Plantation of Peppermint (*Mentha piperita)* Using Water Treated with Low-pressure, Low-temperature Glow Plasma of Low Frequency

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Abstract

After treating peppermint (*Mentha piperita)* plantation with water exposed to low-pressure, low-temperature glow plasma of low frequency (PW) in a 150 days experiment in a greenhouse the growth of leaves and stems was stimulated by 24% and 5%, respectively. Application of PW changed composition of extracts without any negative effect on their bactericidal properties. PW increased the content of chlorophyll, carotenoids and ascorbic acid, decreased the concentration of the Mn(II), Ni(II), Ca and Mg ions in leaves and stems, decreased the concentration of the Pb(II) and Fe(III) ions in leaves and increased their concentration in stems and had no influence of the concentration of the Zn, Cu(II), Cd, Cr(III) and Co(II) ions in both parts of the plant. Simultaneously, PW increased the level of the Na^+ ions, had no influence on the level of the K^+ ions in the leaves and stems. PW considerably reduced the accumulation of sulfates, chlorides and nitrites, slightly reduced the level of phosphates and elevated the level of nitrate

KEYWORDS: bioaccumulation, essential oils, plant growth stimulation**,** plazmed water

Introduction

Recently, Białopiotrowicz et al. (2016) reported the structure and selected physicochemical properties of water exposed to low-pressure, low- temperature glow plasma of low frequency (LPGP). Its Raman spectra appeared identical to the spectra of water subjected to a static magnetic field of induction ~0.5 T.

It was shown that the magnetically treated water considerably stimulated the reproduction and pathogenicity of entomopathogenic fungi (Jaworska, Domański, Tomasik and Znój, 2016). Thus, the effect of water treated with LPGP was tested (Jaworska, Oszczeda and Tomasik, 2018) with success on the same fungi showing also that tap water performed better than distilled water. The effect of watering plantations of wheat with water after treating it with LPGP (Sitarska, 2013) confirmed the benefits of using plazmed water called here PW for stimulation of the growth of the plants in terms of increasing the crop's yield.
In herbs, apart from the stimulation of their growth, the resulting composition and yield of essential oils is also crucial. Hence, in this report the effect of watering peppermint (*Mentha piperita*) growth and essential oil production when using PW, was checked. For its fragrance, biological and pharmacological properties in various species of peppermint are commonly used in many branches of industry. Recently, peppermints are considered as potential food preservatives, stabilizers and food quality improvers (Adaszynska, Swarzewicz, Markowska-Szczupak and Jadczak, 2013; Derwish, Benziane, Taouil, Senhaji and Touzani, 2010; Eteghad, Mirzaei, Pou and, Kahnamui, 2009; Grzeszczuk and Jadczak, 2009; Iscan et al., 2002; Jeyakumar, Lawrance and Pal, 2011; Lawrence, 2007).

Materials and Methods

Materials

Peppermint

Underground stolons of the plant placed into the experimental pots were purchased in 2016 from the Napieralski Enterprise in Lodz, Poland,.

Water

Distilled water or tap water from Boleslawiec with total hardness 129 mg/L $CaCO_3$; pH 7.1; conductivity 334 μS/cm; Fe < 50 μg/L; Mn < 5 μg/L; dissolved oxygen 6.93 mg/L were used.

Microorganisms

Escherichia coli code PCM 2857 and *Staphylococcus aureus* code PCN 2602 were purchased in 2017 from Polish Collection of Microorganisms (Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, Poland).

Methods

Peppermint plantation

The monofactorial experiment was carried out on Nov. 24th 2016 in a greenhouse at Cracow University of Agriculture in the 2016/2017 break. The greenhouse was set for 23°C and automatic additional 16h illumination. Experiments were arranged in six parallel series each containing 20 pots of 10 cm in diameter filled with a universal horticulture soil for flowers purchased from Biovita, Krzeszowice at Cracow, Poland. In order to eliminate the parietal effect, all edges of each collection of the pots were surrounded with double row of pots with peppermint. Each row contained 10 pots. The watering was carried out always at 9:30 a.m. Three of six series of the pots were watered always at 9:30 a.m. with tap water and they were considered as control. The other three another series were watered with plasma treated water (PW). In total the watering was carried out 38 times consuming 395 dm^3 of both tap water PW. When the plants reached 5 – 8 cm in height they were transferred into pots of 15 cm diameter and watering was continued in the same regime with the same volume of water. The experiment terminated on April 24th 2017 when the plants were collected and separated into leaves and stems.

