The estimation of antifungal activity of essential oil and hydrosol obtained from wrinkled-leaf mint (*Mentha crispa* L.)

BARBARA WÓJCIK-STOPCZYŃSKA*, PAULINA JAKOWIENKO,

GABRIELA WYSOCKA
Department of Horticulture, Laboratory of Storage and Processing
West Pomeranian University of Technology in Szczecin
Słowackiego 17
71-434 Szczecin, Poland

*corresponding author: phone: +48 91 449 6427, e-mail: barbara.wojcik-stopczynska@zut.edu.pl

Summary

The effects of essential oil and hydrosol extracted from dried aerial parts of wrinkled-leaf mint (*Mentha crispa* L.), were determined against *Aspergillus fumigatus, Aspergillus parasiticus, Botrytis cinerea, Cladosporium herbarum, Fusarium oxysporum* and *Penicillium cyclopium* growth. The influence of oil was estimated by disc diffusion agar method and expressed (in mm) as inhibition growth zones of tested strains. Furthermore, the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of oil were determined by microdilution method in liquid medium. Chromatography analysis showed that the main components of oil were: piperitenone (29.9%), pulegone (13.9%) and 1,8-cineole (10.5%). The oil proved the highest inhibitory action against *B. cinerea* (MFC≤0.32 $\mu l \cdot ml^{-1}$, inhibition zone 50.0 mm) and *C. herbarum* (MFC=0.63 $\mu l \cdot ml^{-1}$, inhibition zone 59.3mm), and the least – against *A. parasiticus* and *F. oxysporum*, (inhibition zones respectively 7.0 and 12.7 mm, MIC=5.0 $\mu l \cdot ml^{-1}$, and MFC=10.0 $\mu l \cdot ml^{-1}$). Activity of hydrosol was expressed as a percent of inhibition growth of fungi which was calculated on the ground of diameters of colonies growing on agar medium with addition of 10 and 15% hydrosol and diameters of colonies on control medium. Data showed that antifungal activity of hydrosol was weak. It in higher amounts inhibited the growth of *A. fumigatus* (by 8.7 and 15.9%, respectively, at a concentration in medium of 10 and 15%) and stimulated growth of *B. cinerea* and *P. cyclopium*.

Key words: *Mentha crispa L., essential oil, hydrosol, antifungal activity*

INTRODUCTION

Wrinkled-leaf mint (*Mentha crispa* L.) is an aromatic medicinal plant of the family *Lamiaceae*. It has probably come into existence as a result of multiple crossbreeding of different mint species. At present, it is being showed in nomenclature as a separate species – *Mentha crispa* L. [1], or as a variety of spearmint – *Mentha spicata* L. var. *crispa* L. [2].

Wrinkled-leaf mint demonstrates antioxidative activity [3, 4] as well as antimicrobiological activity [4]. These properties are connected with the presence of different active components which, first of all, are present in essential oil. Its main components are carvone, limonene, 1,8-cyneol and trans-sabinene hydrate [5], whereas menthol occurs in trace amounts, similarly as in the essential oil of spearmint (*Mentha spicata* L.) [2, 4, 6]. Furthermore, polyphenolic compounds (mainly flavonoids) and carotenoids [2, 3] are to be found in wrinkled-leaf mint leaves and shoots, as well as tannins and bitters [2, 6].

Hydrosol is a by-product obtained during hydro-distillation of the plant materials essential oils. It is a complex mixture of water-soluble compounds, containing trace amounts of essential oils. Hydrosols, called aromatic or floral water, are used in cosmetic preparations and drinks. Attention has been attracted by their inhibiting impact on the growth of bacteria [7, 8] and fungi [9-11].

In recent years, research has been conducted on the subject of different mint species, varieties and hybrids as well as their antibacterial [12-15] and antifungal activity [13-16]. The effectiveness of peppermint (*M. piperita* L.) [14, 16-21], spearmint (*M. spicata* L.) [8, 14, 16, 22-24], wild mint (*M. arvensis* L.) [25-27] and pennyroyal (*M. pulegium* L.) [15, 25, 28] against microorganisms has been proved. The antimicrobial effect of mint essential oils has been evaluated more frequently [12, 13, 16, 29] whereas there are only few studies illustrating the effect of mint hydrosols [8, 10]. There are no reports in the available literature referring to antimicrobiological activity of both wrinkled-leaf mint essential oil and hydrosol. In this study, research was undertaken which aimed at evaluation of the antifungal activity of essential oil and hydrosol obtained from wrinkled-leaf mint.

