Influence of essential oils from different varieties of peppermint (*Mentha x piperita* L.) on growth of some filamentous fungi

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S u m m a r y

The aim of this study was the estimation of influence of essential oils deriving from two varieties of peppermint: white mint (*Mentha x piperita* L var. *officinalis* Sole f. *pallescens* Camus) and bergamot mint (*Mentha x piperita* L. var. *citrata* Ehrh.), on the growth of some filamentous fungi: *Aspergillus niger, Botrytis cinerea, Eurotium amstelodami, Eurotium chevalieri, Penicillium cyclopium* and *Trichothecium roseum*. The oils were extracted from dried, ground herb by means of hydro-distillation method. The disc diffusion method was used to estimation an antifungal activity of oils (doses of oil: 10 and 5 μl/disc). The diameters of growth inhibition zones were measured and expressed in mm. It was stated that the oils from both varieties of peppermint inhibited the growth of all tested fungi. The activity of oils was significantly differentiated in dependence on kind of oil, dose of oil and species of fungi. The oil from bergamot mint (in both doses) was more effective to most of the tested strains than the oil from white mint. It also possessed a greater content of carvone and pulegone. Usually, the inhibition growth zones at the oil dose of 10 μl/disc were proportionally bigger than at dose of 5 μl/disc. The strains of *P. cyclopium* and *A. niger* were most resistant to the influence of both oils – the average inhibition growth zones were 12.4 and 16.7 mm, respectively. *E. chevalieri* and *E. amstelodami* were most sensitive – average zones of their growth inhibition amounted to 90.0 and 75.9 mm, respectively.

Key words: *white mint, bergamot mint, essential oil, antifungal activity*

INTRODUCTION

The genus *Mentha* L. is represented by the numerous species of plants differing from one another by the shape and the colouring of the sprouts and blossoms. The commonly known species of this genus are: peppermint (*M. x piperita* L.), corn mint (*M. arvensis* L.), spear mint (*M. spicata* L. syn: *M. viridis* L.) and water mint (*M. aquatica* L.) [1]. The peppermint is a mixture generated by crossing spear mint and water mint [2-4]. The mint is a valuable oil producing raw material. The content of the essential oil in the peppermint leaves is within 1.0-3.0%. Its characteristic component is menthol whose share is approximately 50% [2, 5, 6]. Also limonene, menthone, cineole, pulegone, caryophyllene and bourbonene are considered as the basic components of the peppermint oil [2, 7].

The peppermint oil is very popular and widely utilised in the foodstuff, cosmetic and pharmaceutical industries. Apart from the special taste and flavour it also shows antiseptic properties, inhibiting also the spreading of the insects and parasites [2-4, 6].

The investigations of the anti-microorganism properties of the plant essential oils have a long tradition, and they are still useful and continued. They lead to the indication of the species and varieties of plants of highly active oil contents that inhibit the development and spreading of the undesirable microorganism groups. Such plant-origin raw materials are likely to provide the source of the natural, ecological and safe substances which might be used among others for the raw material and ready foodstuff preservation [8].

In the previous investigations more attention has been paid to the antibacterial activities of the peppermint oil [9-13]. Whereas its inhibiting effect on the growth of the mould fungi [7] and yeasts [13, 14] is less known. Particularly rare is the information regarding the activity of the peppermint oil towards fungi of the species *Eurotium* [15], considered as likely to be pathogenic and toxigenic. The data that characterise the antifungal activity of the volatile oils originating from some special varieties of peppermint are also scarce [16].

The purpose of the article has been to evaluate the inhibition of the growth of some mould fungi by the volatile oils obtained from two varieties of the peppermint – the white and the bergamot ones.

