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Organic Cultivation and Deficit Irrigation Practices to Improve Chemical and Biological Activity of *Mentha spicata* Plants

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Abstract: Intensive crop production and irrational use of fertilizers and agrochemicals have questionable effects on the quality of products and the sustainable use of water for agricultural purposes. Organic cultivation and/or deficit irrigation are, among others, well appreciated practices for a sustainable crop production system. In the present study, spearmint plants (*Mentha spicata* L.) were grown in different cultivation schemes (conventional versus organic cultivation, full versus deficit irrigation), and effects on the plant physiological and biochemical attributes were examined in two harvesting periods. Deficit irrigation decreased plant growth, but increased total phenolics, flavonoids, and antioxidant capacity of the plants at the second harvest. Spearmint nutrient accumulation was affected by the examined cultivation practices; nitrogen was decreased in organic cultivation, potassium and sodium were elevated at full-irrigated plants, while magnesium, phosphorus, and copper levels were higher at the deficit-irrigated plants. However, conventional/full-irrigated plants had increased height and fresh biomass at the first harvest. Essential oil content decreased at the second harvest in organic and/or deficit treated plants. Additionally, deficit irrigation affected plant growth and delayed the formation of carvone from limonene. The essential oils were further evaluated with regard to their bioactivity on a major vineyard pest *Lobesia botrana*. Volatile compounds from all essential oils elicited strong electroantennographic responses on female insects antennae, highlighting the role of carvone, which is the major constituent (~70%) in all the tested essential oils. *M. spicata* essential oils also exhibited larvicidal activity on *L. botrana*, suggesting the potential of their incorporation in integrated pest management systems.

Keywords: spearmint; medicinal and aromatic plants; sustained deficit irrigation; insecticidal activity; antioxidant activity; electroantenographic response

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1. Introduction

To provide enough food for an expanding world population, a massive increase in crop production is required in order to meet the food demands of future generations, while preserving the ecological and energy-related resources of our planet [1]. Agricultural production, however, continues to be constrained by a variety of biotic (e.g., pathogens, insects, and weeds) and abiotic (e.g., drought, salinity, cold, frost, and waterlogging) factors that can significantly reduce the quantity and quality of crop production [2]. On top of that, the threat of global warming is likely to increase drought periods in many regions worldwide, drastically affecting crop production [3]. The effect of cultivation practices on crops is well reported in the literature, as the selection of the appropriate ones improves crop quality and productivity [4–6]. The application of an unsuitable practice may reduce the ability of crops to produce high yields, increase the concentration of minerals in the soil up to toxic levels, contaminate waters, and degrade soil quality [7,8]. Farmers are often unilaterally attracted by high yields and to a lesser extent by high quality produce, and thus opt for intensive crop schemes, including conventional crop cultivation of high fertilizer, and phytochemical and water demands. Towards that direction, the selection and the combination of different cultivation practices that demand low inputs (such as the organic cultivation) or a less water demanding crop may not only result in a more eco-friendly and sustainable farming system [9], but when applied to aromatic and medicinal plants, may also reveal improved or new properties of the plant extracts [5]. Additionally, fresh water scarcity in arid and semi-arid regions leads the way to adopting new water-conserving strategies without limiting the dietary features and the biological properties of produced plants [9].

Organic cultivation practices are a promising eco-friendly approach to crop production, able to provide high quality products that are comparable to the conventionally produced ones in terms of nutritional value and properties [10–12]. The environmental benefit from such approaches can be further enhanced if they are combined with cultivation of crops with low water needs or drought-tolerant species to help increase yields, which can be particularly important for areas with limited water reserves [7]. Medicinal and aromatic plants (MAPs) are perfect candidates to such environments and cultivation schemes, due to their low water and minerals demands [13].

In addition to their low environmental footprint, organically grown products are considered to be healthier than those produced conventionally, and numerous studies have been conducted to evaluate this notion, focusing mainly on vegetables, fruits, and animal products [14]. At the same time, consumers' interest in organic herbs and their extracts has been consistently increasing, despite their relatively low yields [12]. There is, however, a limited amount of studies that focus on the comparison between conventional and organic cultivation of medicinal and aromatic plants [15,16], and only a few of them examine the above-mentioned cultivation practices (organic vs. conventional) along with the effect of the applied irrigation scheme [5,14], even though the effects of drought stress or deficit irrigation plans are well-documented [17–21]. Furthermore, several reports mention the effects of the cultivation and fertilization scheme on the plant's growth and biological properties, such as its antioxidant activity, nutrient content in leaves, as well as essential oil yield and richness in particular compounds [5,22,23]. These reports also demonstrated that energy use and carbon footprint were improved in organic compared to conventional spearmint fields, while overall water consumption had no significant differences [12]. Moreover, the application of a deficit irrigation plan may contribute positively to the quality of the produced material [5,20,22,24]. It is evident that the interplay of different farming practices and irrigation schemes as well as their effects on production is complicated and requires further investigation in order to identify key parameters and optimum strategies for sustainable agriculture.

The *Mentha* genus, belonging to the *Lamiaceae* family, includes several species of important herbs, with spearmint (*Mentha spicata*) being one of the most predominant; with a great variety of uses, the worldwide interest in spearmint cultivation is high due to the industrial importance of its essential oil [25]. The economic importance of the plant is derived from its uses in food, perfumery, confectionery, and pharmaceutical industry [26]. Spearmint's essential oils and extracts have been studied for their biological activities, which, most notably, have been reported as antioxidant [27], antibacterial [28], and fungicidal [29]. Another important set of properties of the essential oil of spearmint is its insecticidal and insect repellent activity [30,31], which derives from the presence of compounds with individually strong insecticidal activity as well as their synergistic action [32]. The insecticidal properties of essential oils (EOs) of *Mentha* species offer the prospect of using them as natural pesticides with a commercial value, having social acceptance due to their sustainability and environmentally friendly profile [33], since they are less persistent than conventional pesticides, non-toxic to other organisms when used under controlled manner, and highly effective against resistance [34].

Considering the potential of medicinal plant products as a major class of bio-pesticides, it is important to maintain or increase yield and quality (fresh and dry matter, essential oil yield and composition), while implementing organic farming methods that can meet these targets. Additionally, unlike conventional agricultural production, practices based on a combination of organic agriculture with a customized irrigation schedule can be both beneficial to medicinal plants and less of a burden to the ecosystem. Essential oils from plants have attracted growing interest as contact insecticides and insect repellants or attractants. Electroantennography (EAG) is an effective tool to evaluate and record the olfactory responses to plant odorants [35]. In this context, we evaluated the effects of combining organic farming with a deficit irrigation scheme on *Mentha spicata* plants and their essential oil. In addition, we investigated whether these practices affect the bioactive properties of *M. spicata* essential oil by looking into the effect they exert on the European grapevine moth, *Lobesia botrana* Schiff., which is a major harmful pest for vineyards worldwide.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Uniform size of *Mentha spicata* cuttings (8 cm in height and 6–8 leaves) were purchased from the Cypriot National Centre of Aromatic Plants, Nicosia, Cyprus. Plants were transplanted in soil during spring in a commercial organic farm in Limassol, Cyprus (34°38′ N, 32°56′ E). The pH and the electrical conductivity (EC) of the soil were measured at 8.37 and 0.82 mS/cm, respectively. The mineral composition of the soil was also measured in terms of nitrogen (0.92 g/kg), potassium (0.70 g/kg), sodium (0.16 g/kg), and phosphorus (0.016 g/kg). Other soil properties tested included organic matter (2.97%) and available $CaCO_3$ (22.12%). Local meteorological data of the area were also collected as well during the experiment (Table S1); mean daytime temperature and air humidity were averaged at 32.2 °C and 59%, respectively. Maximum daytime temperature reached 39.3 °C during the early summer period without any rainfall.

