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THE INFLUENCE OF THE SPECIES' CULTIVATION SYSTEM AND THE HARVESTING STAGE ON SOME QUALITY ELEMENTS IN MENTHA SPP.

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ABSTRACT

This paper presents the results of a study conducted on the genus Mentha ssp (Menta piperita and Menta spicata) cultivated in two different periods and in two different systems. Fertilizations were applied to the crop and irrigation was performed as often as the plants needed. The essential oil content and its composition were determined as well as the fresh and dried harvest quantity. After drying, an analysis was carried out by gas chromatography-mass spectrometry (GC-MS) performed on an Agilent Technologies 7890A gas chromatograph, equipped with an HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm), connected in series with an Agilent Technologies 5975C mass spectrometer. The ionization method was electron beam bombardment at 70 eV (EIMS).

INTRODUCTION

Aromatic and medicinal plants are highly valued for their antioxidant and biocidal activities. However, there is a wide variation in these activities, which is determined by the species, climatic conditions (temperature, UV radiation and light intensity), plant age, applied cultivation technology, cultivation system (conventional or organic) and harvest period.

Plants of the Lamiaceae family are well known both in Greece and throughout the world. The genus Mentha is representative, presenting great interest both for fresh and dried consumption, and has 18 to 30 species, which differ significantly in chemical composition (Moldovan et al. 2014; Ahmad et al. 2011; Hadi et al. 2017). However, not all species and varieties of mint are equally valuable.

These plants with a unique and attractive aroma, from which essential oils are obtained, using various extraction methods, are used for medicinal or food purposes. In addition to being classified according to their morphological characteristics, plants are also classified according to: Their usage (pharmaceutical, aromatic, culinary, ornamental); The content of active substances (oils, tannins, etc.); The form (herb, shrub, tree, etc.); The life span (annual, biennial, perennial); The environment in which they grow; The mode of nutrition (autotrophic, heterotrophic, symbiotic) (Pandey et al. 2020)

Aromatic and medicinal plant crops are irrigated when it is necessary and in a manner that serves the crop. The preferred method is drip irrigation, due to the fact that it provides properly distributed moisture to the area where the roots of the cultivated plants are located and not where the weeds are located. Drip irrigation also saves water, although the equipment is more expensive. Drip irrigation was proven to give the highest yield for zucchini plants (Sałata et al. 2012) and also Purwoceng, which reached 511 grams at the age of 70 days after planting (DAT) (Sumarni et al. 2017). Another method is sprinkler irrigation, which is easy to move between fields, because most pharmaceutical and aromatic plant are irrigated 1-2 times each summer. Numerous aromatic and pharmaceutical plants can also be grown without irrigation.

The management of medicinal and aromatic plant cultivation is very important to balance fundamental plant parameters such as biomass and the production of high quality essential oils and extracts with remarkable properties. Essential oils (EOs) have long been recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Tuțulescu et al. 2016). Nowadays, there is a growing interest in industry, academia, agriculture and health for these plants, due to the significant biological properties that are linked to the presence of a series of compounds (phenols, flavonols/flavonoids, alkaloids, polypeptides, vitamins, catechins, phytoestrogens, carotenoids, chlorophyll, minerals, etc.) (Chrysargyris et al. 2021).

For quantitative and qualitative production, medicinal and aromatic plants also need nutrients, but their survival does not depend on the usage of fertilizers. However, this does not ensure the economic viability of the crop. In order for proper growth to occur, which will result in a quality production, the grower must be sure that his plants have the appropriate amount of nutrition. Over the years, and with the contribution of research, knowledge regarding the fertilization of aromatic and pharmaceutical plants has been enriched. Each grower should carry out a chemical soil analysis in order to know in depth the need of his plot and specific nutrients that will contribute to proper and quality production (Ilias, 2018).