MATERIALS AND METHODS

The essential oils from two varieties of pepper mint: white mint (*Mentha x piperita* L var. *officinalis* Sole f. *pallescens* Camus) and bergamot mint (*Mentha x piperita* L. var. *citrata* Ehrh.), constituted material of the research. The plants were cultivated at the Vegetable Experiment Station of West Pomeranian University of Technology in Szczecin, Poland. The herbs (shoots with leaves) were collected just before flowering. They were sorted and then dried in room temperature. Essential oils were

extracted from the dried, ground material by hydro-distillation method, in Deryng's apparatus [17, 18]. The hydro-distillation was carried out with maintaining proportions 1:10 (w/v) between the dried herb and the distillated water. After steam distillation (3h) the oils were isolated and dried over anhydrous sodium sulphate. Before the test, they were stored in dark at 4°C [19]. The composition of oils was analyzed by gas chromatography using Varian 4000 GC/MS/MS apparatus.

The antifungal activity of oils was tested against potential pathogenic and toxigenic six strains: *Aspergillus niger, Botrytis cinerea, Eurotium amstelodami, Eurotium chevalieri, Penicillium cyclopium* i *Trichothecium roseum*. The fungi were isolated from a plant material and identified according to the rules of Klich (2002) [20] and Samson *et al.* (1996) [21]. The sensitivity of strains to the essential oils was determined by the disc diffusion agar method. 20 ml of warm medium (Malt Extract Agar or GC 18 Agar in the case of *Eurotium* strains) was poured into 90 mm Petri dishes [22, 23]. After solidification, 100 μl of fungal spores suspension were spread over the agar plates. The suspensions of spores were prepared using colonies of tested fungi which were incubated at 25°C for 7 days. Each colony was flooded by 5 ml of sterile peptone water with 0.05% of Tween 20 [24]. The suspension was adjusted to concentrations 10^6 CFU·ml^{-1} by dilution with sterile peptone water. A sterile paper disc (6 mm in diameter) was soaked with oil and placed on a surface of inoculated agar plates. Each oil was used in two doses – 10 and 5 μl/disc. The inoculated plates with oil soaked discs were incubated at 25°C for 72 hours. After this time the diameters of inhibition growth zones (including 6 mm of disc diameter) were measured and expressed in millimeters. Sterile distillated water constituted negative control [25]. Actidion (cycloheximid) and potassium sorbate (30μg/disc) were used as positive controls.

Studies were performed in triplicate and mean values were calculated. The obtained results were statistically analysed by 3-way analysis of variance (1st factor – source of oil = pepper mint variety, 2nd factor – dose of oil, 3rd factor – species of fungi). Significant differences between means were assessed using Tuckey's test at significance level P=0.05.

RESULTS

The data characterising the effect of essential oils originating from two varieties of peppermint onto the growth of the investigated mould fungi are shown in table 1. The results obtained indicate that both volatile oils have had inhibiting influence on the growth of all the strains under investigation. However, the size of the fungi growth inhibition zones has been significantly differentiated in relation to the oil kind (variety of peppermint), its dose applied and fungus species towards which the oil has been applied.

While analysing the results obtained, it is not possible to indicate one single volatile oil which would demonstrate higher efficiency than the others in acting

against each of the examined strains. However, the conducted investigations prove that the activity of the bergamot mint has been of generally stronger nature than the oil extracted from white mint – the average diameters of the tested fungi growth inhibition zone have been 49.9 and 43.0 mm, respectively. Under the influence of the oil extracted from the bergamot mint the average growth inhibition zone of *B. cinerea, P. cyclopium* and *E. amstelodami* (52.7, 14.0, 80.7 mm respectively) has been significantly bigger than that obtained while using the white mint oil (accordingly 30.3, 10.7 and 71.0 mm). On the other hand, the white mint oil has inhibited the growth of *A. niger* stronger than bergamot mint oil (inhibition zone 17.8 and 15.5 mm respectively). Nevertheless, both oils similarly affected the growth of *T. roseum* and *E. chevalieri*.