2.2. Insects

A laboratory colony of the European grapevine moth *L. botrana* originating from feral populations from Northern Greece was established at the Chemical Ecology and Natural Products Laboratory of NCSR Demokritos, Athens, Greece. Larvae were reared on an artificial diet. All life stages were kept at a 16:8 (L:D) photoperiod, at 24 ± 1 °C and 60–70% relative humidity.

2.3. Cultivation Plan

The field experiment was conducted using three plots, and each plot having three rows, spaced 30 cm apart. Twelve (12) plants were placed in each row and spaced 30 cm apart. A total of 36 plants were cultivated in each plot, giving a total of 108 plants per treatment. The four different treatments (cultivation practices) applied to the plants were (i) conventional cultivation plan with irrigation at 100% (according to the soil volumetric water content—VWC) (CI), (ii) conventional cultivation plan with a deficit irrigation regime of 50% (CD)l (iii) organic cultivation plan with 100% irrigation (OI); and (iv) organic cultivation plan with deficit irrigation at 50% (OD).

For organic and conventional cultivation, registered organic and conventional fertilizers and pesticides were used accordingly, when needed for powdery mildew, thrips, and white fly (Table S2). Plants were cultivated for four months, and were harvested in two harvesting periods, right before flowering stage. Soil water content measured every five days by a portable field-scout TDR300 apparatus, equipped with 20 cm rods (Spectrum Technologies Inc, Aurora, IL, USA), based on preliminary measures and previous experimentation [5,36–38]. Plants were irrigated approximately every 5–7 days. The amount of water for deficit irrigation treatment was based on the soil volumetric water content (ca. 50% of the VWC) of the full irrigation treatment. Plants were subjected to deficit irrigation for three weeks before the first harvest (early May) and then for three weeks before the second harvest (late June). The aerial parts of the plants were harvested manually (using sharp knives) at 3 cm above soil. Between the two harvests, spearmint was irrigated normally, according to the plant water needs (every four days, based on soil water content measurements), in order to recover the biomass production after the first harvesting. Cultivation of spearmint plants for fresh biomass production (sold as bunches) is a short cultivation where plants are not left to grow more than 30–35 cm in height, and usually this takes place within a few weeks.

2.4. Physiological Measurements and Plant Growth Parameters

During the cultivation period, two harvests took place. Prior to each harvest, leaf chlorophyll fluorescence, SPAD chlorophyll assessment, and stomatal conductance data were collected, using a OS-30p fluoremeter (Opti-Sciences, Hudson, NH, USA), SPAD 502 plus chlorophyll analyser (Konika-Minolta, Osaka, Japan), and ΔT-Porometer AP4 (Delta-T Devices, Cambridge, UK), respectively. Measurements were conducted on three fully expanded leaves per plant, on six different plants per treatment.

Plant growth parameters were assessed in terms of plant height (cm), fresh weight (g), and dry matter content (%), for six plants per treatment, for each harvesting period.

2.5. Nutrient Content in Plant Tissue

Plant tissue was also collected at each harvesting, dried until constant weight (65 °C), milled, burned in an ash furnace at 450 °C, and then subjected to hydrochloric acid (2 N) digestion, for nutrient analysis. The extract was used for the determination of K, Na, P, Mg, Ca, Cu, and Zn [5]. The analysis was conducted using an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). Nitrogen content was estimated using the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Switzerland). Results were expressed as g/kg for macronutrients and mg/kg for micronutrients of dry weight. Samples from each treatment were analyzed in triplicates (one sample was a pool of three different plants).

2.6. Essential Oil Extraction and Compound Identification

After each harvest, fresh spearmint plants were dried at 42 °C in an air oven, until constant weight, approximately 72 h. Hydrodistilation was used for the extraction of the essential oils from plant tissue, using a Clevenger apparatus. The oil yield was calculated as μL of oil per 100 g of dried tissue, and results expressed as percentage (%). Oils were dried using anhydrous sodium sulphate, before analysis. Analysis was performed using a Shimadzu GC20210 gas chromatograph interfaced with a Shimadzu GC/MS QP2010plus mass spectrometer. An aliquot of 2 μL of diluted essential oil in ethyl acetate (1:1000 v/v) was injected into a ZB-5 column (0.25 μm × 30.0 m × 0.25 mm, Zebron, Phenomenex, Torrance, CA, USA), in a 20:1 split mode. The identification of the oil compounds was performed as described by Chrysargyris et al. [39]. Four biological samples (pool from three individual plants) from each treatment were used for the essential oil extraction and analysis.

2.7. Total Phenols and Flavonoids Content and Antioxidant Activity of Plant Extracts

Polyphenols were extracted from three samples (three individual plants were pooled/ sample). Plant tissue (0.5 g) was milled (for 60 s) with 10 mL methanol (50% v/v), and extraction was assisted with ultrasound for 30 min. The samples were centrifuged for 15 min at 4000× g at 4 °C (Sigma 3–18 K, Sigma Laboratory Centrifuge, Newtown, UK). Extracts were stored at −20 °C until analysis of total phenolic and flavonoid compounds and estimation of the antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2′-Azino bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method.

Total phenols content was determined using Folin–Ciocalteu method at 755 nm according to Chrysargyris et al. [21], and results were expressed as equivalents of gallic acid (Scharlau, Barcelona, Spain) per g of fresh weight (mg of GAE/g Fw). Total flavonoid content was assayed using the aluminum chloride colorimetric method [40] and expressed as rutin equivalents (mg Rutin/g of fresh weight).

The activities of DPPH, ABTS, and FRAP were determined as described previously [21]. In detail, DPPH radical scavenging activity of the plant extracts was measured at 517 nm from the bleaching of the purple-colored 0.3 mM solution of DPPH. Standard curve was prepared using different concentrations of trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], and results were expressed as mg trolox/g of fresh weight. ABTS radical scavenging activity of the plant extracts was measured at 700 nm, and the results were expressed as mg trolox/g fresh weight. The antioxidant capacity using the FRAP method was carried out at 593 nm, and results were expressed as mg trolox/g fresh weight.

2.8. Electroantennographic Recordings

The antennal responses of *L. botrana* female adults to *M. spicata* essential oils were evaluated by electroantennography (EAG) using a commercially available electroantennographic system (IDAC-4, Syntech, Hilversum, The Netherlands). Females were targeted to test if compounds present in essential oils can be perceived and thus have the potential to act as attractants or repellents for gravid females.

The antenna of a virgin, two-to-three-days-old female adult was excised from the head close to the scape using micro-scissors and was then mounted between glass micropipette electrodes consisting of silver wire inserted in glass capillaries filled with 0.1 M potassium chloride and 0.1% polyvinylpyrrolidone. The base of the antenna was connected to the indifferent electrode, whilst the distal end was connected to the recording electrode. The signal was amplified 10X by a Universal AC/DC pre-amplifier probe connected to the recording electrode and the analog signal was amplified and detected with a data acquisition controller (IDAC-4, Syntech, Hilversum, The Netherlands).

