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RESPONSE OF ANTIOXIDANT POTENTIAL AND ESSENTIAL OIL COMPONENTS TO IRRIGATION AND FERTILIZATION ON THREE MINT SPECIES (*Mentha* spp. L.)

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ABSTRACT

Mint (*Mentha* spp.), one of the most used plants in traditional medicine, is cultivated since antiquity, being mentioned by ancient writings. Besides its use for improving the human health, mint extracts are found in food, cosmetics, detergents, perfumes, biopesticides, etc. Due to its multiple benefits mint consumption has increased continuously in the last years. Besides the interest in mint yield, another interesting trait of this species is the antioxidant activity and the quality of essential oils. According to some research in the field, using water given by irrigation and fertilization leads to an increase of green mass production, but the level of essential oils remains the same. The aims of the present research were to determine the effects of irrigation, fertilization and species over the quality of mint and to identify the best matches of the experimental factors for increasing the antioxidants and essential oils content. Plant material was represented by *M. spicata, M. piperita* and *M. suaveolens.* Management followed the growing season of mint plants both in non-irrigated and irrigated conditions, with base fertilization and usage of Lignohumate. The analysis of samples was performed using Photochem where photochemical excitation of free radicals is combined with luminometric detection. The composition of the essential oil was determined by Gas chromatography with flame ionisation detector GC-FID. The research concludes that mint quality increased in conditions of no irrigation. For high phytonutrient content it is recommended to cultivate *M. piperita*, because it has the highest antioxidants content. *M. spicata* presented the essential oil with the best content of alcohols, esters and aromatic compounds. *M. suaveolens* reached the best level of esters, aldehydes and aromatic compounds only in the case of fertilization with Lignohumate.

Keywords: *M. spicata*; *M. piperita*; *M. suaveolens*; phenolic substances.

INTRODUCTION

Over the course of history, besides the plants that serve food, man has identified plants with beneficial effects for his health. The beginnings of medicine were born with the use of plants for the improvement of health condition (Muntean et al., 2016). Besides this, aromatic plants were used for gastronomy, beverages, perfumery, etc. (Al-Tawaha et al., 2013; Asghari et al., 2018). Over the last few years, medicinal herbs consumption has increased continuously worldwide (Gerami et al., 2016). The interest in antioxidant and phenolic substances has recently increased (Rowland et al., 2018). Therefore, regarding medicinal plants it is important to determine both the quantity of yield and its quality. Productivity of aromatic plants is conditioned by biological, ecological and technological factors. The biological factors influence both the quantity and the quality of production due to inherited features and the cultural value of the seeds. Soil moisture influences germination, seed growth and growth rate. The lack of water, especially during the critical phases of the growing season, may lead to a change in the quantity and quality of production (Gerami et al., 2016). For aromatic and medicinal plants, fertilization is an important factor for yield. In order to avoid pollution, it is preferable to use organic fertilizers (Muntean et al., 2016). *Mentha* spp. is one of the medicinal plants

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used since the Thracians to nowadays in Romania. Mint has been known since antiquity, being mentioned by Greek and Latin writings. In Romania, mint was among the first aromatic and medicinal cultivated herbs (Muntean et al., 2016). One of the most versatile aromatic plants, mint is used in all types of food (McVicar, 2010). Due to its tonic and refreshing qualities, as well as its antimicrobial properties, mint extracts are found in cosmetics, detergents, drugs, biopesticides, perfumes (Pavela, 2016). Thus, the research activity was interested both in green mass production of mint and its quality. Following the research of Rowland et al. (2018), no irrigation of mint crop determines the improvement of production quality. According to Okwany et al. (2011), reducing the irrigation levels in the case of *M. spicata*, even if there is a decrease in green mass production, the oil content is maintained at the same level. The aims of the present research were:

(i) to determine the effects of irrigation, fertilization and species on mint quality;

(ii) to identify the best option to increase the antioxidants content of plants;

(iii) to determine the essential oil content.