Fresh mint plants have high biological value due to the complex of biologically active substances, such as alkaloids, saponins, organic acids, vitamins, carotenoids, chlorophylls, macronutrients and micronutrients. In particular, in the complex of biologically active substances of mint, phenols, represented by phenolic acids, flavonoids and tannins, take precedence. Other authors have noted the significant antibacterial and fungicidal activity of mint on a wide range of pathogenic microorganisms and fungi (Hutsol, et al. 2023).

The aim of this study was to highlight the importance of peppermint varieties (*Menta piperita* and *Menta spicata*) grown in an irrigated system for essential oil production. It will also highlight the differences between the essential oil content and dry matter of conventional and organic crops and in function of the time of establishment of the crop (May and August).

MATERIALS AND METHODS

The experiment was located in Larissa, located at 39°38.5′N 22°25′E in 2023.

The biological material was represented by 24 rhizomes of Mentha spicata and 24 rhizomes of Mentha piperita, which were purchased from a local agricultural supply store. Each experimental plot has a size of 2x1. Planting was carried out at distances of: 60 cm (~23.62 in) between rows and 30 cm (~11.81 in) between plants

(Figure 1). The experimental plots were delimited with twine. The experiment included the two mint species that were planted in both conventional and organic systems, using early and late varieties (Figure 2). The first planting stage was on June 9, 2023 and refers to the early crop of mint species and includes a1-*Menta spicata* grown in conventional system; a2-*Menta piperita* grown in conventional system; b1- *Menta spicata* grown in organic system; b2-*Menta spicata* cultivated in organic system; Stage aII-took place on August 22, 2023, late crop and includes: c1-*Menta spicata* cultivated in conventional system; c2-Menta piperita cultivated in conventional system and d1-Menta spicata cultivated in organic system; d2-*Menta piperita* cultivated in organic system.

Figure 1. Construction of the experimental plots with a 2 x 1 dimensions.

After planting the rhizomes, a soil conditioner was applied, which came from recycling pre-selected organic matter from the Attica Region. The applied dose was 20 L (676,28 fl. oz) per experimental plot. Regarding the irrigation, umbrella-type mist nozzles were placed along the experimental plots. Irrigation of the experimental plots was carried out 2–3 times per week and always during the evening hours (22:00).

After harvest, the drying of the plants took place in a basement with ideal humidity and temperature conditions. The plants were tied to twine and hung from two pieces of wood for 10 days. They were then stored in basins and the leaves and flowers were separated from the rest of the plant. The distillation of the essential oil was done with the Clevenger hydro-distillation apparatus. Samples of 10 gr (0,35 oz) of dry matter were used, which were mixed with 250 ml (8,45 fl. oz) of water in conical flasks. Then, they were placed in the apparatus and boiled for 105 minutes in order to obtain the essential oil. After receipt, the vials were stored in the laboratory refrigerator to prevent deterioration of their properties.

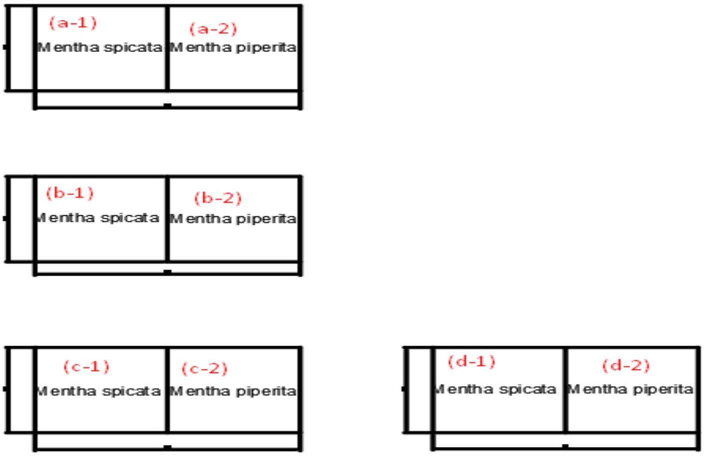

Figure 2. Illustration of experimental plots using AutoCAD2025 software. (a-1) Conventionally grown *Mentha spicata*; (a-2) Conventionally grown *Mentha piperita*; (b-1) Conventionally grown *Mentha spicata*; (b-2) Conventionally grown *Mentha piperita*; (c-1) Organically grown *Mentha spicata*; (c-2) Organically grown *Mentha piperita;* (d-1) Organically grown *Mentha spicata*; (d-2) Organically grown *Mentha piperita.*

