



Research Article



Effect of alginite on some antioxidant indexes in extracts of two variants of *Mentha* and their toxicity

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Article Details:

Received: 2021-08-22

Accepted: 2021-10-20

Available online: 2021-11-30

DOI: <https://doi.org/10.15414/ainhlq.2021.0030>

The experimental work aimed to determine the effect of different applications of alginite on some antioxidant indexes in the extracts of the *Mentha × piperita* L. (cultivars Strawberry Mint and Chocolate Mint), and the acute toxicity of water extracts of the studied mint plants to freshwater invertebrates of the genus *Daphnia* sp. The content of total polyphenols, phenolic acids, and antioxidant activity were determined by spectrophotometric methods. The OECD manual No. 202 was used for the determination of EC₅₀ 48 h in the water extracts of both variants of mint. The content of total polyphenols in all groups of the cv. Chocolate Mint ranged from 3.05 to 3.37 mg CAE/100 g DW. On the other hand, the significantly lower ($p < 0.001$) content of total polyphenols (from 1.01 to 1.38 mg CAE/100 g DW) was found in all groups with the cv. Strawberry Mint. The antioxidant activity determined by the DPPH method was from 115.7 to 119.5 mg TAEC/100 g DW in the cv. Chocolate Mint, but was 2 times lower (from 48.8 to 65.7 mg TAEC/100 g DW) in the cv. Strawberry Mint. The content of phenolic acids in the cv. Chocolate Mint was significantly higher (but only 1.1-fold), than in the cv. Strawberry Mint. The mean acute toxicity after 48 h exposure of the cv. Chocolate Mint to daphnids was nearly 40 times higher than the mean acute toxicity of the cv. Strawberry Mint (EC₅₀ 0.25 mg/L vs. EC₅₀ 9.62 mg/L, respectively). We can conclude, that the cv. Chocolate Mint is a more potent source of substances with antioxidant properties, such as polyphenols and phenolic acids, which is reflected in the significantly increased DPPH radical scavenging activity as compared to the cv. Strawberry Mint. We can only speculate that the higher amounts of the aforementioned active substances in the cv. Chocolate Mint were associated with an increased level of acute toxicity to the tested water invertebrates as compared to the cv. Strawberry Mint. The impacts of alginite application on all studied antioxidant indexes as well on the acute toxicity of the extracts of both variants of mint were not confirmed.

Keywords: mint, alginite, antioxidant, invertebrate, immobilization

Introduction

The current research is focused on the field of organic farming “bioagriculture” production of feedstuffs, which can contribute essentially to animal and human health and are environmentally friendly. One of the main requirements in the bioagricultural production is the usage of organic fertilizers instead of synthetic substances. Alginite is a complex soil aggregate of algae-based biomass from an organo-mineral origin

(Kúšik et al., 2017; Brindza et al., 2021a, b) with high water absorption capacity (Valla et al., 1980), thus can be used as a natural organic fertilizer. The most effective usage of alginite is attributed to agricultural production as a natural fertilizer (Elečko et al., 1998). Slovakia has in Princiná, Lučenec district, one of the largest alginite deposits in Europe suitable for mining (Kulich et al., 2002).

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The plants of the genus *Mentha* belong to the important representatives of the Lamiaceae family. The genus *Mentha* consists of eighteen species and eleven hybrids. Several aromatic species of mint contain significant amounts of essential oil thus are economically important (Tucker and Naczi, 2006). The taxonomic and phylogenetic classification of the genus *Mentha* is very complicated and unclear due to the hyperdiverse of this group of plants (Heylen et al., 2021). Jedrzejczyk and Rewers (2018) summarize the problems with the taxonomic assignment of *Mentha* species to the following points: a) the existence of variation in basic chromosome number, b) frequent interspecific hybridization, c) cytotoxicity, d) polymorphism in morphology, e) essential oil composition under different environmental conditions, f) colonial mutant propagation, and g) the occurrence of polyploids, aneuploids, and nothomorphs.