MATERIAL AND METHODS

Experimental environment

Experiences were conducted on the private land ”the Hodăi body” located at the eastern extremity of Cluj-Napoca on 324 m altitude, 46°48' N latitude and 23°40' E longitude, with a 10-15% slope and southern exposure. Type of predominant soil was stagnic chernozem (CH st) (WRB-SR-19981, World reference base for soil resources, 2014, updated 2015). The experiences were based on the split plot method. Only vegetation stimulation watering (40 m^3 ha^{-1}) was applied in the first year. In the second year (2017) three waterings were applied, with an irrigation rate of 370 m^3 ha^{-1}, and in the third year (2018) an irrigation standard of 255 m^3 ha^{-1}. Basic fertilization, 40 t ha^{-1} of manure, was applied before the growing season. Fertilization with Lignohumate was applied monthly, foliar, 0.02% solution, during the vegetation season (April - October), 120 g ha^{-1}.

Climate conditions

The precipitation and temperature measurements were made from planting/entering vegetation to the last harvest. The data was provided by the Cluj-Napoca weather station, located in the immediate vicinity of the experimental field.

Characterization of experimental years:

- 2016 was excessively rainy (1195.6 mm) with an average temperature of 9.7°C (normal);
- 2017 was slightly rainy (645.1 mm) with an average temperature of 10.21°C (normal);
- 2018 was very rainy (571.6 mm) with an average temperature of 12.08°C (warm).

Genetic materials

M. spicata var. *crispa* “Morroccan”. The cultivar is characterized by the busty appearance of the bushes, the presence of creeping rhizomes, intense green leaves, wrinkled, and white flowers that appear in summer in inflorescences in the form of terminal spices. The height and maximum diameter of the bush are 0.5-1.0 m. It is susceptible to rumination and is relatively less resistant to cold than other varieties (although it can withstand temperatures below -20°C).

M. piperita var. *piperita* “Swiss Mint”. The size of the bushes is 60 x 60 cm, with a port rather inclined to the ground, greenish green leaves and lavender-coloured flowers. Resistant to cold stress (-29°C).

M. suaveolens “Applemint”. The cultivar is characterized by subterranean rhizomes, slightly hairy leaves with rounded shape, and pale pink flowers in dense inflorescences occurring at the end of summer / early autumn. It is less resistant to cold (up to -15 °C).

Determination of antioxidant capacity

a. **Chemicals.** Sample preparation was performed using Methanol 99.8% from SIGMA-ALDRICH and ACW kit from la Analytik Jena.

b. **Sample preparation.** 0.5 g mint sample and 5 ml Methanol were centrifuged for 5 min at 10000 rpm. The supernatant was then extracted further using ACW kit manufacturer specification.

c. **Instrumentation.** PHOTOCHEM® from Analytik Jena AG was used for antioxidant capacity determination.

Determination of essential oils

a. **Chemicals.** Sample preparation was performed using n-Hexane SupraSolv® and n-Methanol SupraSolv® from Merck.

b. **Sample preparation.** Extraction of essential oils were performed using ultrasound assisted extraction with 1:2 hexane:methanol (v/v) on 5 g of mint (*Mentha* spp.) samples.

c. **Instrumentation.** Gas chromatography with flame ionization detector (GC-FID, Agilent Technologies 7890A) with a DB-WAX capillary column (30 m x 0.32 mm x 0.50 µm, Agilent J&W) was used for essential oils separation and quantification.

Data analysis

For statistical analysis of the results, the statistical program POLIFACT variance analysis for fully randomised polyfactorial trials was used. Controls in the statistical analysis were a_1 (non-irrigated) for irrigation, b_1 (basic fertilization) for fertilization and c_1 (*M. spicata*) for the mint variety.

RESULTS AND DISCUSSION

Response of antioxidant activity to irrigation, fertilization and biological material

Plants with higher antioxidant content are more valuable because they are preferred by consumers for their action on human health. Antioxidants reduce the toxic effects of free radicals. Natural antioxidants are preferred instead of synthetic ones due to their reduced carcinogenic potential (Chrysargyris et al., 2017). The antioxidant content was calculated as an average over the three experimental years (2016-2018) and was expressed in ascorbic acid equivalent. The results regarding the influence of technological and biological factors on the antioxidant content are synthesized in Table 1.