Essential oil extracts were diluted in acetone to produce 5 mg/mL solutions. Ten microliter aliquot of each solution was pipetted to a piece of filter paper (7 × 30 mm, Whatman no. 1), and the solvent was allowed to evaporate. Next, the impregnated paper strip (carrying a 50 μg dose of the test stimulus) was inserted into a glass Pasteur pipette (~22.5 cm length, ISOLAB, Germany), and each pipette was sealed with polypropylene tips until use. Stimuli cartridges were similarly prepared for the female sex pheromone component *(E)-7,(Z)-9*-dodecadienyl acetate (*E7,Z9*-12:Ac), as well as 3-octanol, the latter being used as a reference stimulus. The tip of each stimulus pipette was inserted into a small hole in the wall of a glass tube directed towards the antennal preparation. The stimuli were provided as 0.3 s air puffs into a continuous flow of filtered and humidified air. The air flow, at 25 cm^3/s rate, tube diameter 1 cm, was generated by an air stimulus controller (CS-55, Syntech, Hilversum, The Netherlands). At least 1 min was allowed between successive stimulations in order to allow the antenna to recover. Control stimuli consisted of (1) a clean Pasteur pipette (Control 1), (2) a pipette with an untreated filter paper strip (Control 2), and (3) a pipette with filter paper and solvent (acetone). A reference stimulus, consisting of 50 μg dose of 3-octanol, was provided at regular intervals during each recording session. The EAG response to each reference stimulus was defined as 100%, and all responses to the test stimuli between adjacent references were normalized in % relative to the references. All test compounds were measured at a total of 15 antennal preparations.

2.9. Larvicidal Bioassays

M. spicata essential oils extracted from plants of the tested treatments (cultivation practices and harvests) were diluted in acetone to produce solutions at concentrations ranging from 10 to 50 mg/mL. Fifth instar *L. botrana* larvae were placed in a Petri dish (Diameter 5 cm), and aliquots of 2 μL/insect of each solution (20, 40, 60, 80, and 100 μg), were dorsally applied on larvae using a micropipette. After one minute to allow for solvent evaporation, the larvae were transferred to a lidded 24-well plate, each larvae placed in an individual well with a cube of artificial diet. The wells were kept at 24 ± 1 °C and 60–70% RH. Larvae mortality was recorded after 24 h. Control treatments consisted of untreated larvae and acetone treatment only. Ten larvae were used for each treatment, and three independent replicates were conducted.

2.10. Statistical Methods

The analysis of data was accomplished with the use of IBM SPSS vs. 22, where the effects of cultivation practice, irrigation, and harvesting period, as well as their interactions on the plant growth, physiological, biochemical, and nutrient content, and essential oil yield and composition of samples were assessed with three-way ANOVA. Means were compared with one-way analysis of variance (ANOVA) and Duncan's multiple range tests (MRT) at $p < 0.05$. Analyses were performed in four to six biological replications/treatment (each replication consisted of a poll of three individual measures/samples).

The electrophysiological data were subjected to analysis of variance (ANOVA) (SAS Institute, 2000). The means of electrophysiological data were separated using the Duncan's multiple range tests (MRT) at $p < 0.05$. Data obtained from each dose of larvicidal bioassay were subjected to Probit analysis; LC_{50} values and slopes were calculated.

3. Results

Table 1 presents the effects of cultivation practice (conventional vs. organic), irrigation (full vs. deficit), harvesting (first vs. second), and their interaction on plant-related parameters. Cultivation practice affected significantly spearmint N, Na, and Zn content, D-limonene, carvone, monoterpenes, and sesquiterpene hydrocarbons at $p < 0.001$; leaf SPAD, total flavonoids, DPPH, K, and P content at $p < 0.05$. Irrigation practice affected significantly K and Na content at $p < 0.001$; dry matter content, DPPH, D-limonene, and carvone at $p < 0.01$; stomatal conductance and monoterpenes hydrocarbons at $p < 0.05$. Harvesting affected significantly total phenols and flavonoids content, ABTS, FRAP, DPPH N and Na content, D-limonene, eucalyptol, carvone, monoterpenes and sesquiterpene hydrocarbons, and oxygenated monoterpenes and sesquiterpenes at $p < 0.001$; stomatal conductance, chlorophyll fluorescence, and K content at $p < 0.01$; and Ca and Cu content at $p < 0.05$.

Considering the interaction of the examined factors, Cultivation × irrigation practice affected significantly N, K, Mg, and Na content at $p < 0.001$ and DPPH, essential oil yield, and eucalyptol at $p < 0.05$. Cultivation practice × harvesting affected significantly Na content at $p < 0.001$; height, Mg content, and essential oil yield at $p < 0.01$; and stomatal conductance, SPAD, and DPPH at $p < 0.05$. Irrigation practice × harvesting affected significantly N, K, and Na content and D-limonene at $p < 0.001$; Mg content, eucalyptol, and monoterpenes hydrocarbons at $p < 0.01$; and stomatal conductance, SPAD, essential oil yield, carvone, and sesquiterpene hydrocarbons at $p < 0.05$. Cultivation x irrigation × harvesting affected significantly N and K content and monoterpenes hydrocarbons at $p < 0.001$; Mg content, D-limonene, carvone, and sesquiterpene hydrocarbons at $p < 0.01$; and height at $p < 0.05$.

The effect of the different cultivation practices along with the two irrigation regimes on plant growth are illustrated on Table 2. When conventional fertilization was applied together with full irrigation, plant height and fresh weight were higher compared to plants that were cultivated under different cultivation schemes, at the first harvest. At the second harvest, the same treatment resulted in increased biomass (heavier plants), compared to the organic-deficit plan, but the taller plants appeared after the application of organic cultivation together with full irrigation, followed by both conventional treatments (CI and CD). Organic spearmint from deficit irrigation system had higher dry matter content compared to full irrigation regimes (CI and OI), after the first harvest. After the second harvest though, the plants subjected to conventional/deficit plan had increased dry matter, which appeared increased by 11.5% from both organic cultivation plans (OI and OD) and by 27% from the irrigated conventional plan.

Table 1. Effects of cultivation plan (conventional—C or organic—O), irrigation regime (full—I or deficit—D), and harvesting (first harvest or second harvest) on spearmint plant growth, physiology, nutrient content, and essential oil yield and composition.

Three-Way Anova	Cultivation	Irrigation	Harvesting	Cult. × Irrig.	Cult. × Harv.	Irrig. × Harv.	Cult. × Irrig. × Harv.
Height (cm)	ns	ns	ns	ns	**	ns	*
Fresh weight—Fw (g)	ns	ns	ns	ns	ns	ns	ns
Dry matter content (%)	ns	**	ns	ns	ns	ns	ns
Stomatal conductance (cm/s)	ns	*	**	ns	*	*	ns
Chlorophyll fluorescence Fv/Fm	ns	ns	**	ns	ns	ns	ns
SPAD	*	ns	ns	ns	*	*	ns
Total phenols (μmol GAE/g Fw)	ns	ns	***	ns	ns	ns	ns
total flavonoids (mg Rutin/g Fw)	*	ns	***	ns	ns	ns	ns
ABTS (mg Trolox/g Fw)	ns	ns	***	ns	ns	ns	ns
DPPH (mg Trolox/g Fw)	**	**	***	*	*	ns	ns
FRAP (mg Trolox/g Fw)	ns	ns	***	ns	ns	ns	ns
N (g/kg)	***	ns	***	***	ns	***	***
K (g/kg)	*	***	**	***	ns	***	***
P (g/kg)	*	ns	ns	ns	ns	ns	ns
Mg (g/kg)	ns	ns	ns	***	**	**	**
Ca (g/kg)	ns	ns	*	ns	ns	ns	ns
Na (g/kg)	***	***	***	***	***	***	ns
Zn (mg/kg)	***	ns	ns	ns	ns	ns	ns
Cu (mg/kg)	ns	ns	*	ns	ns	ns	ns
Essential oil yield (%)	ns	ns	ns	*	**	*	ns
D-Limonene (%)	***	**	***	ns	ns	***	**
Eucalyptol (%)	ns	ns	***	*	ns	**	ns
Carvone (%)	***	**	***	ns	ns	*	**
Monoterpenes hydrocarbons (%)	***	*	***	ns	ns	**	***
Sesquiterpenes hydrocarbons (%)	***	ns	***	ns	ns	*	**
Oxygenated monoterpenes (%)	ns	ns	***	ns	ns	ns	ns
Oxygenated sesquiterpenes (%)	ns	ns	***	ns	ns	ns	ns

*, **, *** Significant difference at $p \leq 5\%$, 1%, and 0.1% following three-way ANOVA. ns: non-significant.