The genus *Mentha* is characterized by the presence of surface glandular trichomes containing relatively huge amounts of essential oils (Bacílková and Paulusová, 2012). Phenolic acids (rosmarinic and caffeic acids), flavones (luteolin glycoside), and flavanones (eriodictyol glycoside) are probably the main antioxidants in mint (Riachi and De Maria, 2015). The active substances presented in mint are responsible for a broad range of biological activities including digestive, choleric, carminative, antiseptic, antibacterial, antiviral, antispasmodic, antioxidant, anti-inflammatory, myorelaxant, expectorant, analgesic, tonic, and vasodilatation (McKay and Blumberg, 2006). The plants of the genus *Mentha* are known for their strong free radical scavenging and antioxidant activities that lead to the decrease of oxidative stress in the biological system (Nickavar et al., 2008). In the Slovak republic, *Mentha × piperita* L. is widely used as a medicinal plant, fresh leaves of mint are a popular spice and culinary drug, and are used for the preparation of hot drinks mostly in combination with ginger (Eftimová and Habán, 2012).

The cultivar Strawberry Mint (*Mentha × piperita*) is characterized by its shorter stature and small bright green leaves with purple lilac flowers, which have a distinctive sweet aroma that resembles the smell of strawberries. On the other hand, the cultivar Chocolate Mint is a variety of mint that has nuances of chocolate in fragrance and flavour and features rounded to lance-shaped dark green leaves and terminal spikes of small lavender flowers. Both varieties of mint require moist soils that are slightly acidic or neutral in pH and places with a balanced sun/shade regime.

This experimental work aimed to assess the antioxidant activity, the content of polyphenols and phenolic acids

in the extracts of the *Mentha × piperita* (cultivars Chocolate Mint and Strawberry Mint) growing in the soil treated by different applications of alginite, as well as to determine the acute toxicity of the water extract of the dried plant samples to daphnids after 48 h exposure.

Material and methodology

In the experiment, the plants of the *Mentha × piperita* (cultivars Chocolate Mint and Strawberry Mint) were grown on artificially prepared 1 m² plots, on chernozem soil in the village of Hažín, Michalovce district. At the beginning of the experiment, the soil was planted with the seeds of different variants of mint and treated by various applications of alginite: a) control – the plots without the application of alginite, b) group 1 – the plots were treated with the application of powdered alginite in the dose of 0.1 kg/l of water per 1 m² (in total 1 kg of alginate), and group 2 – the plots were treated with the application of powdered alginite mixed into the soil at the dose 1 kg per 1 m². Except for alginite, the soil was not fertilized and no plant protection products were used. Rainwater from a reservoir was used for watering the plants once a day in the morning. At the end of the experiment, the samples of the plants (only leaves) were dried, cut by a mixer, and stored until analysis.

Total polyphenol content

The total polyphenol content in the aqueous plant extracts was measured using Folin-Ciocalteu reagent by the spectrophotometric method according to Singleton and Rossi (1965) modified by Suchý et al. (2013). Briefly, 0.1 mL of each extract was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20 % (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min. in darkness, the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). The total polyphenol content in the aqueous extracts (as caffeic acid equivalent) was calculated from the caffeic acid calibration curve (10–100 µg caffeic acid equivalents (CAE)/L; R² = 0.98) and is expressed in mg CAE/100g DW.

Antioxidant activity (DPPH radical scavenging assay)

The radical scavenging activity in the methanol plant extracts of the samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Ahmad et al., 2014). The extracts (0.5 mL) were mixed with 3.6 mL of DPPH medium (0.025 g of DPPH in 100 mL of ethanol). The absorbance of the sample extract was determined

using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (10–100 mg Trolox/L; $R^2 = 0.99$) was used as the standard for the calibration and the results were expressed in mg Trolox equivalent antioxidant capacity (TEAC)/100g DW.

Total phenolic acid content

Determination of total phenolic acid content in the ethanol plant extracts was carried out using a method of Farmakopea Polska (1999). Briefly, 0.5 mL of extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL of Arnova reagent, 0.5 mL of 1 M sodium hydroxide (w/v), and 0.5 mL of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid was used as a standard for the calibration curve (0.01–1.0 mg caffeic acid equivalents (CAE)/L, $R^2 = 0.99$) and the results are expressed in mg CAE/100 g DW.

Acute toxicity on *Daphnia* sp.

