

THE ACTIVITY OF ESSENTIAL OILS OBTAINED FROM SPECIES AND INTERSPECIES HYBRIDS OF THE *Mentha* GENUS AGAINST SELECTED PLANT PATHOGENIC BACTERIA

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ABSTRACT

Plant essential oils of six aromatic herb species and interspecies hybrids of the family Lamiaceae – chocolate mint (*Mentha piperita* × ‘Chocolate’), pineapple mint (*Mentha suaveolens* ‘Variegata’), apple mint (*Mentha* × *rotundifolia*), spearmint (*Mentha spicata*), orange mint (*Mentha* × *piperita* ‘Granada’) and strawberry mint (*Mentha* × *villosa* ‘Strawberry’) – were investigated for antimicrobial effects against plant pathogenic bacteria: *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *syringae* and *Xanthomonas arboricola* pv. *corylina*. The screening was carried out *in vitro* on agar plates filled with the target organism. All essential oils screened exhibited a higher level of antibacterial activity against *A. tumefaciens* and *X. arboricola* pv. *corylina* than streptomycin used as a standard in all tests. The antimicrobial effect of streptomycin and five mint oils was at the same level for *P. syringae* pv. *syringae*. There were no significant differences in the influence of the chocolate mint oil on the growth inhibition of all bacteria tested. Plant essential oils from pineapple mint, apple mint, spearmint and strawberry mint showed the weakest antimicrobial activity against *P. syringae* pv. *syringae* and the strongest towards *A. tumefaciens* and *X. arboricola* pv. *corylina*. The essential oils from strawberry mint, pineapple mint, spearmint and apple mint had the strongest effect on *A. tumefaciens*, and the lowest inhibitory activity was exhibited by the chocolate mint and orange mint essential oils. *X. arboricola* pv. *corylina* was the most sensitive to the strawberry mint, pineapple mint and spearmint oils. The chocolate mint oil showed the greatest activity against *P. syringae* pv. *syringae*.

Key words: antimicrobial activity, essential oils, mint, phytopathogenic bacteria

INTRODUCTION

Plant protection against bacterial diseases constitutes one of the most important and difficult to resolve issues of plant production, requiring a wide range of actions. Due to a lack of effective chemical

protection and difficulties in obtaining bacteria-resistant cultivars, plant protection against bacteriosis relies strongly on all forms of phytosanitary treatment combined with proper agro-technology.

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The search for new, more efficient and, most importantly, environmentally friendly crop protection methods is thus a crucial task. In recent years, research has been undertaken to the use of alternative plant protection products, i.e. products of plant origin with antimicrobial properties, in plant protection against bacteriosis. Such substances include essential oils, products of plant secondary metabolism. The most important components of essential oils are terpenes, which are organic substances. These compounds are natural oligomers of isoprene and are widespread in nature. Chemically essential oils are multicomponent mixtures of monoterpenes and sesquiterpenes as well as their derivatives, including aromatic ones. These substances comprise alcohols, ketones, aldehydes, esters and ethers. Sometimes their components are chemical compounds containing nitrogen and sulfur. It is common for one essential oil to contain several or even tens of compounds with different concentrations and properties, which is responsible for their wide range of biological activity. The percentage of each component in a given essential oil is variable and dependent on many factors, including the plant species or the cultivar, the environmental conditions of its growth and development as well as the methods of isolation. For the majority of oils, it is possible to identify one main component that is responsible for its specific smell or biological and pharmacological properties [Góra and Lis 2012]. The composition of an essential oil is also influenced by the part of a plant that it originates from. Numerous *in vitro* tests of terpenes isolated from different essential oils have shown that the oils have an antibacterial and bacteriostatic effects. These compounds, being substances of high lipophilicity, are able to easily penetrate the cell walls and membranes of different microorganisms, causing the integrity of these structures to be disrupted and leading to the eventual lysis of bacteria cells [Król et al. 2013].