The response of spearmint's photosynthetic system to the different treatments applied during the cultivation period is presented in Table 3. Right before the first harvest, leaf stomatal conductance was measured higher in conventionally and deficit irrigated plants, compared to the organic/deficit ones. Chlorophyll fluorescence remained unaffected, but levels of chlorophyll in terms of SPAD measurement were higher when conventional fertilizers together were applied with full irrigation, and the lowest levels were measured at the organic/deficit irrigated plants. Before the second harvest, conventionally and irrigated plants had the highest stomatal conductance, followed by organically cultivated and irrigated plants, while the lowest values were found at the deficit irrigated plants of the organic treatment. Organic/deficit irrigated (OD) plants, though, exhibited higher levels of chlorophyll fluorescence compared to the conventional/irrigated ones. SPAD values were also affected after the second cultivation period, and were increased at the conventionally grown plants with the deficit irrigation, compared to both the irrigated cultivation plans.

Table 2. Effect of cultivation plan (conventional—C or organic—O) and irrigation regime (full—I or deficit—D) on spearmint plants growth and essential oils (EO) yields under two harvestings.

Treatment		**Plant Height (cm)**	**Fresh Weight (g)**	**Dry Matter Content (%)**	**EO Yield (%)**
Cultivation/Irrigation Plan		1st harvest			
Conventional/full	CI	44.16 ± 1.46 a	65.31 ± 8.81 a	25.97 ± 0.91 bc	1.96 ± 0.11 a
Conventional/deficit	CD	34.14 ± 1.36 b	45.27 ± 3.70 b	27.37 ± 0.33 ab	2.24 ± 0.12 a
Organic/full	OI	34.41 ± 2.20 b	38.12 ± 3.07 b	24.54 ± 0.47 c	2.47 ± 0.10 a
Organic/deficit	OD	33.50 ± 2.04 b	35.32 ± 3.35 b	28.19 ± 0.69 a	2.54 ± 0.35 a
		2nd harvest			
Conventional/full	CI	26.70 ± 1.63 c	74.59 ± 4.62 a	22.71 ± 0.73 c	2.85 ± 0.06 a
Conventional/deficit	CD	29.66 ± 1.63 c	58.73 ± 6.21 ab	29.11 ± 0.17 a	2.41 ± 0.17 b
Organic/full	OI	43.50 ± 2.12 a	69.78 ± 9.56 ab	26.28 ± 0.94 b	2.12 ± 0.05 b
Organic/deficit	OD	35.83 ± 2.35 b	48.67 ± 5.87 b	26.62 ± 0.93 b	2.38 ± 0.06 b

Values (n = 6 for plant growth; n = 4 for oil yields) in column for each harvest followed by the same letter are not significantly different.

Table 3. Effect of cultivation plan (conventional—C or organic—O) and irrigation regime (full—I or deficit—D) on spearmint plants physiology parameters, under two harvestings.

Treatment		**Stomatal Conductance (cm/s)**	**Chlorophyll Fluorescence Fv/Fm**	**SPAD**
Cultivation/Irrigation Plan		1st harvest		
Conventional/full	CI	0.791 ± 0.07 ab	0.776 ± 0.011 a	46.18 ± 0.99 a
Conventional/deficit	CD	0.940 ± 0.07 a	0.775 ± 0.005 a	47.45 ± 1.77 ab
Organic/full	OI	0.877 ± 0.07 ab	0.775 ± 0.006 a	39.93 ± 1.26 bc
Organic/deficit	OD	0.684 ± 0.08 b	0.788 ± 0.011 a	35.40 ± 2.13 c
		2nd harvest		
Conventional/full	CI	2.451 ± 0.231 a	0.809 ± 0.001 b	42.73 ± 1.79 b
Conventional/deficit	CD	1.393 ± 0.256 bc	0.819 ± 0.007 ab	47.45 ± 0.82 a
Organic/full	OI	1.788 ± 0.190 b	0.810 ± 0.002 ab	42.70 ± 1.11 b
Organic/deficit	OD	0.984 ± 0.113 c	0.823 ± 0.003 a	45.11 ± 0.57 ab

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

The results of leaf nutrient analysis are illustrated in Figure 1A–H for both first and second harvest. At the first harvest, deficit irrigation increased N, P, Mg, and Cu (Figure 1A,C,D,H), but decreased K and Na (Figure 1B,F) content in conventional cultivation practice when compared to the relevant fully irrigated plants, while no difference was observed on Ca and Zn content (Figure 1E,G). Additionally, deficit irrigation decreased N, P, and Na content in organic cultivation when compared to the fully irrigated plants (Figure 1A,C,F). Spearmint grown in organic cultivation revealed higher N, P, Mg, Na, Zn, and Cu, but lower K, when compared to the relevant plants grown in conventional cultivation and applied full irrigation scheme.

At the second harvest, deficit irrigation decreased N, K, and P (Figure 1A–C), but increased Na (Figure 1F) content in conventional cultivation, while deficit irrigation decreased N but increased Na content in organic cultivation (Figure 1A,F). Spearmint grown in organic cultivation revealed decreased N, K, and P, but increased Na and Zn in comparison to the relevant plants grown in conventional cultivation and applied full irrigation scheme. No differences were found on Ca content averaged in 16.02 g/kg and Cu content averaged in 330.91 mg/kg (Figure 1E,H).