MATERIALS AND METHODS

The experimental materials were essential oil and hydrosol gained from aerial part of wrinkled-leaf mint (*Mentha crispa* L.). The plants were cultivated in 2010 at the Vegetable Experimental Station in Dołuje (West Pomeranian University of Technology, Szczecin, Poland). The oil was extracted from the dried and ground herb in Deryng's apparatus [30] by hydro-distillation, while maintaining the ratio of plant material and distillated water 1:10 (w/v) [31]. Hydrosol was identified as by-product of hydro-distillation. It was filtered through filter paper (Whatman No

1) and sterilized, using membrane filter (pores size 0.45 μm) [7, 9]. The oil and hydrosol were stored in sterile dark containers at 4°C before use [7, 9].

The composition of oil was analyzed by gas chromatography (chromatograph Varian Chrompack CP-3800). Conditions of analysis were as follows: temperature of 50°C (held for 1 min.), gradually increasing (by 4°C/min.) to 250°C (held for 10 min.), column – VF-5ms, carrier gas – helium, flow rate 0.5 ml/min., injector – temperature 250°C, split ratio 1:100, injection – 1 μl of sample, detector – Vatran 4000 MS/MS.

The antifungal activity of oil and hydrosol was tested against six strains of potentially pathogenic and toxigenic fungi: *Aspergillus fumigatus, Aspergillus parasiticus, Botrytis cinerea, Cladosporium herbarum, Fusarium oxysporum* and *Penicillium cyclopium*. They can cause diseases and decay of plants and crops. Some of them are pathogenic for human (eg. *A. fumigatus*) and can raise allergic reactions (eg. *Cladopsporium*, *Botrytis).* The strains originated from the collection of Institute of Fermentation, Technical University, Łódź, Poland. They were stored in cool conditions (4±1°C), at slants with Malt Extract Agar (MEA).

The estimation of oil on the growth of filamentous fungi was determined with the disc diffusion agar method. The sterile culture medium (MEA) was poured into Petri dishes (Ø 90 mm) and after solidification 100 μl of fungal spores were spread. The suspensions of spores were prepared using colonies of the tested fungi which were incubated on slants at 25°C for 7 days. The colonies were flooded with 10 ml of sterile peptone water with 0.05% Tween 20 and agitated for 10 min. (shaker MS 3 Digital, 700 rpm/min.). The suspension of spores was adjusted to the concentration of 10^6 CFU·ml^{-1}. A sterile paper disc (6 mm in diameter) was soaked with 10 μl of oil and placed on a surface of inoculated plates. Distillation water (10 μl/disc) was used as a negative control. After 5 days of incubation at 25°C the diameters of inhibition growth zones were measured (including the disc diameter) and expressed in millimeters [32, 33].

In order to the delimitate MIC and MFC of oil microdilution method (with 96 well microplates) was used [33, 34]. Into the well 95 μl of broth medium (Malt Extract Broth – MEB), 5 μl of spores suspension and 100 μl of oil (at determined concentration from 20 to 0.32 μl·ml^{-1}) were added. The first dilution of oil (at concentration 40 μl·ml^{-1}), was prepared with using 10% DMSO – dimethyl sulfoxide. The next dilutions, in MEB medium, were obtained by two-fold dilutions method. Negative control included: 95 μl of MEB, 100 μl distillation water and 5 μl of inoculum. The microplates were incubated at 25°C for 72-120 h, Turbidity of medium was evidence of fungi development. The contents of wells, in which the growth of fungi was not observed, were sifted on the Petri plates with the MEA, focused on verifying the results. The MIC was defined as the lowest concentration of oil inhibiting the growth of fungi and MFC – the concentration, which caused total inhibition of mycelium growth [34].