The *Mentha* genus of the Lamiaceae family includes many plants used as spices and plants used to obtain essential oils. It consists of nineteen different species and thirteen interspecies hybrids. Plants of the *Mentha* genus, in addition to their taste, possess biological and medical properties and are used as raw material in medicine as well as

cosmetics and food industries [Kumar et al. 2011, Góra and Lis 2012, de Sousa Baros et al. 2015]. According to the research performed, the essential oils obtained from these plants as well as their components, including menthol, have a strong antimicrobial effect. It has been reported that essential oils obtained from peppermint (*Mentha × piperita*), chocolate mint (*Mentha × piperita* ‘Chocolate’), spearmint (*Mentha spicata*) and horsemint (*Mentha longifolia* spp. *longifolia*), show strong activity against human pathogenic fungi and bacteria, e.g. *Candida albicans*, *Bacillus subtilis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *P. ovale*, *P. acnes*, *P. fluorescens*, *Salmonella enteritidis*, *Serratia marcescens*, *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus pneumoniae* [McKay and Blumberg 2006, Gulluce et al. 2007, Kusiak et al. 2010, Tsai et al. 2013, Adaszyńska et al. 2013, Ceylan et al. 2014, Mahboubi and Kazempour 2014, Singh et al. 2015].

We are also interested in the research investigating the antibacterial properties of the essential oils obtained from plants of the *Mentha* genus against plant pathogenic bacteria. Iscan et al. [2002] determined that peppermint essential oil was characterized by the highest activity against *Pseudomonas syringae* pv. *phaseolicola*. The essential oils obtained from squaw mint (*Mentha pulegium*) effectively inhibited the growth of *Clavibacter michiganensis* subsp. *michiganensis* [Daferera et al. 2003] and *Erwinia amylovora* [Kokoskova and Pavela 2007]. In a later study by Kokoskova et al. [2011], it was found that *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae* and *Pantoea agglomerans* had considerably higher sensitivity to the essential oil from field mint (*Mentha arvensis*) than to the oil from squaw mint. According to the study of Yanmis et al. [2012] on the influence of the horsemint essential oil on bacteria species isolated from apricot (*Agrobacterium tumefaciens*, *Bacillus pumilus*, *Erwinia chrysanthemi*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *tabaci*, *Ralstonia solanacearum*), peach (*Pseudomonas cichorii*, *Pseudomonas syringae* pv. *pisii*), cherry (*Enterobacter intermedius*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv.

tomato), tomato (*Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas corrugata*, *Xanthomonas vesicatoria*), bean (*Pseudomonas syringae* pv. *phaseolicola*) and pepper (*Xanthomonas axonopodis* pv. *campestris*), all these bacteria showed sensitivity to the essential oil, with the most sensitive bacterium *Clavibacter michiganensis* subsp. *michiganensis*. Gulluce et al. [2007] stated that the essential oil obtained from *Mentha longifolia* subsp. *longifolia* had no antibacterial effect against *Xanthomonas campestris*, *Pseudomonas michiganensis* or *Pseudomonas syringae* pv. *tomato*.

The aim of this study was to evaluate the antimicrobial activity of essential oils obtained from plants of the *Mentha* genus against *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *syringae* and *Xanthomonas arboricola* pv. *corylina*.

MATERIAL AND METHODS

In the research, three strains of bacteria were used: *Agrobacterium tumefaciens* (strain C58), *Pseudomonas syringae* pv. *syringae* (strain 760) and *Xanthomonas arboricola* pv. *corylina* (strain R1PF-x13). They were obtained from the Collection of the Bacteriological Laboratory of the Research Institute of Horticulture in Skierniewice. Nutrient agar (NA) medium was used for maintenance of the bacteria tested.

The essential oils (EOs) used in this study were extracted from the air-dried material from chocolate mint (*Mentha × piperita* ‘Chocolate’), pineapple mint (*Mentha suaveolens* ‘Variegata’), apple mint (*Mentha × rotundifolia*), spearmint (*Mentha spicata*), orange mint (*Mentha × piperita* ‘Granada’) and strawberry mint (*Mentha × villosa* ‘Strawberry’). The composition of essential oils was investigated using gas chromatography and mass spectrometry analysis (GC and GC-MS). The extraction and analysis were carried out as described by Staniek [2016].

The agar disc diffusion method was used for determination of antimicrobial activity of the essential oils. A suspension of bacteria tested (100 µl of 24-hour-old cultures of concentration 10⁷ CFU/ml) was spread on solid media dishes. Sterile Petri dishes (10 cm in diameter) were filled with the growth

medium – *Pseudomonas* agar medium (Merck company) with 1% glycerol. Two sterile filter paper discs (6 mm in diameter) were individually impregnated with 10 µl of essential oils and one was immersed in a streptomycin solution (200 ppm) and placed on the inoculated agar. Three discs were placed on each plate and for each bacterial strain, 4 plates were used. The plates were incubated at room temperature for 24 h. The inhibitory effect of each treatment and bacterium tested was determined by measuring the diameter of the inhibition zones (in millimeters) encompassing the paper discs.