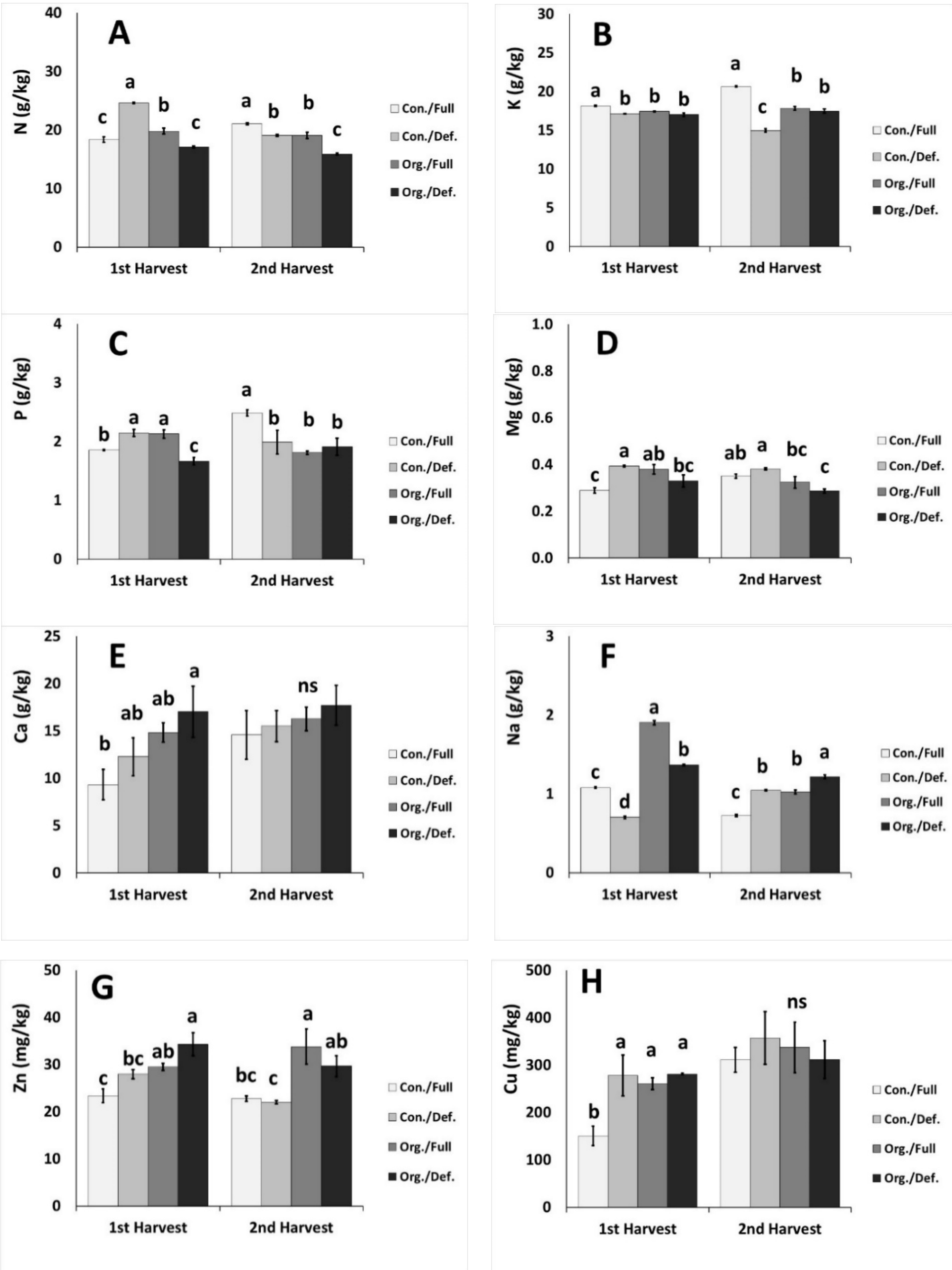

Figure 1. Effect of cultivation (conventional/organic) and irrigation (full/deficit irrigation) plans on the concentration of nutrients in spearmint leaves. Values represent mean (±SE) of measurements made on four independent replications per treatment. (**A**) nitrogen—N, (**B**) potassium—K, (**C**) phosphorus—P, (**D**) magnesium—Mg, (**E**) calcium—Ca, (**F**) sodium—Na, (**G**) zinc—Zn, and (**H**) copper—Cu. Mean values followed by the same letter do not differ significantly at $p \geq 0.05$ according to Duncan's MRT. ns indicates non-significant.

Total phenolic content, flavonoids, and antioxidant activity of spearmint's leaf extracts are presented in Table 4. Extracts from plants of the first harvest revealed no significant differences in total phenolic compounds, total flavonoids, and antioxidant activity when assayed with ABTS and FRAP. The DPPH assay though, showed that deficit irrigation plan of the organic cultivation resulted in the higher antioxidant activity of the tested extracts at 60.11 mg Trolox/g Fw, followed by the deficit/conventional plan (43.69 mg Trolox/g Fw). Full irrigated spearmint plants had the lowest activity in terms of DPPH, at 23.65 and 26.87 mg Trolox/g Fw, at the conventional and organic plan, respectively. After the second harvest, phenolic, flavonoid compounds and antioxidant activity followed a uniform trend; organic cultivated plants appeared to have the highest content compared to conventionally cultivated plants, regardless of the irrigation regime (with the exception of the DPPH in which deficit irrigation increased the DPPH values compared to the full irrigation under organic cultivation plan).

Table 4. Effect of cultivation (conventional—C or organic—O) and irrigation regime (full—I or deficit—D) practices on spearmint 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 harvesting periods.

Compound		Total Phenols	Total Flavonoids	ABTS	DPPH	FRAP
				1st harvest		
Conventional/Full	CI	384.17 ± 45.34 a	24.99 ± 1.63 a	45.81 ± 5.06 a	23.65 ± 4.41 c	176.70 ± 30.76 a
Conventional/Deficit	CD	399.45 ± 11.83 a	24.30 ± 1.13 a	36.19 ± 1.35 a	43.69 ± 4.00 b	150.49 ± 10.41 a
Organic/Full	OI	414.56 ± 48.56 a	27.96 ± 1.95 a	35.44 ± 1.30 a	26.87 ± 3.96 c	169.15 ± 7.83 a
Organic/Deficit	OD	444.72 ± 28.67 a	29.18 ± 1.29 a	37.83 ± 3.62 a	60.11 ± 4.81 a	200.36 ± 8.47 a
				2nd harvest		
Conventional/Full	CI	75.62 ± 2.34 b	7.39 ± 0.20 b	8.94 ± 0.30 b	53.40 ± 6.74 c	27.20 ± 1.61 b
Conventional/Deficit	CD	75.36 ± 2.60 b	7.15 ± 0.36 b	8.20 ± 0.47 b	62.87 ± 1.16 c	28.12 ± 1.25 b
Organic/Full	OI	95.22 ± 1.89 a	11.15 ± 0.41 a	10.77 ± 0.39 a	82.01 ± 2.36 b	43.54 ± 3.16 a
Organic/Deficit	OD	90.69 ± 5.60 a	9.88 ± 0.81 a	10.61 ± 0.38 a	102.83 ± 7.58 a	37.87 ± 1.23 a

Values (n = 4) in column for each harvest followed by the same letter are not significantly different.

Essential oil yield of spearmint plants of the first harvest was not affected by the different cultivation (organic or conventional) and irrigation (full or deficit) practices applied during the experiment, and was averaged at 2.30% (Table 2). Irrigated/conventionally cultivated plants from the second harvest had the highest oil yield (2.85%), compared to the other treatments of the second harvest, averaged at 2.30% (Table 2).

Essential oil compound composition is presented on Table 5. For both harvesting periods, the major components (>1%) of the spearmint oil were carvone, limonene, eucalyptol, β-pinene, and β-caryophyllene. The compounds of the essential oils from plants from the first harvest were affected by the cultivation plan; carvone had higher participation in the oil profile at the conventionally cultivated plants (at both irrigation regimes), while limonene had the lowest percentage, compared to the organic plants. Deficit irrigation increased eucalyptol content when compared to the full irrigation treatment for the conventionally grown plants; however, eucalyptol levels did not differ in the organic oils in both irrigation plans. As for the second harvest, carvone's percentage had its lowest value at the deficit irrigated/organic plants, compared to the oils from plants cultivated under all other regimes, while the reverse was revealed for limonene. In addition, eucalyptol was higher at the fully irrigated organic plants.