Drying peppermint

The plant was dried at 105°C for 4 hours

Separation of essential oils

Sample of the dry plant (1g) was steam distilled in a Deryng apparatus with a closed water circulation. The collected oil was transferred to a closed vial then analyzed chromatographically.

Treating water with LPGP (PW)

Either distilled or tap water (1000 mL) was placed in the chamber of the reactor (Oszczeda, Elkin and Strek, 2009) and exposed to plasma for 30 min. Plasma of 38°C was generated at $5x10^{-3}$ mbar, 600 V, 50 mA and 280 GHz frequency. The produced water was stored at ambient temperature in 100 mL closed Teflon containers.

Gas chromatographic analyses

An Agilent 7890A gas chromatograph (Agilent Technologies, Inc., Santa Clara, Cf. USA) was equipped with a Supelcowax-10 30cm x 0.32mm x 0.25 μm column. An injector was maintained at 270°C. Initial temperature of 40°C was maintained for 1 min, then rose to 220°C with a rate of 4°C/min. Helium (0.5 mL/min) was used as the gas. Analyzed sample mass ranged from 33 to 333Da. Temperature of the ion generator was maintained at 220°C. SPMR injections were performed in a splitless manner.

Analyses for cations

Samples were mineralized in a microwave oven (MarsXpress CEM company). Samples (0.5 g) were digested with nitric acid 65% analytical grade (10 mL). Determination of metals content was performed with atomic absorption spectrometry with electrothermal device (AA Varian 240 instrument). A palladium standard solution (1000 mg/dm^3) was used as a modifier.

Anion analyses with ion chromatography

A DX500 microbore (2 mm) ion chromatograph with a CD20 conductivity detector and GP40 gradient pump (Dionex, California) was used for ion separation and detection. Commercially available Ionpac CG12A guard and CS12A analytical columns (Dionex, California) with carboxylic–phosphonic acid functional groups were used for cation analysis. Ionpac AG14 guard and AS14 analytical columns (Dionex, California) with quaternary ammonium functional groups were used for anion separation. Eluents were stored in vessels pressurized at 8 p.s.i. using high purity argon (BOC gases), and flow-rates were maintained at 0.45 ml/min for anions and 0.40 ml/min for cations using a GP40 gradient pump (Dionex, California). Samples were loaded from an AS40 automated sampler (Dionex, California).

Determination of chlorophylls and carotenoids

Either leaves or stems of peppermint (200 mg) were homogenized for 2 min in a cooled mortar then homogenized for further an additional 2 min with the acetone/ammonia (0.05 mol/dm^3) 8/2 blend (5 mL) cooled to 0 – 5^{o}C. The extraction was continued for 2 more min. by addition of a subsequent 5 mL of extracting acetone/ammonia blend. The resulting suspension of well disintegrated sample was transferred into 25 mL measuring cylinder, the mortar was washed with extracting blend (10 mL) and the wash was combined with the extract. The extract was then either filtered or centrifuged for 10 min at 5000 rpm, and decanted. The volume of the extract was increased to 25 mL by adding the extracting blend.

The absorbance (A) of resulting extract was taken at 470, 647 and 664 nm. The content of chlorophylls a and b in mg/g was estimated from the formulas (1) and (2), respectively

$$\mathbf{chl.a} = \frac{\mathbf{25 \cdot a}}{\mathbf{m}} \qquad (1)$$

where $a = 11.78\ A_{664} - 2.29\ A_{647}$

$$\mathbf{chl.b} = \frac{\mathbf{25 \cdot b}}{\mathbf{m}} \qquad (2)$$

where $b = 20.05\ A_{647} - 4.77\ A_{664}$

The content of carotenoids (β-carotene and xanthophyll) was calculated from formulas (3)

$$\mathbf{car.} = \frac{\mathbf{25 \cdot c}}{\mathbf{229 \cdot m}} \qquad (3)$$

where $c = 1000\ A_{470} - 3.27a - 104b$

In Eqs. (1) – (3) m denotes the weight (mg) of the fresh plant material

Test for bactericidal properties of essential oils and extracts

The Blanc method (Blanc, Lugeon, Wenger and Siergist,1994) was followed. Filter paper circles, ϕ = 6 mm, were soaked for 1 – 2 min in essential oil or extract (10 μl) and immediately transferred on inoculated plate which was then incubated for 24 h at 37 °C. The area of suppressed growth was measured with a caliper. The experiments were run in triplicates. The results were statistically calculated using Excel 97 software.