One-way analysis of variance (ANOVA) was performed. The analysis was prepared for the following combinations: a) reactions (inhibition of growth) of each strain of bacteria of streptomycin and each of the six essential oils tested, b) influence of each of the six essential oils and streptomycin on the inhibition of growth of three bacterial strains. The homogeneous groups were identified for each combination using the Newman-Keuls test at the significance level of P = 0.05. All calculations were made using Statgraphics Plus 4.1.

RESULTS AND DISCUSSION

In this study, no statistically significant differences in the influence of streptomycin on the growth of inhibition of three bacterial strains were noted. In the case of *Agrobacterium tumefaciens* and *Xanthomonas arboricola* pv. *corylina*, each of the six essential oils used showed a stronger bacteriostatic effect than streptomycin (Tab. 1).

The influence of EOs and their components is sometimes known to be stronger than the influence of antibiotics. The peppermint essential oil proved to be an effective bacteriostatic agent against chloramphenicol-resistant *Xanthomonas campestris* pv. *phaseolicola*, *X. campestris* pv. *campestris*, *Pseudomonas syringae* pv. *phaseolicola*, *P. syringae* pv. *tomato* and *P. syringae* pv. *syringae* [Iskan et al. 2002]. According to Kokoskova et al. [2011], the growth inhibition zones of *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae* and *Pantoea agglomerans* were much larger after using field mint (*Mentha arvensis*) oil than after using streptomycin. However, there were cases where the activity of EOs was lower than that of an antibiotic. This

was reported by Gulluce et al. [2007], who studied the influence of *Mentha longifolia* subsp. *longifolia* essential oil and netilmicin on *Xanthomonas campestris*, *Pseudomonas michiganensis* and *Pseudomonas syringae* pv. *tomato*. Vasinauskiene et al. [2006] stated that the activity of peppermint essential oil was at the same level as the activity of streptomycin. Similar results were obtained in the present study. For *Pseudomonas syringae* pv. *Syringae*, the antimicrobial effect of streptomycin and five mint oils was at the same level (Tab. 1).

During the analysis of the influence of each of six EOs used on the growth inhibition of the three bacterial strains, significant differences were not detected only in the activity of the chocolate mint oil. The remaining oils, except for the orange mint oil, had the weakest inhibitory effect on *Pseudomonas syringae* pv. *syringae* and the strongest on *Agrobacterium tumefaciens* and *Xanthomonas arboricola* pv. *corylina* (Tab. 1).

When assessing the average values of the growth inhibition zones of each of the three bacterial strains using each of the six EOs applied, the strongest effect on *Agrobacterium tumefaciens* was detected for essential oils from strawberry mint,

pineapple mint, spearmint and apple mint. The lowest sensitivity of bacteria was observed when using the chocolate mint and orange mint essential oils. *Xanthomonas arboricola* pv. *corylina* was the most sensitive to the strawberry mint, pineapple mint and spearmint oils. The greatest activity against *Pseudomonas syringae* pv. *syringae* was found for the chocolate mint oil (Tab. 1).

Differences in the antimicrobial activity of the essential oils depend largely on the bacterium species. *In vitro* tests showed in particular that the Gram-positive bacteria are more sensitive to the essential oils than Gram-negative bacteria, which is believed to be due to the differences in the structure of their cell walls [Helander et al. 1998]. Other researchers have not noted visible differences in the size of the growth inhibition zones resulting from the use of the essential oils from horsemint [Yanmis et al. 2012] and salvia [Hać-Szymańczuk et al. 2014] between Gram-positive and Gram-negative bacteria. According to Papadopoulos et al. [2006] and Bouhdid et al. [2008], bacteria of the *Pseudomonas* genus, e.g. *P. fluorescens* and *P. aeruginosa*, were among the most antibiotic-resistant ones.