Table 5. Chemical composition (%) of essential oils of spearmint 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 Pinene	933	0.847 b	0.944 ab	0.999 a	0.980 a	0.899 a	0.772 b	0.856 a	0.870 a
Camphene	948	0.066 a	0.073 a	0.068 a	0.067 a	0.046 a	0.041 c	0.044 ab	0.042 bc
Sabinene	973	0.628 a	0.681 b	0.687 b	0.675 b	0.651 a	0.580 b	0.629 a	0.605 b
β Pinene	977	**1.301 b**	**1.376 ab**	**1.390 a**	**1.386 a**	**1.201 a**	**1.088 b**	**1.204 a**	**1.159 b**
β Myrcene	989	0.712 b	0.741 ab	0.767 a	0.709 b	0.727 a	0.626 b	0.634 b	0.619 b
3 Octanol	1003	0.168 a	0.133 b	0.139 b	0.102 c	0.105 a	0.098 ab	0.102 a	0.085 b
α Terpinene	1005	0.044 a	0.040 a	0.042 a	0.044 a	-	-	-	-
D-Limonene	1028	**11.687 b**	**12.253 b**	**14.603 a**	**13.354 a**	**15.140 c**	**16.149 b**	**15.670 c**	**18.594 a**
Eucalyptol	1031	**6.042 b**	**6.869 a**	**6.516 ab**	**6.545 ab**	**4.764 b**	**4.626 b**	**5.004 a**	**4.517 b**
β Ocimene	1036	0.142 a	0.142 a	0.129 ab	0.127 b	0.062 a	0.054 ab	0.051 b	0.056 b
trans β Ocimene	1046	0.024 b	0.059 a	0.043 ab	0.041 ab	0.063 b	0.069 b	0.066 b	0.082 a
γ Terpinene	1058	0.102 a	0.085 a	0.094 a	0.100 a	0.044 a	0.035 a	0.036 a	0.022 a
cis Sabinene hydrate	1067	0.384 b	0.365 ab	0.333 b	0.407 a	0.171 a	0.161 a	0.145 a	0.162 a
iso Menthone	1164	0.069 b	0.088 a	0.078 ab	0.069 b	0.133 b	0.144 a	0.133 b	0.149 a
Borneol	1166	0.372 a	0.391 a	0.333 b	0.313 b	0.198 a	0.184 a	0.170 ab	0.155 b
Menthol	1175	-	-	-	-	0.156 c	0.252 b	0.185 c	0.332 a
Terpinen 4 ol	1178	0.315 a	0.313 a	0.295 ab	0.247 b	0.150 a	0.114 ab	0.125 a	0.058 b
α Terpineol	1191	0.226 b	0.259 a	0.251 a	0.218 b	0.219 a	0.200 a	0.211 a	0.163 b
Dihydro carveol	1993	0.511 b	0.290 c	0.292 c	0.621 a	-	-	-	-
neo Dihydro carveol	1995	0.460 b	0.414 c	0.409 c	0.505 a	0.290 a	0.269 b	0.294 a	0.300 a
trans Carveol	1219	0.495 a	0.293 b	0.236 b	0.172 b	0.024 a	0.000 a	0.000 a	0.000 a
cis Carveol	1231	0.569 b	0.475 bc	0.384 c	0.764 a	-	-	-	-
Pulegone	1240	0.525 ab	0.535 a	0.509 ab	0.475 b	0.818 d	1.066 b	0.868 c	1.188 a
Carvone	1244	**70.290 a**	**69.285 a**	**67.626 b**	**68.260 b**	**71.582 a**	**71.049 a**	**71.051 a**	**68.173 b**
iso Dihydro carveol acetate	1326	0.064 b	0.040 bc	0.027 c	0.159 a	-	-	-	-
cis Carvyl acetate	1361	0.100 b	0.088 b	0.059 b	0.234 a	-	-	-	-
β Bourbonene	1386	0.679 a	0.757 a	0.684 a	0.677 a	0.463 c	0.511 b	0.536 ab	0.564 a
β Elemene	1393	0.217 a	0.221 a	0.215 a	0.184 a	0.106 a	0.074 b	0.061 b	0.078 b
β Caryophyllene	1425	**1.098 a**	**1.061 a**	**1.093 a**	**1.057 a**	**0.832 a**	**0.749 a**	**0.830 a**	**0.827 a**
Germacrene D	1497	0.537 a	0.439 a	0.475 a	0.388 a	-	-	-	-
Bicyclogermacrene	1512	0.230 a	0.207 a	0.237 a	0.178 a	0.138 a	0.069 b	0.074 b	0.068 b
Germacrene A	1519	0.075 a	0.048 a	0.072 a	0.054 a	-	-	-	-
trans Calamene	1534	0.204 a	0.218 a	0.204 a	0.206 a	0.227 a	0.231 a	0.253 a	0.249 a
Cubenol 1,10 di epi	1617	0.124 a	0.122 a	0.103 a	0.132 a	0.086 a	0.094 a	0.095 a	0.105 a
α Cadinol	1657	0.136 a	0.113 a	0.099 a	0.102 a	0.049 a	0.059 a	0.039 a	0.049 a
Mint sulfide	1737	-	-	-	-	0.093 a	0.064 a	0.078 a	0.063 a
Total Identified		99.509 ab	99.425 b	99.495 ab	99.498 a	99.452 a	99.424 a	99.451 a	99.340 b
Monoterpenes hydrocarbons		15.603 c	16.395 bc	18.822 a	17.485 ab	18.834 b	19.406 b	19.192 b	22.056 a
Sesquiterpenes hydrocarbons		3.041 a	2.952 a	2.981 a	2.747 a	1.775 a	1.634 a	1.755 a	1.783 a
Oxygenated monoterpenes		80.271 a	79.579 a	77.263 b	78.600 ab	78.508 a	78.068 a	78.188 a	75.199 b
Oxygenated sesquiterpenes		0.260 a	0.235 a	0.203 a	0.235 a	0.135 a	0.153 a	0.134 a	0.155 a
Others		0.332 b	0.262 bc	0.225 c	0.496 a	0.198 a	0.162 ab	0.181 ab	0.148 b

Values (n = 4) in rows for each harvest followed by the same letter are not significantly different, $p \leq 0.05$. Bold highlighted values represent components with average ≥1.0%. RI represents the retention indices of the compounds on a ZB-5 column in reference to n-alkanes (C8–C20).

Finally, the levels of several of the remaining constituents of the EOs were also affected by the different cultivation and irrigation practices implemented in this study, but their percentage was too low (less than 1%) to alter the total oil profile. The most abundant group of components in all cases was the oxygenated monoterpenes group, followed by monoterpenes hydrocarbons. At the essential oils of the first harvest, the total percentage of the oxygenated monoterpenes was higher at the conventionally cultivated plants and exhibited the lowest values at the organically/full irrigated plants. The percentage of the monoterpenes hydrocarbons followed the opposite trend, where they had the lowest values at the conventionally cultivated plants. As for the second harvest, oxygenated monoterpenes had the lowest value of 75.199% at the organically grown plants that were cultivated under deficit irrigation, whilst exhibiting the highest value (22.056%) of the total monoterpenes hydrocarbons.

3.1. Electroantennographic Response of Female L. botrana Adult Insects

Volatile compounds from all EOs elicited strong EAG response on female *L. botrana* antennae (Figure 2). There was no significantly distinct difference noted among them. Their responses were at the same level as that of octanol, which was used as a reference stimulus (F = 19.591, df = 10, p = 0.000). R-(–)-carvone alone, which is the major constituent (~70%) in all EO, elicited a level of response that was roughly half of that of the EOs responses and was comparable to the one caused by the female sex pheromone. the observed antennal responses to non-host plant constituents were strong enough to potentially disrupt the olfactory process in finding a suitable host by masking or exerting a deterrent or repellent effect.