Although the irrigation regime led both to an increase in green mass production and dry matter content in each of experimental years, the antioxidants content was reduced. Lignohumate fertilization did not substantially affect the antioxidant content, and the effects on the amount of yield were also poor. The greatest influence on the antioxidant content was due the mint species. Highly significant differences from the other species were found for *M. piperita*. Thus, for high phytonutrient content, it is recommended to cultivate this variety.

Table 1. Antioxidant content of mint plants response to irrigation (A), fertilization (B) and species (C)

A		B		C	
Experimental condition	Antioxidant content ($\mu g\ mg^{-1}$ acid ascorbic equivalent)	Experimental condition	Antioxidant content ($\mu g\ mg^{-1}$ acid ascorbic equivalent)	Experimental condition	Antioxidant content ($\mu g\ mg^{-1}$ acid ascorbic equivalent)
Non-irrigated	15827.45	Base fertilization	14122.04	*M. spicata*	9866.44
Irrigated	8215.72	Base fertilization x Lignohumate	9921.13^{0}	*M. piperita*	17789.24***
				M. suaveolens	8409.07
LSD (p 0.05) = 8098.29 LSD (p 0.01) = 18701.40 LSD (p 0.001) = 59513.02		LSD (p 0.05) = 3519.35 LSD (p 0.01) = 5823.39 LSD (p 0.001) = 10899.87		LSD (p 0.05) = 3859.72 LSD (p 0.01) = 5316.22 LSD (p 0.001) = 7318.90	

Note: LSD = Least Significant Difference

The interaction of the studied experimental factors showed statistically significance only in case of *M. piperita* with irrigation system and base fertilization (Table 2). In this case antioxidants content evolved negatively. Rowland et al. (2018) identified that for some culinary herbs, including mint, crop quality can be increased using non-irrigation system. Under optimal environmental conditions of irrigation and growth stimulation, plants are concentrating their energy to produce green mass; thereby the antioxidants content is less.

Table 2. Interaction of the experimental factors on the antioxidant content of mint plants

Experimental condition	Antioxidant content ($\mu g\ mg^{-1}$ acid ascorbic equivalent)
$a_1\ b_1\ c_1$	14160.84
$a_2\ b_1\ c_1$	6732.78
$a_1\ b_1\ c_2$	33667.69
$a_2\ b_1\ c_2$	14953.43^{00}
$a_1\ b_1\ c_3$	9449.24
$a_2\ b_1\ c_3$	5768.24
$a_1\ b_2\ c_1$	12707.29
$a_2\ b_2\ c_1$	5864.83
$a_1\ b_2\ c_2$	14116.82
$a_2\ b_2\ c_2$	8419.02
$a_1\ b_2\ c_3$	10862.81
$a_2\ b_2\ c_3$	7555.98
LSD (p 5%) = 10279.28; LSD (p 1%) = 18288.92; LSD (p 0.1%) = 43157.25	

Note: a_1 = non-irrigated; a_2 = irrigated;
b_1 = base fertilization; b_2 = base fertilization + Lignohumate;
c_1 = *M. spicata*; c_2 = *M. piperita*; c_3 = *M. suaveolens*.

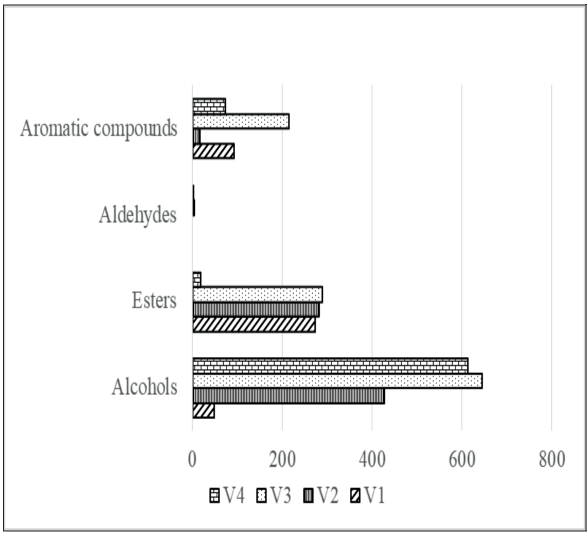
Response of essential oil composition to irrigation, fertilization and biological material