The antifungal activity of hydrosol was estimated by the plate method, using the MEA medium with addition of 10 and 15% (v/v) of hydrosol [8, 9]. The medium

without hydrosol was a control. The plates were inoculated on the middle of surface by mycelium (square, the side about 2 mm) cutting with peripheral zone of fungi colony. Incubation was kept at 25°C for 5 days, and then diameters of colonies were measured (in mm). Inhibition of mycelium growth was calculated in percents according to the following formula published by Sağdiç and Özcan (2003) [8] as well as Boyraz and Özcan (2005) [9]:

$$I = [(C - T)/C] \times 100$$

where: I – inhibition of growth [%]
C – colony diameter of control Petri plate [mm]
T – colony diameter of practice Petri plate [mm]

The experiments were performed in triplicate with the same conditions. The obtained results were submitted to analysis of variance. Significant differences between means were assessed using Tuckey's test at significance level $p=0.05$.

RESULTS

Table 1 presents the chemical composition of essential oil isolated from wrinkled-leaf mint. Its basic component was piperitenone (29.86%). Moreover, it was distinguished by pulegone (13.85%) and 1,8-cyneol (10.48%) contents. Other identified components of this essential oil, arranged according to the descending percentage (from 6.92 to 2.17%) are as follows: isopiperitenone, E-caryophyllene, limonene, D germecrene, terpinene-4-ol, menthol, myrcene and sabinene hydrate. Carvone occurring in minute amounted to 0.12%.

Data characterising the antifungal activity of essential oil obtained from wrinkled-leaf mint are presented in table 2. The obtained results show that its essential oil inhibits the growth of all tested filamentous fungi. Growth inhibition zone diameters as well as MIC and MFC were within a range of 7.0 to 59.3 mm as well as <0.32 to 5.0 $\mu l \cdot ml^{-1}$ and ≤ 0.32 to 10.0 $\mu l \cdot ml^{-1}$, respectively.

The size of fungal growth inhibition zones was significantly diversified depending on fungi species towards which the essential oil had been used. The essential oil had the strongest inhibiting effect on the growth of *C. herbarum* (inhibition zone of 59.3 mm), followed by *B. cinerea* (inhibition zone of 50.0 mm), whereas the smallest one on the development of *A. parasiticus* (mean growth inhibition zone of 7.0 mm). The abovementioned results were confirmed by the MIC and MFC values for the examined essential oil determined for these fungi species. They were <0.32 and ≤ 0.32 $\mu l \cdot ml^{-1}$, respectively, for *B. cinerea* while 0.32 and 0.63 $\mu l \cdot ml^{-1}$, respectively, for *C. herbarum*. On the other hand, *A. parasiticus* and *F. oxysporum* required the highest concentrations of essential oil to obtain the inhibiting and cidal effect for which MIC amounted to 5.0 and MFC to 10 $\mu l \cdot ml^{-1}$.

Table 1.

Chemical composition of wrinkled-leaf mint essential oil

No.	Component	Retention time	Yield [%]
1	Piperitenone	22.87	29.86
2	Pulegone	19.34	13.85
3	1,8-Cineole	11.83	10.48
4	2-Cyclohexen-1-one,3-methyl-6-1(methylethenyl), (S) (isopiperitenone)	20.47	6.92
5	E-caryophyllene	25.29	4.18
6	Limonene	11.69	3.99
7	D Germacrene	27.22	3.56
8	Terpinen-4-ol	17.26	3.41
9	Menthol	17.12	2.73
10	Myrcene	10.25	2.31
11	cis-Sabinene hydrate	13.25	2.17
12	cis-Muurola-4 (14),5-diene	26.63	1.44
13	beta-Pinene	9.74	1.24
14	gamma-Terpinene	12.75	1.04
15	Bicyclogermacrene	27.68	0.99
16	Carvone	19.66	0.12
		Total:	88.29

Table 2.

Antifungal activity of wrinkled-leaf mint essential oil

Tungi	The inhibition growth zones (mm)	MIC (μl•ml-1) [a]	MFC (μl•ml-1) [b]
Aspergillus fumigatus	16.7±1.0	2.5	5.0
Aspergillus parasiticus	7.0±0.0	5.0	10.0
Botrytis cinerea	50.0±0.0	Lower than 0.32	0.32 or lower
Cladosporium herbarum	59.3±1.2	0.32	0.63
Fusarium oxysporum	12.7±0.6	5.0	10.0
Penicillium cyclopium	18.7±1.2	2.5	5.0
Mean	27.4±0.7		
LSD 0,05	2.045		

[a] MIC – minimal inhibitory concentration of growth of fungi
[b] MFC – minimal fungicidal concentration
negative control – water – no inhibition effect