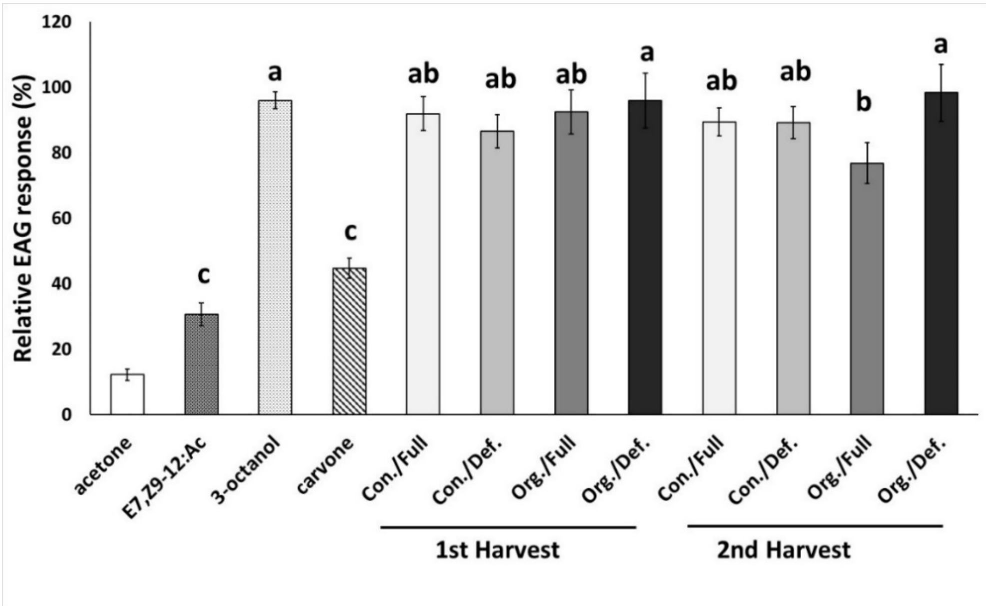

Figure 2. Mean electrophysiological responses of male *L. botrana* antennae to spearmint essential oils, E7,Z9-12:Ac, 3-octanol, and carvone. Spearmint plants were subjected to different cultivation (conventional/organic) and irrigation (full/deficit irrigation) plans. All stimuli were tested at a 50 μg dose. Means (± SE) followed by the same letter are not significantly different (Duncan's multiple range tests (MRT) at $p < 0.05$, F = 19.591, df = 10, p = 0.000).

3.2. Larvicidal Bioassays

The average weight of the fifth instar larvae used in the bioassays was 10.42 ± 0.35 mg. Results revealed that *M. spicata* EOs had larvicidal activity on *L. botrana*, displaying a significant LD_{50} value on topical application that ranged from 47 to 57.7 μg (Table 6). All EOs displayed the same level toxic effects on larvae.

Table 6. Larvicidal activity of the EOs recorded at 24 h after topical application. LD values are expressed in µg; they are considered significantly different when 95% CL fail to overlap. Since goodness-of-fit test is significant ($p < 0.05$), a heterogeneity factor is used in the calculation of confidence limits (CL).

Essential Oil		LD_{50}	CL 95%	Slope ± SE	Intercept ± SE	x^2	*p*
				1st harvest			
Conventional/Full	CI	52.11	41.23–61.75	0.03 ± 0.001	−1.45 ± 0.09	147.20	0.000
Conventional/Deficit	CD	53.29	44.76–61.90	0.03 ± 0.001	−1.70 ± 0.83	159.16	0.000
Organic/Full	OI	50.84	42.45–59.02	0.03 ± 0.001	−1.67 ± 0.08	151.53	0.000
Organic/Deficit	OD	57.72	52.96–62.40	0.03 ± 0.001	−1.73 ± 0.08	53.78	0.000
				2nd harvest			
Conventional/Full	CI	51.26	39.73–61.61	0.03 ± 0.001	−1.44 ± 0.08	225.08	0.000
Conventional/Deficit	CD	47.03	33.49–53.59	0.03 ± 0.001	−1.35 ± 0.08	244.57	0.000
Organic/Full	OI	51.96	42.00–61.86	0.04 ± 0.001	−1.86 ± 0.09	235.51	0.000
Organic/Deficit	OD	51.43	44.99–57.36	0.03 ± 0.001	−1.46 ± 0.08	79.50	0.000

4. Discussion

Results of the present study demonstrate that cultivation practices diversely affect the growth, physiology, and quality of spearmint plants and their secondary metabolites biosynthesis. Growth parameters were affected by the cultivation scheme. Plants were higher and had increased fresh weight mass when they were fully irrigated at a conventional cultivation scheme. The same trend for the fresh biomass was observed at the second harvest as well, while fully irrigated organically grown plants exhibited increment in biomass production, indicating the importance of the sufficient irrigation on plant growth performance and nutrient absorption. On the other hand, Osakabe et al. [41] reported that water stress causes signaling changes in abscisic acid, changes in ion transport, and decreases in leaf stomatal conductivity. These reports are confirmed by our observations in this work for the second harvest, where stomatal conductance was reduced in deficit-treated compared to fully irrigated plants. Similarly, stomatal conductance decline has been reported when *Sideritis perfoliata* plants were subjected to deficit irrigation [5]. Under water-stressed conditions, stomatal conductance decreases due to the closure of stomata to maintain the leaf water status. Stomata play a key role in regulating the flow of water in the soil–plant system. Stomatal adjustments help to maintain plant water status under varying soil moisture and atmospheric conditions. Nevertheless, there are reports on mechanism responsible for stomatal closure, with studies endorsing that chemical or hydraulic signals are responsible for the stomatal closure, or the combination of both in our case [9]. As for the chlorophyll content, medicinal plants respond differently, as increases in content have been mentioned for *Matricaria chamomilla* (L.) when subjected to mild water stress [42], while *Salvia fruticosa* (L.) exhibits a decline in chlorophyll content when exposed to a mild water stress [21].

As for the antioxidant activity and the total phenolic content, they did not differ significantly after the cultivation practices applied at the first harvest. These findings are in agreement with previous studies on *Lavandula angustifolia* where plants were subjected to different levels of water stress [21] or peppermint and cinnamon plants grown in conventional and organic farming systems [14], but results in literature are contradicting. Samples collected at the second harvest revealed a significant higher antioxidant activity and phenolic and flavonoid content of the extracts from plants that were grown organically, whereas DPPH activity was increased in organic deficit irrigation compared to full irrigation scheme, being in accordance with previous studies in *S. fructicosa* when subjected to severe water stress [21]. There are reports that indicate an increase in phenolic compounds and antioxidant activity in organically grown medicinal plants such as rosemary and lemon balm [43,44] and this increase is attributed to the fertilization plan and available nutrients to the plants. In organic farming, nitrogen is provided mainly in organic form, and only a part of that is bioavailable to support plants, indicating N-deprived levels for the plants that account for poor plant development, chlorophyll reduction, and low photosynthetic rate [45]. According to the C/N theory [46], the increased nitrogen may produce more metabolites with high nitrogen content in the form of proteins and free amino acids, while the production of compounds as flavonoids and phenolics may be reduced [44]. On this basis, the conventionally grown spearmint had less bio-compounds as measured by total phenols, flavonoids, and antioxidant capacity of the plants, compared to the relevant plants grown under organic farming. The decreased levels of N found in the conventionally grown plants are reflected in the increased levels of phenolics, flavonoids, and antioxidants found in the plants of the second harvest. Nitrogen shortage drives plants to diversification, increasing secondary metabolism including the overproduction of defense components as phenolics and antioxidant compounds [47].

Nutrient accumulation in plant tissue is associated to several growth-related factors, including the available water in soil and the climatic conditions, as both cations and anions absorption by the plant depend on those parameters. Monovalent cations, such as K^+ and Na^+, are quite mobile compared to higher valency cations (Ca^{2+}, Mg^{2+}, etc.) and were accumulated at a higher quantity in plant tissue when the plants were subjected to full compared to deficit irrigation, under a conventional scheme, while other elements/nutrients such as Mg, N, P, and Cu were accumulated more in deficit compared to the full irrigation treatment.

