)**	**MIC (mg/ml)**	**IC_{50} (mg/ml)**
First harvest				
ConFI	4.68 ± 0.00b	12.30 ± 0.66b	1.17 ± 0.00b	6.76 ± 0.46b
ConDI	4.68 ± 0.00b	9.26 ± 1.26b	1.17 ± 0.00b	4.17 ± 0.28c
OrgFI	9.37 ± 0.00a	17.76 ± 2.50a	2.34 ± 0.00a	10.76 ± 1.47a
OrgDI	9.37 ± 0.00a	17.58 ± 1.34a	2.34 ± 0.00a	12.52 ± 0.38a
streptomycin	0.0016 ± 0.0000c	0.0134 ± 0.0002c	0.0008 ± 0.0000c	0.0103 ± 0.0006d
Second harvest				
ConFI	4.68 ± 0.00b	14.08 ± 3.50a	1.17 ± 0.00b	8.17 ± 2.80a
ConDI	4.68 ± 0.00b	11.59 ± 1.11a	2.34 ± 0.00a	10.30 ± 2.40a
OrgFI	9.37 ± 0.00a	14.06 ± 0.23a	1.17 ± 0.00b	10.34 ± 0.34a
OrgDI	4.68 ± 0.00b	10.98 ± 2.69a	2.34 ± 0.00a	11.29 ± 1.83a
streptomycin	0.0016 ± 0.0000c	0.0134 ± 0.0002c	0.0008 ± 0.0000c	0.0103 ± 0.0006d

Note. Data is reported as a means of three measurements $(n = 3) \pm SE$. Different letters (a–d) indicate significant difference between extracts ($p < .05$). Treatments followed by the same letter in each column are not significantly different.

Chrysargyris et al., 2019; Litskas et al., 2019) These cultivation practices can be combined with low irrigation to help increase yields, especially in areas with limited water reserves (Chrysargyris et al., 2021). At the second harvest, conventional/deficit treatment also affected the cell viability of HCT-116 cells; however, the IC50 value was similar to that of organic/full. Finally, extract from conventional/full, was the only one that did not show any difference between the two different harvests on HCT-116 cells. Our findings are consistent with those of Mohammadi et al. (2016), who showed that treatment of highly aggressive and metastatic HCT-116 cells with a dichloromethane extract of *U. dioica*, resulted in cell apoptosis and arrest at the G2/M phase of the cell cycle. However, in this study, we showed that the different ethanolic extracts from the leaves of *U. dioica*, did not show cytotoxic effect on the low invasive HT-29 cells and the observed IC_{50} values were more than 1,000 μg/ml. Interestingly, Ghasemi et al. (2016) showed that ethanolic extract of *U. dioica* roots decreased cell viability of HT-29 cells, with IC_{50} value of 24.7 μg/ml after 72 hr exposure. Overall, our findings showed that the treatment of cells with organic/deficit extracts significantly reduced cell viability in HCT-116 cells (Table 5).

Conventional farming is often an intensive agricultural farming system involving the utilisation of chemical fertilisers and pesticides. This may enhance plants biomass production, but it doesn't always enhance the quality and safety of the commodities that are consumed or used, as mineral fertilisers reduce the level of bioactive compounds and hence the nutritive value of plants (Kazimierczak et al., 2015). Jolliet et al. (2003) reported that fertilisation accounted for 43% of the total adverse environmental effects, followed by watering (36%), maintenance (10%), and transplant (6%) on a hectare basis, highlighting the importance of the present study on using less fertilisers and less water for *U. dioica* cultivation in Mediterranean basin.

Table 5. Effect of cultivation (conventional-Con or organic-Org) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on stringing nettle plants antimicrobial properties. IC_{50} value of *U. dioica* extract and its groups on HT-29 and HCT-116 colon cancer cell lines.

	IC_{50} values (μg/ml)	
Treatment	**HT-29**	**HCT-116**
First harvest		
ConFI	>1,000	>1,000a
ConDI	>1,000	>1,000a
OrgFI	>1,000	>1,000a
OrgDI	>1,000	657.70 ± 28.11b
Second harvest		
ConFI	>1,000	>1,000a
ConDI	>1,000	676.98 ± 44.71b
OrgFI	>1,000	793.93 ± 58.69b
OrgDI	>1,000	334.15 ± 28c

Note. Data is reported as a means of three measurements $(n = 3) \pm SE$. Different letters (a–c) indicate significant difference between extracts ($p < .05$). Treatments followed by the same letter in each column are not significantly different.

Conclusion

The present investigation looked at how water scarcity and production practices affected the *U. dioica* growth, biochemical characteristics, phytochemical profile, and biocidal properties of plant extracts, including antimicrobial and cytotoxic properties. In both conventional and organic cultivation practices, deficit irrigation decreased plant growth and yield. Plants reacted to DI by closing the stomata to reduce the water losses through evapotranspiration as evidenced in both harvesting periods. Moreover, plants grown in organic cultivation, for both full and deficit irrigation applications, had decreased nitrogen, phosphorus, and potassium content, with more profound effects at first harvesting. Deficit irrigation and/or organic cultivation affected plants' calcium content, with decreased values at the first harvest and increased values at the second harvest, compared with the full-irrigated and conventionally grown plants. The DI increased plants total phenolics, flavonoids, and antioxidants, especially at the first harvest. Plants grown under organic practices stimulated Hydroxytyrosol and decreased Rutin content, as opposed to conventionally full irrigated plants at the first harvesting. At the second harvesting, Fraxin content was increased, while Hydroxytyrosol, Chlorogenic acid, and Citric acid were decreased with the DI treatment in both conventionally and organically grown plants. The conventional cultivation practices resulted in plants with higher antibacterial activities against *E. coli* and *S. aureus*, with more profound effects at the first harvesting period. In oppose, plants grown under organic cultivation had stronger cytotoxic properties against two human colorectal cancer cell lines, with more profound effects with the DI applications, providing new insights on cultivation management for *U. dioica*. The application of water stress and organic culture, with less available minerals and water affected the biocidal properties of *U. dioica*. This was directed with putative antimicrobial and anticancer properties, which were mirrored to the changes of phenolic components. This induced plant defence mechanisms and affecting the relevant phenolic components, such as Rutin, Chlorogenic acid, and Hydroxytyrosol, across the two harvesting times. Future research needs to focus on tailoring the mineral application and examined various stress conditions, i.e., salinity in order to optimise the plant responsiveness and possible stimulation of important phenolic components with various biocidal activities.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

Data will be available from authors on request.

Author contributions

Antonios Chrysargyris (Methodology, Investigation, Formal analysis, Writing—original draft, Writing—review & editing), Panayiota Xylia (Methodology, Investigation, Formal analysis, Writing—original draft), Kalia Kyriakou (Investigation, Writing—original draft), Konstantina Papastavropoulou (Methodology, Investigation, Writing—original draft), Panagiotis-Loukas P. Gialouris (Methodology, Investigation), Eleni S. Nastou (Methodology, Investigation), Panagiota Miltiadous (Investigation, Resources, Writing—original draft), Nikolaos S. Thomaidis (Methodology, Validation, Supervision, Writing—review & editing), Charalampos Proestos (Conceptualisation, Methodology, Validation, Supervision, Writing—review & editing), and Nikolaos Tzortzakis (Conceptualisation, Project administration, Resources, Investigation, Methodology, Supervision, Funding acquisition, Writing—original draft, Writing—review & editing)

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Conflicts of interest

The authors declare no conflict of interest.

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