Table 3 presents the results illustrating the effect of wrinkled-leaf mint hydrosol on the colony growth of the examined fungi. The statistical analysis of results demonstrated that the hydrosol effect significantly depended on its concentration in the medium and the examined strain. In general, hydrosol applied in a 15% dose demonstrated stronger effect on the growth of fungi. However, it was found that its effect on the development of the examined fungi differed. Hydrosol stimulated the growth of *B. cinerea* and *P. cyclopium* colonies in both applied doses, whereas it demonstrated a weak inhibiting effect in relation to other strains. The highest colony growth inhibition (8.7% and 15.9% at a hydrosol concentration in the medium of 10% and 15%, respectively) was characteristic for *A. fumigatus*.

Table 3.

Percentage inhibition of fungi growth by wrinkled-leaf mint hydrosol

Fungi	Concentration of hydrosol in culture medium		Mean
	10%	15%	
Aspergillus fumigatus	8.7±0.0 [a]	15.9±1.3	12.3±0.7
Aspergillus parasiticus	5.5±1.6	6.4±1.6	6.0±1.6
Botrytis cinerea	–68.9±0.0 [b]	–68.9±0.0	–68.9±0.0
Cladosporium herbarum	6.3±0.0	0.0±0.0	3.2±0.0
Fusarium oxysporum	3.3±1.2	3.3±1.2	3.3±1.2
Penicillium cyclopium	–9.9±0.0	–19.6±2.1	–14.8±1.1
Mean	–9.2±0.5	–10.5±1.0	
$LSD_{0,05}$ for	1 – concentration	0.759	
	2 – strain	1.969	
	1 x 2 interaction	1.858	

[a] mean value ± standard deviation (n=3)
[b] minus before value marks stimulation function

DISCUSSION

The antifungal activity of wrinkled-leaf mint essential oil demonstrated in this study corresponds to the results of studies showing that essential oils of other mint species have also an inhibiting effect on the colony growth of filamentous fungi [13, 14, 17, 22, 28, 35]. It was proved that peppermint essential oil had an inhibiting effect on the fungi species such as *A. flavus*, *A. niger*, *Mucor rouxii*, *Mucor* spp. and *T. rubrum* [18, 36], spearmint essential oil on *A. flavus*, *A. niger*, *A.*

ochraceus and *Fusarium moniliforme* [22, 37], whereas wild mint essential oil on *A. flavus*, *A. vericolor*, *Fusarium nivale*, *F. roseum*, *Alternaria alternata*, *Trichoderma viride* and *Monilia* sp. [27].

It was demonstrated in the experiment that wrinkled-leaf mint essential oil inhibited the growth of *Cladosporium herbarum, Botrytis cinerea, Penicillium cyclopium, Aspergillus fumigatus, Fusarium oxysporum* and *Aspergillus parasiticus* (in the descending order depending on the size of inhibition zone). Bansod and Rai [22] proved that the growth of *A. fumigatus* was also inhibited by spearmint essential oil. Depending on a dose of the examined essential oil, they obtained growth inhibition zones being within a range of 10.0 to 16.0 mm (thus being similar to those obtained in this study), whereas the MIC value for the essential oil towards that strain amounted to 0.5 % (v/v) and 0.12 % (v/v), depending on the research method applied. Khan and Ahmad [36] demonstrated the susceptibility of *A. fumigatus* and *F. oxysporum* to peppermint essential oil obtaining inhibition zone diameters for their growth amounting to 37.33 and 19.66 mm, respectively. Kumar et al. [27] found that inhibition of the growth of *F. oxysporum* by wild mint essential oil amounted to 89.1%. On the other hand, this essential oil inhibited completely *A. fumigatus* and *Penicillium* sp. development.