The oils extracted from both varieties of the peppermint applied in the 10 μl dose have been more effective (generally in ca 50%) in inhibiting the growth of the tested fungi than the same applied in 5 μl dose. However, in case of the *E. chevalieri* strain both oils, in both doses, have fully inhibited its development (inhibition zone of 90.0 mm). The 5 μl dose of bergamot mint oil has also been sufficient to completely inhibit the growth of *E. amstelodami* and significantly limit the growth of *B. cinerea,* approximating that obtained with 10 μl dose (inhibition zone 50.3 and 55.0 mm, respectively).

Amongst the tested strains the highest sensitivity to the activities of both oils has been displayed by *E. chevalieri*, next *E. amstelodami* (average inhibition zone of their growth: 90.0 and 80.8 mm). The highest resistance has been demonstrated by *P. cyclopium* and *A. niger*. The average inhibition zones of their growth have been 12.3 and 16.7 mm.

In the experimental conditions the activities of the oils extracted from both varieties of the pepper mint have been stronger than within the positive control samples. Potassium sorbate failed to show the activity in inhibiting the growth of the fungi under investigation and actidione (cycloheximide) has had the inhibiting effect on the growth of *B. cinerea, E. amstelodami* and *E. chevalieri* strains only.

Most probably it has been their contents (table 2) that influenced the variations in the antifungal activities of both oil varieties disclosed in the investigation conducted. In the oils extracted from both varieties of the peppermint carvone dominated. Its content in the bergamot mint oil has been, however, significantly larger (77.61%) than in the white mint oil (63.31%). The oil extracted from the bergamot mint has also distinguished itself by the content of pulegone (8.04%) which has not been found in the white mint oil. On the other hand, the white mint oil has been characterised by the higher content – than in case of bergamot mint oil – of the following: E – caryophyllene, limonene, cis – dihydro-carvone, germacrene D, 1,8-cineole, β-bourbonene and β-pinene. While considering the results obtained, it can be however assumed that these specified components, differentiating the oil extracted from the white mint, have had no visible effect on the increase of its antifungal activities.

Table 1.

Average diameters (mm) of inhibition growth zones of fungi in dependence on kind of oil, dose of oil and strains of fungi

kind of oil	dose of oil	*A. niger*	*B. cinerea*	*P. cyclopium*	*T. roseum*	*E. amstelodami*	*E. chevalieri*	Means
white mint oil	10 µl	24.0	40.0	15.3	48.3	90.0	90.0	51.3
	5 µl	11.7	20.7	6.0	27.3	53.0	90.0	34.8
bergamot mint oil	10 µl	25.0	55.0	16.0	50.0	90.0	90.0	54.3
	5 µl	6.0	50.3	12.0	24.7	90.0	90.0	45.5
means for variety (1 fact.)	White mint	17.8	30.3	10.7	37.8	71.5	90.0	43.0
	Bergamot mint	15.5	52.7	14.0	37.3	90.0	90.0	49.9
means for oil dose (2 fact.)	10 µl	24.5	47.5	15.7	49.2	90.0	90.0	52.8
	5 µl	8.8	35.5	9.0	26.0	71.5	90.0	40.1
means for strain (3 fact.)		16.7	41.5	12.3	37.6	80.8	90.0	
$LSD_{0.05}$ for	1 – pepper mint variety	0.632	LSD for interaction:		1 x 2	0.378		
	2 – dose of oil	0.267			2 x 3	0.728		
	3 - strain	0.515			1 x 3	0.728		

Inhibition zones for controls: water – no inhibition effect; potassium sorbate – no inhibition effect; actidion – *B. cinerea* (21.7 mm), *E. amstelodami* (14.5 mm), *E. chevalieri* (12.0 mm), no inhibition effect against *A. niger*, *P. cyclopium*, *T. roseum*

Table 2.