The acute toxicity in the aquatic extracts of dried plant samples of both variants of mint was evaluated by the OECD manual No. 202: *Daphnia* sp.: Acute immobilisation test (OECD, 2004). The mobility of freshwater invertebrates exposed to the different concentrations of the substance is monitored during a 48-hour ecotoxicological test. Determination of the half-maximal effective concentration (EC_{50}) at 24 hours and/or 48 hours is recommended. The experiment was performed with some deviations from the manual, which do not affect the endpoints of the test. Briefly, young forms of daphnids were transferred to each test vessel from inert material. A volume of 10 mL of tested concentration for 5 daphnids per test vessel was used (20 daphnids per group). The dried samples of mint leaves were diluted in a hot water for 15 min. (the form of decoction) to the concentration of 0.5 mg/L, 1.0 mg/L and 5.0 mg/L. The control group was only with natural spring water.

Statistical analysis

All data were analyzed by the one-way analysis of variance (ANOVA) with the Tukey post-hoc test

($p < 0.05$). The ecotoxicological results were analyzed by the Probit statistical analysis and the Interpolation statistical method. Association between reported total polyphenols and antioxidant activity were examined by Spearman's correlation.

Results and discussion

The genus *Mentha* is a rich source of different biologically active substances, such as essential oils. The main group are monoterpenes with the largest proportion up to 60 % presented by menthol, followed by isomenthone, neomenthyl acetate, and menthone only up to 5 % (Makkar et al., 2018). The amounts of active substances in the plants of peppermint differ widely from variety to variety. The wild mint contains up to 33 % of menthol, up to 29 % of menthone, to 18 % of pulegone, up to 11 % of 1,8-cineole, and less than 5 % of terpineol-4 and piperitone (Hajlaoui et al., 2008; Mkaddem et al., 2009).

Flavonoids, gentians, bitter tastants, triterpenes, and tannins are present in the leaves of mint. Sesquiterpenes, such as cadinene and β -caryophyllene, and other compounds (e.g. isoamyl alcohol, amyl alcohol, hexenol, acetaldehyde, and isovaleraldehyde) are also present in these plants but in small amounts (Bulkova, 2011). Growing evidence indicates that these polyphenols can act as potent natural antioxidants (Kanatt et al., 2007; Rita et al., 2016). The contents of total polyphenols in the aqueous extract of the *Mentha* \times *piperita* (cultivars Chocolate Mint and Strawberry Mint) treated by the different applications of alginite are presented in Table 1.

Different laboratory methods for the determination of polyphenols from plants have been applied to study the antioxidant properties in these herbal matrixes. For example, Abootaleb et al. (2016) expressed the amounts of polyphenolic compounds in methanol extracts as tannic acid equivalents (TAE) which ranged from 50.0 to 67.0 mg TAE/g DW. The total phenolic content in *M. spicata* extract was nearly 26.0 mg of catechin equivalent per g DW as was reported by Kanatt et al. (2007). Scherer et al. (2013) used gallic acid equivalents (GAE) and reported that the methanolic extracts of *M. spicata* contained around

Table 1 Content of total polyphenols in the samples of mint treated by the different application of alginite

Cultivar chocolate mint	mg CAE/100 g DW	Cultivar strawberry mint	mg CAE/100 g DW
Control	3.37 ABCDE	Control	1.23 CFIX
Group 1	3.08 bIJK	Group 1	1.38 EGKM
Group 2	3.05 aFGH	Group 2	1.01 DHJxM

Notes: the same letters indicate statistical significance between the two respective groups: xx = $p < 0.05$; Xx = $p < 0.01$; XX = $p < 0.001$

76.0 mg GAE per gram of DW. Benabdallah et al. (2016) analyzed the amounts of total polyphenols in five members of the genus *Mentha* spp. The authors found that *M. aquatica*, *M. arvensis*, and *M. piperita* are rich sources of the total polyphenols, while *M. pulegium*, *M. rotundifolia*, and *M. villosa* have low total polyphenol contents. The total phenolic content in Medina mint and Hasawi mint was 2.64 mg GAE/g DW and 1.54 mg GAE/g DW, respectively (Brown et al., 2019). Brahmi et al. (2015) compared the phenolic composition in three Algerian *Mentha* species: *M. spicata* L., *M. pulegium* L., and *M. rotundifolia* L., and the highest content of polyphenols (12.0 mg GAE/g DW) was revealed in *M. spicata*. On the other hand, the same authors (Brahmi et al., 2014) published the phenolic content in *Mentha pulegium* to be much higher around 55.0 mg GAE/g DW.