Researches show that particular strains within a species may demonstrate different resistance to the effect of essential oil. This results from the properties of both microorganism itself and essential oil, including in particular its chemical composition [37]. In the chemical composition of the essential oil used in the experiment, piperitenone percentage was the highest, followed by that of pulegone and 1,8-cyneol. Limonene and sabinene hydrate were also present in it. Gherman et al. [5] report that 1,8-cyneol, limonene and sabinene are characteristic components of wrinkled-leaf mint essential oil but its predominant component is carvone which was found in a minute amount in the essential oil examined in this study. In the opinion of Kokkini et al. [38] as well as Wyk and Vink [2] wrinkled-leaf mint essential oil should contain a considerable amount of carvone, similarly as the essential oil obtained from spearmint. On the other hand, Kumar et al. [39] report that high carvone content in the essential oil is characteristic, as a rule, of three mint species, i.e. the above-mentioned spearmint (*M. spicata* L.), horse mint (*M. longifolia* (L.) Huds.) and apple mint (*M. suaveolens* Ehrh.). It should be also stressed that researches of many authors show the chemical composition of the essential oil obtained from the same plant species to undergo significant quantitative and qualitative changes under the effect of diversified geographical and agrotechnical conditions and vegetation conditions [39-42].

It can be stated that all components found in the examined essential oil were identified in the essential oils of plants of the family *Mentha* L., including pennyroyal [15], peppermint [2, 4, 18, 43, 44], spearmint [42] and round leaf mint (*M. rotundifolia* (L.) Huds.) [12].

The demonstrated antifungal activity of the examined essential oil may be combined with a considerable percentage of such components like pulegone

(menthone derivative) and 1,8-cyneol. Gulluce et al. [13] proved a strong antimycological effect of horse mint essential oil which, in the opinion of these authors, was connected with high content of pulegone (15.5%). Soković et al. [16] demonstrated a considerable effect of 1,8-cyneol and limonene on fungal growth reduction. However, attention should be put to the fact that essential oil properties are being also developed by secondary substances with a smaller percentage in the general profile of its components [25].

The hydrosol obtained from wrinkled-leaf mint was characterised by weaker antifungal activity than that of essential oil, which probably results from differences in the chemical composition of these two derivatives. Usually, other predominant components are being found in hydrosols than in essential oils, with lower percentages [45]. The examined hydrosol showed, depending on the strain, both the stimulating and the inhibiting effect of the fungal growth. Boyraz and Özcan [9] and Özcan [11] reported a similar diverse effect (inhibiting and stimulating) of hydrosols from different herbs of the family *Lamiaceae*. The inhibition of fungal growth by the hydrosol evaluated in this study may be defined as low. Inouye et al. (2009) [10] also found a weak inhibiting effect of peppermint hydrosol, as well as a moderate one of spearmint hydrosol, on the growth of *Candida albicans*. Other researches show that mint hydrosol induced inhibition of the growth of filamentous fungi [11] but sometimes did not show any microbiological activity [8].

In the examinations of antimicrobial activity shown by plant substances, a potential opportunity of using them in the role of natural preservatives or botanical pesticide is being noticed [29]. The obtained results point to the possibility of wrinkled-leaf mint essential oil application as a natural fungicide, in particular being effective towards such harmful fungi as *B. cinerea* and *C. herbarum*.

CONCLUSIONS

1. The wrinkled-leaf mint essential oil has the higher antifungal activity than hydrosol extracted from this plant. Piperitenone, pulegone and 1,8-cineole were the main components of the oil.
2. The oil inhibited and ceased the growth of all the tested strains, but its activity was varied significantly depending on a species of a fungi. *B. cinerea* and *C. herbarum* were the most sensitive to the oil, whereas *A. parasiticus* and *F. oxysporum* were most resistant to it.
3. Activity of hydrosol depended significantly on a dose and its influence on the fungi was differentiated – it inhibited the growth of *A. fumigatus, A. parasiticus, C. herbarum* and *F. oxysporum* and stimulated the growth of *B. cinerea* and *P. cyclopium*.