Percentage composition of essential oils of white mint and bergamot mint

No.	components	yield [%]	
		white mint oil	bergamot mint oil
1.	carvone	63.31	77.61
2.	E-caryophyllene	5.59	1.57
3.	limonene	5.03	2.83
4.	cis-dihydro carvone	4.77	1.78
5.	germacrene D	4.20	0.52
6.	1,8-cineole	3.99	0.65
7.	β-bourbonene	1.75	0.85
8.	pulegone	tr.	8.04
9.	β-elemene	0.13	1.25
10.	menthone	–	0.62
11.	iso-menthone	tr.	0.23
12.	β-pinene	0.28	0.19

tr. – trace = less than 0.05%
– absent

DISCUSSION

The results presented in this article are compatible with the studies of the other authors, indicating that the essential oil extracted from the peppermint has an inhibitive effect on the growth of the mould fungi colonies, germinating of the fungi spores [7, 15, 26] and the development of yeast [4, 13, 14]. It has been proven that the peppermint oil inhibits the growth of among others: *Aspergillus niger, A. flavus, A. versicolor, A. terreus, Penicillium ochrochloron, P. corylophilum, Trichoderma viride* and *Fusarium tricinctum* [7, 15] and the yeast: *Candida albicans* [13, 14]. The previous reports regarding the activities of the peppermint oil against *Eurotium* species fungi are scarce [15]. They indicate that the colonies: *E. amstelodami, E. herbariorum, E. repens*, *E. rubrum*, while growing on the substrate with the addition of peppermint oil have reached the diameter within 0 and 52.7 mm. The most sensitive was the strain *E. amstelodami*, the least – *E. herbariorum*. The results of this paper also confirm the sensitivity of the *E. amstelodami* strain toward the activity of the peppermint oils. It should be noted that the sensitivity of a microorganism is a result of the properties of both microorganism as well as the oil. Within a species, also individual strains may be characterised by various sensitivity to effect of the oil [8].

Fungi belonging to genus *Eurotium* can grow in products which are characterised by a relatively low level of moisture. Therefore, their growth can decrease the quality of stored seeds [15]. The results of this work indicate potential possibility to use both

tested oils (especially bergamot mint oil) as natural antifungal agents against *Eurotium* strains.

The researches presented in this article have proven that the examined essential oils have demonstrated stronger antifungal effect that substances used in control samples. Potassium sorbate, recognised as a preservative strongly acting against yeast and moulds [27], has failed to inhibit the growth of any strains under examination. Whereas Abdel-Hafez and El-Said (1997) [28] have proven that another commonly applied preservative – sodium benzoate in the concentration 1.0; 1.5 and 2.0% (w/v) has completely inhibited the growth of the mould fungi examined by them. The application of actidione in this study was also less effective than the investigation references might suggest [29].

The anti-microorganism activity of a herbal derivative depends on the applied concentration [30, 31]. A specific oil dose may have inhibiting effect both in relation to the growth of the mycelium, colonies quantity or any possible production of mycotoxins [32-34]. Usually the highest antimycological activity of an oil is recorded while its bigger dose is applied [8, 35, 36], which is confirmed also by the results of the presented experiment. However, the application of twice lower oil dose not always caused twice as low inhibition of the growth of a given strain. The similar lack of relationship has been observed by Singh *et al.* (2006) [36], while examining the antifungal effectiveness of the volatile oil extracted from fennel (*Foeniculum vulgare* L.) applied in 2, 4 and 6 μl/disc concentrations.

The results of the study have proven that the essential oils extracted from two varieties of peppermint demonstrated varying degrees of antifungal activity. This probably results from the differences in the chemical contents of these oils. The results of the investigations of other authors indicate that the percentage participation of the individual oil components and its antimicrobiological activity depend on numerous factors, including the species and variety of a plant (with the particular consideration of the plant's genotype and chemotype) [6, 8, 37]. Many authors, while describing or examining the contents and antimicrobiological activities of various peppermint derivatives draws attention to the fact that the strong influence of these preparations is related with their high content of carvone, menthone or menthol [4, 7, 11, 38-40].