Essential oils (EOs) are secreted by plant cells and tissues. They are the result of secondary metabolism of plants. The importance of EOs is determined by their action: analgesic, stomachic, choleretic, antiemetic, cardiotonic, antispastic, antibacterial, antidiabetic, and antiseptic (Muntean et al., 2016; Bayan et al., 2018). Singh and Pandey (2018) highlighted the importance of different mint species EOs for the antifungal, insecticidal, repellent and antifeedant effects. The quantity and quality of EOs may be influenced by type and amount of fertilizers (Chrysargyris et al., 2017). The identified components are presented in Table 3.

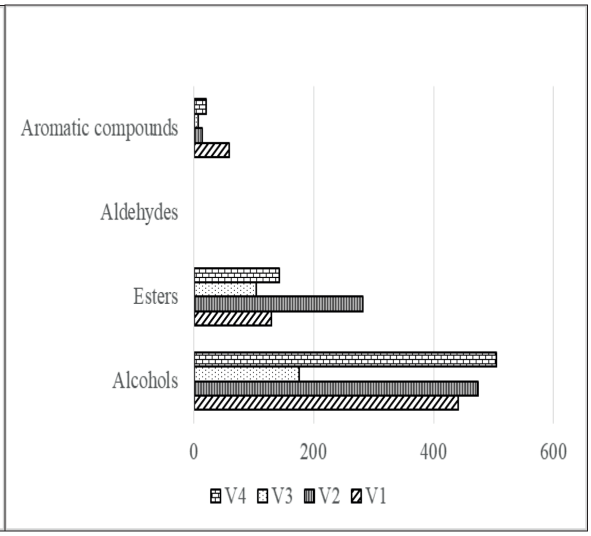
Table 3. Essential oil compounds identified in mint samples

Essential oil compounds	
Alcohols	isobutanol, 3-methyl-2-butanol, 4-methyl-2-pentanol, 5-hexen-1-ol, alpha-terpineol, 1-decanol, 1-phenylethanol, 2-phenylethanol
Aldehydes	benzaldehyde, phenyl-ethyl-acetate
Esters	isobutylacetate, ethyl hexanoate, ethyl nonanoate, ethyl decanoate, diethyl succinate
Aromatic compounds / hydrocarbs	2-methoxypyrazine, 2-isobutyl-3-methoxypyrazine

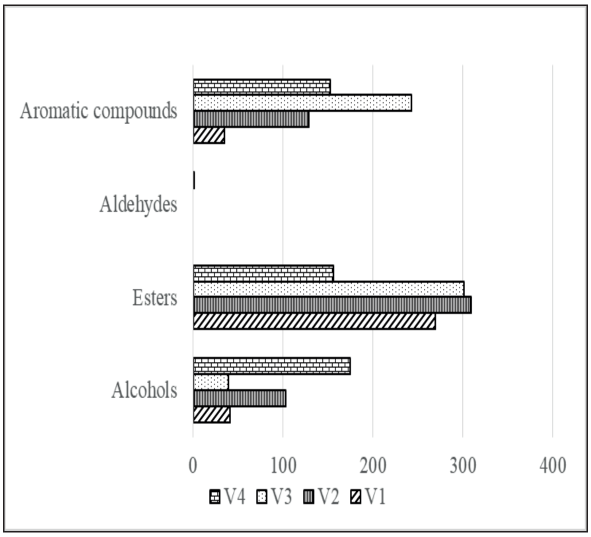
Figure 1 presents the interactions of irrigation and fertilization on aromatic compounds, aldehydes, esters and alcohols, for each of the three species of mint. The level of EOs compounds in four experimental conditions was determined: non-irrigation with base fertilization (V1), non-irrigation with base fertilization and Lignohumate (V2), irrigation with base fertilization (V3), irrigation with base fertilization and Lignohumate (V4).