Table 1. Effectiveness of plant oils against *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*

Bacteria	Average of bacterium inhibition zone diameters (mm)						
	S	M1	M2	M3	M4	M5	M6
<i>Agrobacterium tumefaciens</i>	7.3 a*/A**	11.4 b/A	16.9 c/C	14.1 c /B	14.7 c/B	9.6 b/A	17.0 c/B
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	9.5 a/A	12.9 b/A	9.9 a/A	10.3 a/A	10.8 a/A	8.4 a/A	9.6 a/A
<i>Xanthomonas arboricola</i> pv. <i>corylina</i>	8.3 a/A	13.0 bc/A	14.0 cd/B	12.9 bc/B	14.5 cd/B	11.5 b/B	15.3 d/B

M1 – chocolate mint, M2 – pineapple mint, M3 – apple mint, M4 – spearmint, M5 – orange mint, M6 – strawberry mint, S – streptomycin

* Means within a row followed by the same lowercase letter do not differ significantly by the Newman-Keuls test (P = 0.05)

** Means within a column followed by the same uppercase letter do not differ significantly by the Newman-Keuls test (P = 0.05)

Table 2. Chemical composition of essential oils from different mint plants

Chemical compound	Retention time (min)	Content of peak surface in ion current of chromatogram (%)					
		mint plants					
		M1	M2	M3	M4	M5	M6
α -pinene	6.38	0.28	0	0.14	0.20	0.21	0.12
β -pinene	7.36	0.66	0.38	0.36	0.43	0.22	0.22
eucalyptol	8.55	4.24	0	1.69	0.93	2.43	0
limonene	8.62	0	0.92	5.86	3.54	0	0.55
β -ocimene	8.81	0.13	0	0	0	0	0.42
α -terpineol	9.12	0	0	0.47	0	1.65	0
β -terpineol	9.30	2.02	0.16	0.34	0.56	0	0
linalool	10.06	0.18	0	0	0.26	34.68	0.34
menthone	10.92	28.95	0.16	0	0	0.21	0.22
isomenthone	11.01	2.66	0.16	0	0	0	0
menthofuran	11.06	0.69	0	0	0	0	0
neomenthol	11.13	1.54	0	0	0	0	0
dihydrocarvone	11.39	0	0	0	1.30	0	0
menthol	11.51	40.81	0	0	0	0	0
carvone	12.17	0.27	0	51.45	73.38	0	0
ocimene	12.62	0	0	0	0	24.56	0
eucarvone	13.62	0	0.16	0	0	0	21.60
oxide caryophyllene	14.05	0	64.32	0	0	0	53.73
geraniol	14.24	0	0	0	0	0.75	0
jasmone	14.33	0	0.15	0	0	0	0.13
α -myrcene	14.57	0	0.18	0	0	0	0
caryophyllene	14.93	1.2	0	0.93	1.10	0	0
β -farnesene	15.36	0.28	0.72	0	0	0.24	0
β -cubebene	15.69	3.07	4.70	0	1.43	1.16	0
germacrene D	15.72	0	0	2.53	0	0	0.45
germacrene B	15.83	0	0	0.12	0	0	0
gamma-gurjunene	16.90	0	0	0	0	1.17	0
ledol	16.92	0	0	0.23	0	0	0
Total (%)		87.40	72.01	64.12	83.13	67.28	77.78

M1 – chocolate mint, M2 – pineapple mint, M3 – apple mint, M4 – spearmint, M5 – orange mint, M6 – strawberry mint

Differences in the antimicrobial activity of essential oils against bacteria may also depend on the origin of the raw plant or some of the chemical components, which may be related to their properties and bioactivity [Iscan et al. 2002, Kokoskova et al. 2011, Yanmis et al. 2012]. The quantitative and qualitative composition of the essential oils and therefore their biological activity are generally subject to different types of variability: genetic, ontogenetic and environmental [Nurzyńska-Wierdak 2015]. The essential oils used by present authors contained various chemical compounds (Tab. 2). Majority of the compounds in the pineapple mint, apple mint, spearmint, orange mint and strawberry mint essential oils were monoterpenes. They were also found to contain sesquiterpenes. *In vitro* tests carried out using monoterpenes from different essential oils showed that they have a bactericidal and bacteriostatic effect. These compounds are highly lipophilic and can easily penetrate the cell walls and cell membranes of many microorganisms, causing the integrity of these structures to be disrupted. One of the proposed mechanisms of toxicity of the essential oils or their terpenic components to bacteria could be the coagulation of cytoplasm and the permeabilization of the cell membrane. This would contribute to an excessive loss of hydrogen and potassium ions and lower the potential of the cell membrane. Damage of the cell wall and membrane would eventually result in lysis of the bacterial cells [Król et al. 2013].