Carvone has prevailed in the contents of the oils extracted from the examined varieties of peppermint. From the literature references is can be concluded that the dominance of carvone in the contents of the oil is also typical for the oil extracted from spear mint [7, 39]. The comparison of our results to the studies of other authors analysing the peppermint oil contents indicates that the oils from tested varieties of pepper mint had low amounts of menthol and menthone [4, 7, 38]. Only bergamot mint oil has been characterised by a minor content of menthone (0.62%) and iso--menthone (0.23%) and the presence of pulegone (8.04%) – a derivative of menthone. The bergamot variety has contained nearly 15% more carvone than the white variety and ca 8% of pulegone which has been found only in trace amounts in white mint oil. The bergamot mint oil has been also characterised with strong antifungal activity. The higher inhibition effect of this oil is probably related with the significant content of

the aforesaid components. The high antifungal efficiency of carvone – exceeding that of menthone, 1,8-cineole and limonene – has been proven by Soković *et al.* (2009) [7]. In the opinion of Gulluce *et al*. (2007) [1], the high antimicrobiological activity of horsemint oil (*M. longifolia* L. Huds.) has been related with the large content of pulegone (15.5%) in this oil.

CONCLUSIONS

1. The essential oils extracted from the investigated varieties of peppermint – the white and the bergamot ones – have had the inhibiting effect on the growth of all the tested fungi. The oil inhibiting effect has been varied depending on the origin and dose of oil and on the strain species.

2. The inhibiting effect of the bergamot mint oil in both doses applied has been generally stronger than the oil extracted from the white mint. The higher antifungal activity can be justified in the significant content of carvone and presence of pulegone in this oil.

3. The application of higher concentrations of pepper mint oils usually caused increase of the growth inhibition of tested fungi.

4. The highest sensitivity to the oils extracted from both varieties of peppermint has been demonstrated by *E. chevalieri* and *E. amstelodami*, whereas *P. cyclopium* and *A. niger* were the most resistant.

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WPŁYW OLEJKÓW ETERYCZNYCH Z RÓŻNYCH ODMIAN MIĘTY PIEPRZOWEJ (*Mentha x piperita* L.) NA WZROST NIEKTÓRYCH GRZYBÓW PLEŚNIOWYCH

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

Celem pracy była ocena wpływu olejków eterycznych z dwóch odmian mięty pieprzowej: białej (*Mentha x piperita* L var. *officinalis* Sole f. *pallescens* Camus) oraz cytrynowej (*Mentha x piperita* L. var. *citrata* Ehrh.), na wzrost szczepów grzybów strzępkowych z gatunków: *Aspergillus niger, Botrytis cinerea, Eurotium amstelodami, Eurotium chevalieri, Penicillium cyclopium* i *Trichothecium roseum*. Olejki pozyskano z suszonego, rozdrobnionego ziela metodą hydrodestylacji. Aktywność przeciwgrzybową olejków określano za pomocą metody krążkowej (dawka olejku 10 i 5 μl/krążek), mierząc w mm strefy zahamowania wzrostu kolonii szczepów. Stwierdzono, że olejki z obu odmian mięty pieprzowej działały hamująco na wzrost wszystkich badanych grzybów. Aktywność przeciwgrzybowa olejków była istotnie zróżnicowana w zależności od rodzaju olejku (źródła pochodzenia), dawki olejku oraz gatunku grzyba. Wobec większości szczepów silniejsze działanie, w obu dawkach, wykazywał olejek uzyskany z odmiany cytrynowej. Wyróżniał się on wyższą, niż olejek z mięty białej, zawartością karwonu i pulegonu. Przy dawce olejku wynoszącej 10 μl strefa zahamowania wzrostu grzybów była zwykle proporcjonalnie większa niż przy dawce 5 μl. Na działanie obu olejków najwyższą odporność wykazywały szczepy gatunków *P. cyclopium* i *A. niger* (średnia strefa zahamowania ich wzrostu, wynosiła odpowiednio 12,4 i 16,7 mm), natomiast najbardziej wrażliwe były szczepy *E. chevalieri* i *E. amstelodami* (średnia strefa zahamowania wzrostu, odpowiednio 90,0 i 75,9 mm).

Słowa kluczowe: *mięta biała, mięta cytrynowa, olejek eteryczny, aktywność przeciwgrzybowa*

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