In our experiment, the highest content of total polyphenols (3.37 mg/100 g DW) was determined in the aqueous extract of the cv. Chocolate Mint in the control group. In addition, the amounts of total polyphenols in all samples of the cv. Chocolate Mint were significantly higher as compared to all samples in the cv. Strawberry Mint. We can state that the cv. Chocolate Mint is a richer source of polyphenols (nearly 3 times higher amount) than the cv. Strawberry Mint. On the other hand, we can conclude that the application of alginite did not affect the total polyphenol content in the plants of mint. Nevertheless, the cv. Strawberry Mint treated with the application of alginite mixed into the soil had the lowest amount of total polyphenol, only 1.01 mg CAE/100g DW, but this phenomenon was not revealed in the cv. Chocolate Mint (Table 1).

The usage of peppermint plants in traditional “folk” medicine is attributed to the high amount of biologically

active compounds. The polyphenols and lipophilic flavonoids were found to possess a strong antioxidant potential and are the most important pharmaceutically active secondary metabolites in mint (Mimica-Dukic and Bozin, 2008). Growing evidence indicates that the biotic and abiotic factors can influence the composition of biologically active substances in the mint plants thus designating the antioxidant properties in the extracts (Gouvea et al., 2012; Rahimi et al., 2018; Brahmi et al., 2020).

In our study, a Spearman correlation analysis was performed to identify the association of the amount of total polyphenols with the antioxidant activity determined by the DPPH method. The radical scavenging ability (the antioxidant activity determined by the DPPH radical production) was positively correlated with a higher content of total polyphenols in the extract ($r = 0.75$, $*p < 0.255$). These results are in accordance with the previous articles published by Benabdallah et al. (2016). The antioxidant activity was determined by the inhibition % of DPPH in the ethanol extracts of the cultivars Chocolate Mint and Strawberry Mint (Table 2).

Similarly, the statistical differences in the antioxidant activity between the groups of both variants of mint (Control, Group 1 and Group 2) were not found, but the antioxidant activity in all groups of the cv. Chocolate mint was 2 times higher in comparison to the groups with the cv. Strawberry mint (Table 2). The antioxidant activities in the methanol extracts of the *Mentha × piperita*, L. and *Mentha spicata*, L. were 84.81 and 52.66 %, respectively (Martiš and Eftimová, 2019). In this study, around 40 % of antioxidant activity by the DPPH method was found in the cv. Chocolate Mint and

Table 2 Antioxidant activity in the samples of mint treated by the different applications of alginite

Cultivar chocolate mint	mg TEAC/100 g DW	Inhibition % of DPPH	Cultivar strawberry mint	mg TEAC/100 g DW	Inhibition % of DPPH
Control	115.7ABC	39.2 %	Control	65.7ADG	21.9 %
Group 1	119.5DEF	40.5 %	Group 1	48.8BEH	16.1 %
Group 2	117.6GHI	39.8 %	Group 2	55.7CFI	18.5 %

Notes: the same letters indicate statistical significance between the two respective groups: XX = $p < 0.001$

Table 3 Content of phenolic acids in the samples of mint treated by the different applications of alginite

Cultivar chocolate mint	mg CAE/100 g DW	Cultivar strawberry mint	mg CAE/100 g DW
Control	1.57AbC	Control	1.49adg
Group 1	1.57DeF	Group 1	1.51beh
Group 2	1.53GhI	Group 2	1.49cfi

Notes: the same letters indicate statistical significance between the two respective groups: xx = $p < 0.05$; Xx = $p < 0.01$; XX = $p < 0.001$

Table 4 Effect of water extracts of the *Mentha × piperita* L. (cv. Chocolate Mint) on daphnids mobility in the control group

Parameter	Concentration (mg/L)			
	0.0	0.5	1.0	5.0
Affected after 24 h. (%)	0	20	35	70
Affected after 48 h. (%)	0	70	90	95
Endpoint (mg per liter)				
EC ₅₀ after 24 h &	2.16 mg/L (CI: 0.97–4.81 mg/L)			
EC ₅₀ after 48 h *	0.25 mg/L (CI: 0.26–2.32 mg/L)			