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on an Agilent Technologies 7890A gas chromatograph, equipped with an HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm), connected in series with an Agilent Technologies 5975C mass spectrometer. The ionization method was electron beam bombardment at 70 eV (EIMS). A 1 μl aliquot of a 1% solution of essential oil in pentane was injected into the chromatograph with a split ratio of 30:1. The inert gas helium ($_2He$) (1.2 mL/min) was used as the mobile phase. The thermal program used in the analyses of essential oils had an initial temperature of 60 °C (140 °F) where it remained constant for 3 min and then, with a rate of increase of 4 °C/min (39,2 °F/min), it reached 260 °C (500 °F) where it remained constant for 10 min. The inlet temperature was 240 °C (464 °F) and the detection temperature was 250 °C (482 °F). The identification of the chemical components was performed by the comparison of the retention time of each component in relation to the retention times of standard compounds and by studying the mass spectra with the assistance of libraries (Wiley library spectra, NIST/NBS) and literature data (Adams, R. P. 2007). The quantification of the components was based on the total number of component fragments, as detected by the mass spectrometer.

RESULTS AND DISCUSSION

After obtaining the oil, the main components of the oil were determined, such as: n-nonane, α-pinene, sabinene, β-pinene, limonene, 18-cineole, carvone, cubebene, neryl formate, ocimene, myrcene, depending on the species, the cultivation system and the era of establishment of the crop. The results are presented in tables 1-9 and figures 1-3.

Table 1

Results of analysis of Mentha piperita essential oil (1st cycle) using a gas chromatograph.

Mentha piperita 1st cycle		0.020/19.5				
T(exp)	T(adams)	Compound	KI	MW	ΔT	%
5,1	4,93	n-nonane	900	128	0,17	tr
6,06	5,85	α-pinene	939	136	0,21	tr
7,22	6,91	Sabinene	975	136	0,31	0,58
7,31	7,04	β-pinene	979	136	0,27	0,62
7,75	7,43	Myrcene	990	136	0,32	1,67
8,83	8,59	Cymene	1024	136	0,24	tr
8,97	8,69	Limonene	1029	136	0,28	0,98
9,06	8,76	1,8-Cineole	1031	154	0,3	5,8
11,46	11,32	Linalool	1096	154	0,14	30,95
21,07	19,32	Neryl formate	1282	182	1,75	2,88
24,11	24,04	Cubebene	1351	204	0,07	0,75

The analysis of *Mentha piperita* essential oil of the 1st cycle (July collection) showed that the three main components are Linalool (30.95 %), 1,8-Cineole (5.80 %) and Neryl Formate (2.88 %), covering a total percentage of 39.63 % (Table 1).

Table 2

Results of analysis of Mentha piperita essential oil (2nd cycle) using a gas chromatograph.

Mentha piperita 2nd cycle		0.020/13.5				
T(exp)	T(adams)	Compound	KI	MW	ΔT	%
5,11	4,93	n-nonane	900	128	0,18	5,44
7,22	6,91	Sabinene	975	136	0,31	tr
7,31	7,04	β-pinene	979	136	0,27	tr
7,75	7,43	Myrcene	990	136	0,32	1,74
8,97	8,73	Limonene	1029	136	0,24	0,79
9,07	8,76	1,8-Cineole	1031	154	0,31	3,44
9,64	9,42	Ocimene	1037	136	0,22	0,68
11,41	11,32	Linalool	1096	154	0,09	21,78

The analysis of Mentha piperita essential oil of the 2nd cycle (November collection) showed that the three main components are Linalool (21.78 %), n-nonane (5.44 %) and 1,8-Cineole (3.44 %), covering a total percentage of 30.66 % (Table 2).