(A) *M. spicata*



(B) *M. piperita*



(C) *M. suaveolens*

Figure 1. Variation of essential oil composition depending on irrigation and fertilization in case of *M. spicata* (A), *M. piperita* (B) and *M. suaveolens* (C)

For *M. spicata* (Figure 1A) the most important effect on the quality of the essential oil was found in V3 (the highest content in alcohols, esters and aromatic compounds). For *M. piperita* (Figure 1B) the highest alcohol content was reached on V4 and the highest content of esters on V2; *M. Piperita* reached the highest value of aromatic compounds in V1. For *M. suaveolens* (Figure 1C) V4 increased the alcohols content, V2 had the greatest influence on the content of esters and V3 increased the level of aromatic compounds.

The essential oil with the highest content of alcohols, esters and aromatic compounds was obtained in *M. spicata*, followed by *M. piperita* (Figure 2). *M. suaveolens* had the highest esters content in the not irrigated system, but fertilized with Lignohumate.

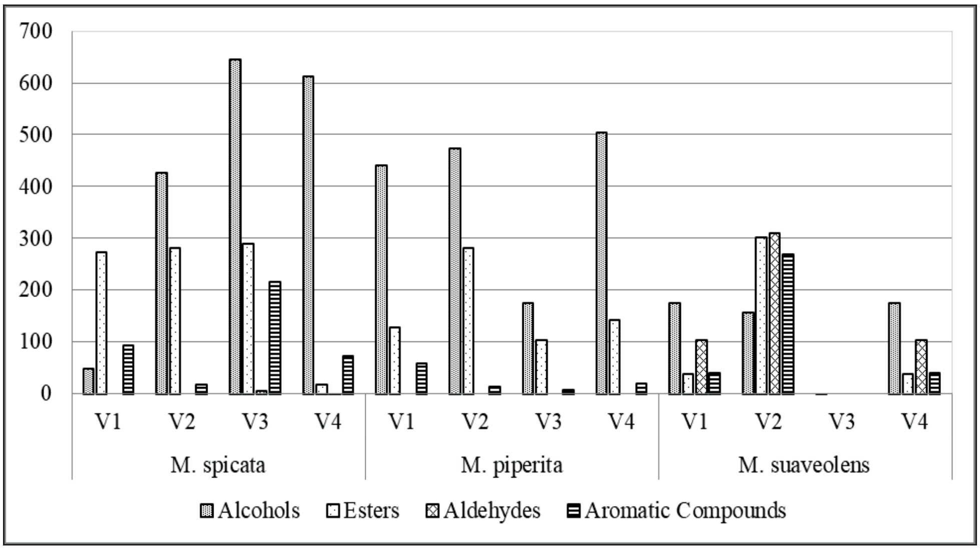


Figure 2. Essential oil composition according to mint variety

Knowing the response of plants to growing conditions allows controlling the quantity and quality of EOs. This is very important because the changes that may occur in EOs chemical compositions can affect the commercial value of the oil (Aghayari and Darvishi, 2011), due to the fact that the multiple uses of the oils depend on the chemical composition (Kizil et al., 2010). Many studies of EOs are related to the antimicrobial activities, these oils having very good effects against microorganisms including gram (-) and gram (+) bacteria (Sevindik et al., 2017). *M. piperita* EO is primarily recommended as minor antispastic and local analgesic due to the active principles represented particularly by menthol (50%) and menthona (5-25%) (Fit et al., 2009; Tsai et al., 2013). The main components of *M. spicata* are carvone (50%) and limonene (16%) (Kizil et al., 2010). Its oil has a high antibacterial activity, particularly against gram (-) bacteria (Niksic et al., 2018). The EO of *M. suaveolens* also has the main components carvone (50%) and limonene (31%) with great antibacterial properties (El-Sayeda et al., 2014).

CONCLUSIONS

The research concluded that:

(i) the quality of mint production increases in conditions of no irrigation;

(ii) the highest antioxidants content was determined in *M. piperita*, hence for high phytonutrient content, it is recommended to cultivate this species;

(iii) *M. spicata* had the essential oil with the highest content of alcohols, esters and aromatic compounds; *M. suaveolens* reached the highest level of esters, aldehydes and aromatic compounds on fertilization with Lignohumate.

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