Results and Discussion

One may see from Table 1 that watering peppermint with PW stimulates the growth of the plant. In case of leaves there was the 24% increase in the dry mass whereas in case of stems that increase reached only 5%. The comparison of the crop yield expressed in grams for plants watered with water and PW showed that there was reduced demand for watering when PW was used. The same volume of PW provided more dry crop than did tap water

The chromatograms showed that the stimulation of the growth of peppermint with PW was accompanied by essential changes in the composition of extracts from leaves (Table2) and stems (Table 3).

Table 2 collects data solely for the peaks areas of whose were higher from 100 pA x s. One could see that watering peppermint with PW resulted in a higher intensity of the peaks of the retention time of 2.08, 3.50 and 3.99 min. In contrast to that the peaks of the retention time of 3.07 and from 1.53e4 17.55 min were considerably higher in the extracts from control

plants, that is, those watered with tap, non-plazmed water. Particularly, the peak belonging to menthol, the major peak in chromatogram (Adaszynska, Swarzewicz, Markowska-Szczupak and Jadczak, 2013) at 11.05 min faced tremendous decrease in the extract from plant watered with PW. Instead, in that extract components with the retention time of 16.28 , 43.83 and 44.59 appeared solely in the extract from the plant watered with PW.

Extracts from stems of the plant watered with PW contained numerous components of lower retention time from 3.53 to 17.56 min. Among them the peak at the retention time of 10.99 min likely belonged to menthol. These components were absent in the extracts from control plants. The latter extracts were rich in the components of the retention time from 37.75 to 49.14 min. Except relatively small amount of the component of the retention time of 44.68 min they were absent in the extracts from plant watered with tap water.

The characterization of the components will be presented in a subsequent paper.

Essential oil from the plant watered with PW exhibited higher bactericidal properties against *Escherichia coli* and *Staphylococcus aureus* than the oil from control plant. The oil from stems was slightly more bactericidal than the oil from the leaves and these from PW watered plants were slightly more bactericidal. Always these oils performed better for *S. aureus.* Extracts were less bactericidal than the oil regardless their origin.

Watering with PW influenced accumulation of metal ions in the plant (Table 5). Watering with PW decreased the concentration of the Mn(II) and Ni(II) ions in leaves and stems, decreased the concentration of the Pb(II) and Fe(III) ions in leaves and increased their concentration in stems and had no influence of the concentration of the Zn, Cu(II), Cd, Cr(III) and Co(II) ions in both parts of the plant. Simultaneously, PW increased the level of the Na^+ ions, had no influence on the level of the K^+ ions and decreased the level of the Ca^{2+} and Mg^{2+} ions in leaves and stems.

Watering peppermint with PW influenced also the anion uptake (Table 6). PW the most considerably reduced the accumulation of sulfates in leaves and stems. Also the accumulation of chlorides in both parts of the plant after watering with PW significantly declined. At the same time, after watering with PW, the level of phosphates was relatively slightly reduced whereas the level of nitrates tremendously increased.

Watering with PW reduced the content of chlorophyll a but simultaneously, stimulated synthesis of chlorophyll b to a such extent that the total chlorophyll content increased. At the

same time, syntheses of carotenoids and ascorbic acid were also stimulated (Table 7). Chlorophyll a synthesis is light stimulated whereas the synthesis of chlorophyll b proceeds in plants cultivated in the shadow (Chatterjee and Kundu, 2015). The increase in the chlorophyll b in the peppermint watered with PW could be stimulated by the clathrates of singlet oxygen in PW quenching the near UV radiation at 195 and 230 nm[1].

Presented results show a positive role of PW in enhancing the crop yield of peppermint plantations. Although watering the plantations with PW reduced the content of menthol in the plant the total composition of essential oil and extract from leaves and stems retains their bactericidal properties on the same level. Instead of components of essential oils content of which is reduced, watering with PW offers enhanced level of total chlorophyll, carotenoids and ascorbic acid. The watering with PW increases practical application of the plant stems as in contrast to stems of the plant watered with plasma untreated water they contain considerable amount of some components of essential oil including menthol (see Table 3).

Conclusions

Watering peppermint (*Mentha piperita*) with water treated with low-pressure, low temperature glow plasma of low frequency (PW) stimulated the growth of the plant and reduced its demand for water. Simultaneously, the composition of essential oil and extracts from leaves and stems significantly changed. However, with no significant effect upon their bactericidal properties, such watering elevates the total yield of carotenoids, ascorbic acid and total chlorophyll with domination of chlorophyll b. Watering with PW influences accumulation of metal ions and their counterions in the plant.

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