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REFERENCES

4. Kołodziej B. (ed.). Uprawa ziół. Poradnik dla plantatorów. Poznań 2010.
5. Wyk B-E, Wink M. Rośliny lecznicze świata. Wrocław 2008:206-7.
6. Grzeszczuk M, Jadczak D. Estimation of biological value of some species of mint (*Mentha* L.). Herba Pol 2009;53(3):193-9.
7. Kohlmünzer S. Farmakognozja. Warszawa 2010.
8. Gherman C, Culea M, Cozar O. Comparative analysis of some active principles of herb plants by GC/MS. Talanta 2000; 53:253-62.
9. Volák J, Stodola J. Rośliny lecznicze. Warszawa 1987:200.
10. Sağdiç O. Sensitivity of four pathogenic bacteria to Turkish thyme and oregano hydrosols. Lebensm-Wiss u-Technol 2003; 36:467-73.
11. Sağdiç O, Özcan M. Antibacterial activity of Turkish spice hydrosols. Food Control 2003; 14:141-3.
12. Boyraz N, Özcan M. Antifungal effect of some spice hydrosols. Fitoterapia 2005; 76:661-5.
13. Inouye S, Takahashi M, Abe S. Inhibitory activity of hydrosols, herbal teas and related essential oils against filament formation and the growth of *Candida albicans*. Jpn J Med Mycol 2009; vol. 50:243-51.
14. Özcan M. Effect of spice hydrosols on the growth of *Aspergillus parasiticus* NRRL 2999 strain. J Med Food 2005; 8:275-8.
15. Derwich E, Benziane Z, Boukir A. Antibacterial activity and chemical composition of the leaf essential oil of *Mentha rotundifolia* from Morocco. Electronic Journal of Environmental, Agricultural and Food Chemistry 2010; 9(1):19-28.
16. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. Food Chem 2007; 103:1449-56.
17. Hammer K A, Carson C F, Riley T V. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999; 86:985-90.
18. Mahboubi M, Haghi G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J Ethnopharmacol 2008; 119:325-7.
19. Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, van Griensven LJLD. Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. Molecules 2009; 14:238-49.
20. Giamperi L, Fraternale D, Ricci D. The in vitro action of essential oils on different organisms. J Esst Oil Res 2002; 14(4):312-18.
21. Kumar Tyagi A, Malik A. Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapour phase against food spoiling microorganisms. Food Control 2011, 22:1707-14.
22. Saeed S, Tariq P. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. Pak J Bot 2005; 37(4): 997-1001.
23. Singh R, Shushni M, Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. Arabian J Chem 2011. (in press).
24. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control 2010; 21:1199-18.
25. Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. World Journal of Medicinal Sciences 2008; 3(2):81-8.

26. Özcan M, Erkmen O. Antimicrobial activity of essential oils of Turkish plant spices. Eur Food Technol 2001; 212:658-60.
27. Shapiro S, Meier A, Guggenheim B. The antimicrobial activity of essential oil and essential oil components towards oral bacteria. Oral Microbiol Immunol 1994; 9:202-8.
28. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. Food Chem Toxicol 2008; 46:446-75.
29. Johnson M, Wesely E G, Kavitha M S, Uma V. Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L. Asian Pacific J Trop Med 2011;4(3):196-200.
30. Kumar A, Shukla R, Singh P, Singh A K, Dubey N K. Use of essential oil from *Mentha arvensis* L. to control storage moulds and insects in stored chickpea. J Sci Food Agric 2009; 89:2643-9.
31. Daferera D J, Ziogas B N, Polissiou M G. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *Michiganensis*. Crop Protection 2003; 22:39-44.
32. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. Intern J Food Microbiol 2004; 94:223-53.
33. PN-ISO 6571:2001. Przyprawy i zioła. Oznaczanie zawartości olejku eterycznego.
34. Karwowska K, Przybył J. Suszarnictwo i przetwórstwo ziół. Warszawa 2005:109.
35. Peéiulytë D. Effect of tea tree essential oil on microorganisms 2. Evaluation of fungal reaction to tea tree oil under different conditions. Biologia 2005; 2:21-28.
36. Adiguzel A, Ozer H, Kilic H, Cetin B. Screening of antimicrobial activity of essential oil and methanol extract of *Satureja hortensis* on foodborne bacteria and fungi. Czech J Food Sci 2007; 25(2):81-9.
37. Şahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G, Özer H. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. Food Control 2004; 15:549-57.
38. Soliman K M, Badeaa R I. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 2002; 40:1669-75.
39. Khan M S, Ahmad I. Antifungal activity of essential oils and their synergy with fluconazole against drug-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum*. Appl Microbiol Biotechnol 2011; 90:1083-1094.
40. Kalemba D. Przeciwbakteryjne i przeciwgrzybowe właściwości olejków eterycznych. Post Mikrobiol 1998; 38(2):185-203.
41. Kokkini R, Balta M, Hanlidou E, Kokkini S. „Mints", smells and traditional uses In Thessaloniki (grece) and other Mediterranean countries. J Ethnopharmacol 1995; 109:248-57.
42. Kumar P, Mishra S, Malik A. Insecticidal properties of *Mentha* species: A review. Industrial Crops Prod 2011; 34:802-17.
43. Karousou R, Balta M, Hanlidou E, Kokkini S. „Mints", smells and traditional uses In Thessaloniki (Greece) and other Mediterranean countries. Journal of Ethnopharmacology 2007; 109:248-57.
44. Lorenzo D, Paz D, Dellacassa E, Davies P, Vila R, Cañigueral S. Essential oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. Brazilian Arch Biol Technol 2002;45(4):519-24.
45. Telci I, Demirtas I, Bayram E, Arabaci O, Kacar O. Environmental variation on aroma components of pulegone/pieritone rich spearmint (*Mentha spicata* L.). Industrial Crops Prod 2010; 32:588-92.
46. Aflatuni A. The yield and essential oil content of mint (*Mentha ssp.)* in Northern Ostrobothnia. Oulu 2005:50.
47. İşcan G, Kirimer N, Kürkcüoğlu M, Hüsnü can Başer K, Demirci F. Antimicrobial screening of *Mentha piperita* essential oils. J Agric Food Chem 2002; 50:3943-6.
48. Inouye S, Takahashi M, Abe S. A comparative study on the composition of forty four hydrosols and their essenial oils. Int J Ess Oil Ther 2008;2(3):89-104.