Table 3

Results of analysis of Mentha spicata essential oil (1st cycle) using a gas chromatograph.

Mentha spicat 1st cycle		0.020/15.5				
T(exp)	T(adams)	Compound	KI	MW	ΔT	%
5,11	4,93	n-nonane	900	128	0,18	1,5
6,06	5,85	α-pinene	939	136	0,21	0,76
7,22	6,91	Sabinene	975	136	0,31	0,55
7,31	7,04	β-pinene	979	136	0,27	0,96
8,97	8,69	Limonene	1029	136	0,28	11,05
9,07	8,76	1,8-Cineole	1031	154	0,31	3,33
18,42	17,53	Carvone	1243	150	0,89	70,5
24,11	24,04	Cubebene	1351	204	0,07	0,83

The analysis of Mentha spicata essential oil of the 1st cycle (July collection) showed that the three main components are Carbone (70.50%), Limonene (11.05%) and 1,8-Cineole (3.33%), covering a total percentage of 84.88% (Tab. 3).

Table 4

Results of analysis of Mentha spicata essential oil (2nd cycle) using a gas chromatograph.

Mentha spicata 2nd cycle		0.020/15.5				
T(exp)	T(adams)	Compound	KI	MW	ΔT	%
5,11	4,93	n-nonane	900	128	0,18	0,55
6,06	5,85	α-pinene	939	136	0,21	0,75
7,22	6,91	Sabinene	975	136	0,31	1
7,32	7,04	β-pinene	979	136	0,28	1,07
7,75	7,43	Myrcene	990	136	0,32	0,81
8,97	8,69	Limonene	1029	136	0,28	7,52
9,07	8,76	1,8-Cineole	1031	136	0,31	7,65
9,29	8,96	Ocimene	1037	136	0,33	0,72
10,27	10,2	Sabinene hydrate	1221	196	0,07	2,32
18,42	17,53	Carvone	1243	150	0,89	55,59

The analysis of *Mentha spicata* essential oil of the 2nd cycle (November collection) showed that the three main components are Carbone (55.59 %), 1,8-Cineole (7.65 %) and Limonene (7.52 %), covering a total percentage of 70.76 % (Table 4).

Regarding the first season of planting, a total of 612 grams (21,58 oz) of fresh (*Menta spicata*) and 168 grams (5,92 oz) of dry product and 450 grams (15,87 oz) of *Menta piperita* resulting from 121 grams (4,26 oz) of dry product.

During the 2nd collection, the fresh and dry weight (after drying) of the products were:

- Conventionally grown mint (*Menta spicata*):
  Fresh Weight at 450 gr (15,87 oz)
  Dry Weight at 78 gr (2,75 oz)
- Organically grown mint (*Menta spicata*):
  Fresh Weight at 1180 gr (41,62 oz)
  Dry Weight at 78 gr (2,75 oz)

- Conventionally grown spearmint (*Menta piperita*)
  Fresh Weight at 1030 gr (36,33 oz)
  Dry Weight at 145 gr (5,11 oz)
- Organically grown spearmint (*Menta piperita*):
  Fresh Weight at 350 gr (12,34 oz)
  Dry Weight at 74 gr (2,61 oz)

The essential oil was obtained from the production of dried mint, in several stages of distillation depending on the harvesting (collection) stage, species, and the cultivation system (tables 5-6)