At the first harvest, the essential oil yield remained unaffected by the different treatments. Similar findings have been previously reported for *M. spicata* plants, where the essential oil yield was not affected when the plants were subjected to different abiotic stresses [24]. At the second harvest, conventionally grown plants at full irrigation regime exhibited the highest essential oil yield, compared to the deficit irrigated or the organically cultivated plants, followed by the N content and fresh biomass increases that were found in the same treatment. However, the increased crop yield of MAPs under conventional intensive farming is not always reflected in increased product quality [44]. Different levels of fertilizers may have an impact on the essential oil yield in spearmint, as it has been described previously [23]. Results are in agreement with reports on peppermint from Okwany et al. [48], in which essential oil yield remained unchanged across different irrigation regimes at the first harvest, but the deficit regimes revealed a decrease in yield, potentially due to the decreasing trend in fresh mass production.

The major constituents identified among *Mentha* species are R-(–)-carvone, limonene, 1,8-cineole (eucalyptol), pulegone, menthone, menthol, and β-caryophyllene, with R-(–)-carvone being the predominant compound of spearmint (*M. spicata*) essential oil, ranging from 35–65% of the total oil composition and being responsible for the distinctive smell of the herb [33,49–51]. Even though it is mentioned in the literature that irrigation may affect the content of carvone in spearmint oil [52], this was evident only at the second harvest, for the organically grown plants. Indeed, deficit irrigation seems to delay the formation of carvone from limonene (through *trans*-carveol), delaying the terpenes biosynthesis and giving at the same time the highest content of limonene. At the first harvest, the delay in the formation of carvone comes from the fertilization plan. As we have demonstrated in previous studies [24], spearmint plants subjected to abiotic stresses delay the formation of carvone. In any case, the carvone content was averaged at 68.8% at the first harvest and 70.1% at the second harvest, a high carvone content that represents an oil of high quality [53]. An improvement of carvone's content in oil, which will have a direct impact on oil's biological properties [23], has been proven that can be achieved by optimizing the fertigation of spearmint plants [54], as is highlighted in the present study.

Phytochemicals are important olfactory cues for the location of hosts by moths, such as *L. botrana*, one of the most harmful pests for vineyards worldwide with major economic impact on the viticulture industry. These insects need to accurately discriminate different chemical profiles according to seasonal progression and make the correct behavioral selection for their progeny. Limonene, eucalyptol, and β-caryophyllene have been found to elicit electrophysiological responses and attract *L. botrana* adults [55,56]. It has also been shown that this attraction effect is stronger when β-caryophyllene is part of a blend than as a pure compound, reflecting the behavioral flexibility in oviposition preference of a polyphagous insect such as *L. botrana* [56–58].

M. spicata constituents may also have insecticidal or repellent effects on *L. botrana*. Several studies reported that *M. spicata* essential oil shows excellent fumigant toxicity against several storage insect species of the order *Coleoptera* [59–61], as well as repellent, larvicidal, and ovicidal activity on Dipteran insects, largely from the *Culicidae* family and the *Drosophila* genus [62,63]. Similar activities of *M. spicata* oil have been reported for *Lepidopteran* species, such as *Plutella xylostella*, *Ephestia kuehniella* (Zeller), and *Plodia interpunctella* (Hübner) [64]. Notably, some of these reports have linked the richness of *M. spicata* oil in R-(−)-carvone with insect repellent, fumigant, and contact toxicity, often in a synergistic mode with other compounds such as pulegone, menthone, or eucalyptol [59,60,65]. Limonene, pulegone, menthone, menthol, and eucalyptol are also well known for their insecticidal and repellent activity [33,60,65–69].

It has been established that *M. spicata* EO constituents have insecticidal properties. It has also been shown that EO volatiles are well perceived by the antennae of adults. Since many of the compounds present in the blend have been reported to attract *L. botrana* adults, it may be suggested that this herb has the potential to assist in vineyard protection in two possible modes: (1) as a cover crop, where some of the volatiles constituents attract egg-laying females and encourage oviposition, while others contribute to egg and larval mortality, thus reducing infestation in grapevines, as has been reported for other Lepidoptera species [70] or (2) as an essential-oil-based product aiming to contribute to pest management. In the context of increasing the adoption of sustainable agricultural practices such as integrated pest management, organic cultivation, or deficit irrigation schemes, the use of other plant species (intercropping, side-cropping, etc.), such as *M. spicata*, can potentially reinforce the efforts to protect vineyards against the grapevine moth with greener and environmentally friendly approaches, either by increasing biodiversity or by using essential oil-based products. Ideally, organically produced spearmint plants combined with a deficit irrigation scheme would contribute towards a sustainable strategy for vineyard protection against *L. botrana*. In this work, we demonstrated that different cultivation and irrigation treatments, as well as harvesting periods, had an effect on the levels of several bio-active constituents in *M. spicata* essential oils. Our electrophysiological and toxicological results showed no significant differences among EOs of differently treated *M. spicata* plants.

Although the exact modes of action on both the olfactory response and toxicity of *M. spicata* essential oil constituents remain unclear, varying levels of the major components of the oil made no difference to the overall insect response. Perhaps, R-(−)-carvone, which is the predominant component in all samples (~70%), may be the main or sole contributor to the observed electrophysiological responses and larvicidal effects on *L. botrana*, thus providing an explanation for the uniformity of results. In any case, these findings suggest that, despite the differences in essential oil yield and composition between different cultivation and irrigation regimes, organically grown *M. spicata* plants combined with a deficit irrigation plan can be as good an option as conventional ones, as part of a strategy to protect vineyards in a more sustainable and environmental-friendly manner.

5. Conclusions

The present work examined the performance of spearmint plants under different cultivation practices, the conventional vs. organic and the full vs. deficit irrigation practices on the plant growth and physiology, and the biosynthesis and bioactivity of their secondary metabolites. Differences between cultivation schemes were observed only at the second harvest, where the total phenols and flavonoids, as well as the antioxidant capacity, were increased in the organically grown plants, regardless of the irrigation plan, highlighting a possible induced stress caused by the organic and/or deficit irrigation practices. Moreover, nutrient accumulation in plant tissues is affected, with N losses in organic cultivation, while other nutrients, i.e., K, Na, Mg, P, and Cu were affected by the irrigation scheme applied (full vs. deficit). However, conventional and full-irrigated plants had a beneficial effect on plant growth-related parameters, i.e., height and fresh biomass at the first harvest. Essential oil content was affected at the second harvest with decreased content in organic and/or deficit treated plants. Deficit irrigation seems to delay plant development and terpene biosynthesis for the formation of carvone from limonene.

The effects of plant extracts and/or essential oils from MAPs subjected to different cultivation management under the pressure of climate change are of increasing interest and are thus important to be further evaluated. In addition, MAPs may possess bioactive properties that could be used to contribute to integrated pest management. Therefore, combining spearmint intercropping or side-cropping in vineyards with organic cultivation or deficit irrigation schemes can potentially reinforce the efforts to protect vineyards against the grapevine moth with greener and environmentally friendly approaches. Our findings have shown that MAPs (such as *M. spicata*), which are low demanding crops if grown organically in combination with a deficit irrigation plan, can be as good an option as conventional ones for a more sustainable farming management strategy.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/3/599/s1, Table S1: Climatic conditions during the experimental study, Table S2: Fertilizers and crop protection means applied during the experimental study.

Author Contributions: A.C. carried out the crop cultivation, physiology assessments, and essential oil and nutrient analysis; P.X. carried out the biological protocols; E.K. and A.F. carried out the insect rearing, the bioassays, and the electroantennographic recordings; M.K. supervised the entomological analysis; N.T. supervised the agronomical/biochemical analyses. N.T. conceived and designed the experiments. A.C., E.K., M.K. and N.T. contributed to the writing of the paper. All authors have read and agreed to the published version of the manuscript.

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