OCENA AKTYWNOŚCI ANTYGRZYBICZEJ OLEJKU ETERYCZNEGO I HYDROZOLU UZYSKANYCH Z MIĘTY KĘDZIERZAWEJ (*MENTHA CRISPA* L.)

BARBARA WÓJCIK-STOPCZYŃSKA*, PAULINA JAKOWIENKO, GABRIELA WYSOCKA

Katedra Ogrodnictwa
Pracownia Przechowalnictwa i Przetwórstwa
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie
ul. Słowackiego 17
71-434 Szczecin, Polska

*autor, do którego należy kierować korespondencję: tel.: +48 91 449 6427,
e-mail: barbara.wojcik-stopczynska@zut.edu.pl

S t r e s z c z e n i e

Określono działanie olejku eterycznego i hydrozolu, otrzymanych z wysuszonych, nadziemnych pędów mięty kędzierzawej (*Mentha crispa* L.), na wzrost grzybów: *Aspergillus fumigatus, Aspergillus parasiticus, Botrytis cinerea, Cladosporium herbarum, Fusarium oxysporum* i *Penicillium cyclopium*. Wpływ olejku zbadano metodą krążkową, mierząc (w mm) strefy zahamowania wzrostu grzybów. Ponadto wyznaczono dla olejku minimalne stężenie hamujące (MIC) oraz grzybobójcze (MFC), stosując metodę mikrorozcieńczeń w podłożu płynnym. Analiza chromatograficzna wykazała, że głównymi składnikami olejku były: piperytenon (29,9%), pulegon (13,9%) i 1,8-cyneol (10,5%). Olejek wykazywał najwyższą aktywność wobec *B. cinerea* (MFC ≤0,32 $\mu l \cdot ml^{-1}$, strefa zahamowania 50,0 mm) oraz *C. herbarum* (MFC=0,63 $\mu l \cdot ml^{-1}$, strefa zahamowania 59,3 mm), natomiast najmniej ograniczał wzrost *A. parasiticus* i *F. oxysporum* (strefy zahamowania odpowiednio 7,0 i 12,7 mm, MIC=5,0 $\mu l \cdot ml^{-1}$, a MFC=10,0 $\mu l \cdot ml^{-1}$). Aktywność hydrozolu wyrażano jako procent zahamowania wzrostu grzybów, który obliczano na podstawie wielkości średnic kolonii rosnących na podłożu agarowym z dodatkiem 10 i 15% hydrozolu oraz średnic kolonii na podłożu kontrolnym. Badania wykazały, że działanie przeciwgrzybicze hydrozolu było słabe. Najbardziej hamował on wzrost *A. fumigatus* (o 8,7 i 15,9%, odpowiednio przy stężeniu w podłożu 10 i 15%), a stymulował wzrost *B. cinerea* i *P. cyclopium*.

Słowa kluczowe: *Mentha crispa* L.*, olejek eteryczny, hydrozol, aktywność przeciwgrzybicza*