Table 5

Results of distillation of essential oil of the first (1st) collection

1st collection of experimental (No. 1)	
distillation of *Mentha piperita* **organic** (60 gr, dry matter, 24/10/2023)	
Sample	Essential oil (ml)
Average	0,34
distillation of *Mentha spicata* **organic** (30 gr, dry matter, 25/10/2023)	
Sample	Essential oil (ml)
Average	0.24
distillation of Mentha spicata **conventional** (50 gr, dry matter, 26/10/2023)	
Sample	Essential oil (ml)
Average	0.25
distillation of *Mentha piperita* **conventional** (40 gr, dry matter, 02/11/2023)	
Sample	Essential oil (ml)
Average	0.32
distillation of *Mentha spicata* (20 gr, dry matter, 15/11/2023)	
Sample	Essential oil (ml)
Average	0.1

Table 6

Results of distillation of essential oil of the second (2nd) collection.

2nd collection of experimental (No. 2, No. 3, No. 4)	
Organic *Mentha piperita* (50 gr, 10/01/2024)	
Sample	Essential oil (ml)
Average	**0,18**
Conventional *Mentha piperita* (50 gr, 29/03/2024)	
Sample	Essential oil (ml)
Average	0,16
Organic *Mentha spicata* (50 gr, 30/03/2024)	
Sample	Essential oil (ml)
Average	**0,14**
Conventional *Mentha spicata* (50 gr, 13/04/2024)	
Sample	Essential oil (ml)
Average	0.11

The data in table 5 demonstrate that Menta piperita recorded a higher amount of oil in the organic cultivation system while Menta spicata in the conventional system. Also from the data in this table it is observed that as the harvested material ages, the content in essential oils decreases (Menta spicata). For the second harvesting stage (table 6) the highest amount of oil was obtained in the organic system for both species.

Tables 7-8 present a summary of the essential oil content for each mint species depending on the harvest stage and the cultivation system (organic), and it is found that more oil is obtained in the first harvest than in the second, for both mint species.

Table 7

Average distillation yields of organically grown Mentha piperita.

Distillation yields of organic Mentha piperita (Average)	
1st Harvest (July)	3.36 %
2nd Harvest (November)	1,84 %

Table 8

Average distillation yields of organically grown Mentha spicata

Distillation yields of organic Mentha spicata (Average)	
1st Harvest (July)	2,19 %
2nd Harvest (November)	1,44 %

Table 9 presents the essential oil content in the conventional culture system and it is noted that it is much lower than in the organic system.

Table 9

Average distillation of Mentha piperita and Mentha spicata plants of conventional cultivation

Distillation yields from the 2nd harvest of conventionally grown Mentha spicata and Mentha piperita (Average)	
Mentha spicata	1,18 %
Mentha piperita	1,60 %

CONCLUSIONS

Regarding the analysis of essential oils in the gas chromatograph of Mentha piperita, the main component was Linalool. A decrease in the Linalool content in the essential oil obtained from the late crop (November) in the organic cultivation system was observed by 9.20 %, compared to the essential oil of the early organic mint crop (30.95 % vs. 21.75 %). In the case of spearmint, the main component is Carvone. Similarly, in mint, between the early and late organic cultivation, a decrease in the percentage of Carvone by 14.91 % (70.5 % vs. 55.59 %) is observed. It can be said that the early organic cultivation system provides an essential oil with a higher intensity in components.

In terms of green production yield and dry matter, organically grown mint provides a higher production and a correspondingly higher amount of dry matter, compared to mint grown in the conventional system.

Regarding the essential oil yield, comparing the two species cultivated early in the organic system, it was found that Menta spicata obtained a higher yield than Menta piperita.

Comparing the essential oil from the same species (Peppermint) from the organic culture with that from the conventional culture, it was found that the organic system was more productive than the conventional one.

Comparing the essential oil of Peppermint from the conventional system with that from the organic system, the one from the organic is more productive than the conventional peppermint.

This information could be useful for focusing measures for the sustainable exploitation of plants occurring in areas of special interest, but also for improving species- and ecosystem-based conservation management.

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