According to our results, chocolate mint essential oil contained menthol (40.81%) and its menthone content (28.95%) was higher than that in the essential oils from strawberry mint (0.22%), orange mint (0.21%) and pineapple mint (0.16%) (Tab. 2). This oil was characterized by the greatest activity against the *Pseudomonas syringae* pv. *syringae* bacterial strain used in this study. Iscan et al. [2002] and Sokovic et al. [2009] reported that the peppermint EO, the main components of which were menthol and menthone, had a strong antimicrobial effect against aerobic bacteria, i.e. *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella pullorum*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Comamonas terrigena*. Moreover, Iscan et al. [2002] reported that

peppermint essential oil showed the greatest activity against phyto-bacteria of the *Pseudomonas* genus as well. According to Schelz et al. [2006], the strong antibacterial activity of the peppermint EO as well as its component, menthol, is associated with elimination of the plasmid resistance to antibiotics of the bacteria. It was determined that this process consisted of the replication of a given plasmid, which was observed for the *E. coli* K12LE140 strain with the F⁺lac plasmid.

The authors of this article argued that carvone (monoterpene) was the most common component of apple mint essential oil (51.45%). Carvone was also the most commonly found component in spearmint EO (73.38%) (Tab. 2). De Carvalho and da Fonseca [2006] stated that carvone is an important monoterpene, which can be used as antimicrobial resistance agent against *Listeria monocytogenes*, *Enterococcus faecium* and *Escherichia coli*. According to the analyses performed by Staniek [2016], the orange mint essential oil used by the authors was the only EO out of the six essential oils tested to contain monoterpene β -ocimene (24.56%), and its linalool content was 100 times higher than in the chocolate mint, spearmint and strawberry EOs. The pineapple mint essential oil was characterized by a high content of the sesquiterpene caryophyllene oxide (64.32%). We reported that strawberry mint essential oil contained eucarvone (21.60%), while its main component was caryophyllene oxide (53.73%) (Tab. 2). The latter also applied to the pineapple mint EO. According to Kędzia et al. [2000], essential oil from *Melaleuca alternifolia* containing, among others, linalool and β -caryophyllene, was characterized by activity against bacteria from the *Escherichia*, *Bacillus*, *Salmonella* and *Staphylococcus* genera.

To conclude, it can be mentioned that the essential oils of different species and interspecies hybrids of mint presented in this paper showed varied antimicrobial activity against the Gram-negative phyto-bacteria tested, which has also been reported by Iscan et al. [2002] for *Mentha* \times *piperita* essential oils, by Kokoskova et al. [2011] for *Mentha arvensis* essential oils and Yanmis et al. [2012] for *Mentha longifolia* spp. *longifolia* essential oils. Other researchers have also described noticeable inhibi-

tion of phytobacterial growth after the use of essential oils from different plant species of the Lamiaceae family. Saad et al. [2008] and Mikiciński et al. [2012] reported that the essential oils obtained from thyme (*Thymus vulgaris*) and salvia (*Salvia fruticosa*), respectively, had an influence on *Agrobacterium tumefaciens*. The antimicrobial activity has been proved by results of the research on *Pseudomonas syringae* pv. *syringae* and essential oils from oregano (*Origanum vulgare*) by Vasinauskiene et al. [2006] and Kokoskova et al. [2011] as well as the research on *Origanum compactum* and lemon balm (*Melissa officinalis*) essential oils by Kokoskova et al. [2011].

The research results presented in this paper constitute the first report on the activity of essential oils obtained from chocolate mint, pineapple mint, apple mint, spearmint, orange mint and strawberry mint against phytobacteria. The authors selected preliminarily only three bacterial species for testing: *Agrobacterium tumefaciens*, *P. syringae* pv. *syringae* and *Xanthomonas arboricola* pv. *corylina*.

Nevertheless, the essential oils screened in the study showed promising potential as new pesticide products or templates for new, more effective compounds. However, further *in vivo* studies are necessary to confirm the safety of these oils as well as their toxicity towards plants.

CONCLUSIONS

1. No differences in the influence of streptomycin on the growth of inhibition of the three bacterial strains were observed.

2. It was established that the essential oils from chocolate mint, pineapple mint, apple mint, spearmint, orange mint and strawberry mint presented varied antimicrobial activity against *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *syringae* and *Xanthomonas arboricola* pv. *corylina*.

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