Notes: CI – confidential interval, & probit statistical analysis. * Interpolation statistical method, EC₅₀ – half-maximal effective concentration

Table 5 Effect of water extracts of the *Mentha × piperita* L. (cv. Strawberry Mint) on daphnids mobility in the control group

Parameter	Concentration (mg/L)			
	0.0	0.5	1.0	5.0
Affected after 24 h. (%)	0	10	10	35
Affected after 48 h. (%)	0	15	10	40
Endpoint (mg per liter)				
EC ₅₀ after 24 h &	25.18 mg/L (CI: 5.31–119.52 mg/L)			
EC ₅₀ after 48 h &	9.62 mg/L (CI: 3.13–29.57 mg/L)			

Notes: CI – confidential interval, & probit statistical analysis, EC₅₀ – half-maximal effective concentration

lower antioxidant activity of only up to 22 % in the cv. Strawberry Mint.

Hydroxycinnamic and hydroxybenzoic acids are the main groups of phenolic acids in these plants (Tanase et al., 2019). The study performed by Tahira et al. (2011) revealed that the major polyphenolic acids in the genus *Mentha* are rosmarinic acid, caffeic acid, ferulic acid, and eugenol. The content of phenolic acids in the ethanolic extracts of the cultivars Chocolate Mint and Strawberry Mint is presented in Table 3. All ethanol extracts of the cv. Chocolate Mint, had a higher content of phenolic acids as compared to the ethanol extracts of the cv. Strawberry Mint. No statistical differences in this index were observed between the groups within the same variant of mint.

It is difficult to compare the acute toxicity of mint extracts with the literature because only a very limited number of ecotoxicological studies concerning the acute toxicity of various substances present in the *Mentha* species were published so far. Recently, Miura et al. (2021) evaluated the toxicological effects of essential oils from different plants, including *Mentha arvensis* L. and *Mentha piperita* L. The authors revealed that the half-maximal effective concentration (EC₅₀) in daphnids after 48 hours of exposure (48 h) was 43.7 mg of *Mentha piperita* essential oil per litre of water medium and *Mentha arvensis* essential oil was less toxic. On the other hand, the EC₅₀ 48 h for menthol,

the predominant monoterpene presented in the *Mentha* species (Eftekhari et al., 2021), is estimated to be 26.6 mg/L (OECD, 2003).

In our study, only the leaves were used for the preparation of water extracts. Based on our findings, we could presume that the daphnids included in our experiment were affected by different substances (only the amounts of polyphenols and phenolic acids were determined in this study) which are naturally presented in the plant of mint and were extracted into the water extracts during their preparation. Overall, the water extract of the cv. Chocolate Mint was nearly 40 times more toxic to daphnids after 48 h of exposure than the water extract of the cv. Strawberry Mint (Table 4 and 5). Within the groups of the same variant of mint, the effect of alginate application on the toxicity of water extracts was not revealed. According to the acute toxicity rating scale for aquatic organisms provided by the U.S. Fish and Wildlife Service as published by El-Harbawi (2014), the water extract of the cv. Chocolate Mint is highly toxic, whereas the water extract of the cv. Strawberry Mint belongs to the group of moderately toxic substances.

Conclusion

We can conclude, that the *Mentha × piperita* (cv. Chocolate mint) possesses more potent antioxidant properties, due to the higher content of total polyphenols and

phenolic acids, as compared to the cv. Strawberry Mint. Furthermore, the toxicity of the cv. Chocolate Mint is much higher than the toxicity of the cv. Strawberry Mint. This phenomenon is probably due to a higher amount of some active substances in the cv. Chocolate Mint as was confirmed by the statistically increased amount of all studied biologically active substances as compared to the cv. Strawberry Mint. The beneficial effect of alginite application on all studied parameters has not been demonstrated in this experiment, but the highest content of total polyphenols (3.37 mg/100g DW) was determined in the aqueous extract of the cv. Chocolate Mint in the control group, and the lowest amount of total polyphenol, only 1.01 mg CAE/100 g DW, was found in the water extract of the cv. Strawberry Mint treated with the application of 1 kg of alginite mixed directly into the soil.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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