

THE INFLUENCE OF PLANT ESSENTIAL OILS ON *in vitro* GROWTH OF *Pectobacterium* AND *Dickeya* spp. BACTERIA

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ABSTRACT

The activity of essential oils from *Eucalyptus globulus*, *Pinus silvestris*, *Lavandula angustifolia*, *Juniperus virginiana*, *Rosmarinus officinalis* and *Citrus paradise* against the soft-rot pathogens *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, *Pectobacterium parmentieri* and *Dickeya solani* was determined *in vitro*. The antibacterial activity of the essential oils will be evaluated using the disk-diffusion method by Kirby-Bauer [Bauer et al. 1966]. It was found that all the presented essential oils varied in antimicrobial activity against the four bacterial strains. No differences in the influence of streptomycin on inhibition of growth of the four bacterial strains were observed. Among six tested plants, essential oils from *P. silvestris* had the strongest inhibitory effect on the growth of soft rot bacteria from *Pectobacterium* genus. This paper constitute the first report on the activity of the essential oils obtained from *J. virginiana* against soft rot bacteria. They are also the first report on the activity of the essential oils obtained from *E. globulus*, *P. silvestris*, *L. angustifolia* and *C. paradisi* against *P. atrosepticum*, *P. parmentieri* and *D. solani* as well as on the activity of the *R. officinalis* essential oils against *P. atrosepticum* and *P. parmentieri*.

Key words: antimicrobial activity, plant essential oils, soft rot bacteria

INTRODUCTION

Blackleg and soft rot are among the most damaging bacterial diseases of potato. Their occurrence is observed worldwide and can cause a 10–25% loss of potato yield. Additional losses occur in storage, lowering the market value of tubers. The etiology of these diseases is complex, as potato tuber soft rot can be spread during both cultivation and storage by several species of pectolytic bacteria in the genera *Pectobacterium* and *Dickeya*. The dominant pathogen species in this storage rot complex is often determined by ther-

mal conditions and the availability of oxygen [Goto 1992, Gardanet et al. 2003, Duarte et al. 2004, Samson et al. 2005, Sławiak et al. 2009, Toth et al. 2011].

In Poland, a major percentage of potato infections during cultivation and storage are caused by *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum* [Sledz et al. 1998], *Pectobacterium carotovorum* subsp. *brasiliense*, *Dickeya dianthicola* and *Dickeya solani* [Sławiak et al. 2009] as well as *Pectobacterium parmentieri* [Lebecka 2017].

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In addition to yield losses in the field and during storage, significant costs are incurred due to degradation of seed plantings. Currently the use of healthy propagating material is a key factor in the protection of potatoes against bacterial diseases. In addition, seed potatoes should be planted in warm, moderately humid, well-drained soil, and fertilised properly with N and Ca. It is also important to remove and destroy diseased plants in seed plantations. Cutting seed potatoes is advised against. The potato harvest should be performed during dry weather and the tubers collected should be stored in conditions that decrease the incidence of tuber soft rot [Wójtowicz and Mrówczyński 2017]. Because there are no chemical options to protect against pectolytic bacteria of potato, the search for alternative protection methods is of great significance. For example, development of new potato cultivars with increased tuber resistance to pectolytic bacteria has provided partial suppression of these diseases [Zimnoch-Guzowska et al 2000, Barzic et al. 2012, Lebecka 2017].

Recently, many research centres in Europe, Asia and Africa have conducted intensive studies on the use of natural substances in plant protection against bacterial diseases [Işcan et al. 2002, Papadopoulos et al. 2006, Vasinauskiene et al. 2006, Bouhdid et al. 2008, Kokoskova et al. 2011, Mikiciński et al. 2012, Yanmis et al. 2012, Gakuubi et al. 2016]. Among natural substances with antimicrobial properties, oil extracts constitute a group with chemical structures and properties. These essential oils are obtained through steam distillation. Chemical groups in essential oils include monoterpenes (acyclic, monocyclic, dicyclic), sesquiterpenes, phenols (simple, phenol alcohol), and polyacetylene. They possess many valuable biological properties, including antibacterial activity [Kędzia and Kędzia 2009, Hołderna-Kędzia 2010, Adaszyńska and Swarczewicz 2014].

Essential oils are present in over 2000 plant species from about 60 families [Gakuubi et al. 2016]. In recent years, a wide range of plant essential oils and their constituents have been investigated for their antibacterial properties against such plant pathogenic bacteria as *Xanthomonas vesicatoria*, *Pseudomonas marginalis* pv. *marginalis*, *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato* [Vasinauskiene et al. 2006], *Pseudomonas savastanoi* pv. *phaseolicola*, *Xan-*

thomonas axonopodis pv. *phaseoli* and *Xanthomonas axonopodis* pv. *manitobis* [Gakuubi et al. 2016].

The antibacterial activity of the different essential oils against soft rot bacteria has been reported by many authors [Vasinauskiene et al. 2006, Almashahi et al. 2010, Neshad et al. 2012, Badawy and Abdalgaleil 2014, Fernández et al. 2014, Mehrosorosh et al. 2014, Jamshidi and Adargani 2016, Elansary et al. 2018, Popović et al. 2018, Salem et al. 2018, Okla et al. 2019]. Given the fact that soft rot disease is one of the limiting factors in global potato crop production and the fact that current control measures are insufficient, the aim of this work is to evaluate the inhibitory potential of some essential oils on the growth of the following soft rot bacteria: *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, *Pectobacterium parmentieri* and *Dickeya solani*.

MATERIALS AND METHODS

The four strains of bacteria that were used in assays: *Pectobacterium carotovorum* subsp. *carotovorum* (strain Pcc2 M15), *Pectobacterium atrosepticum* (strain Pba3M14), *Pectobacterium parmentieri* (strain Pw1M13) and *Dickeya solani* (strain IFB0099) – were obtained from the Laboratory of Phytopathology of the Department of Potato Genetics and Parental Lines in Młochów Research Center, Plant Breeding and Acclimatization Institute-National Research Institute, Poland.

Composition of essential oils was determined by An Agilent Technology 6890N gas chromatograph equipped with a 30 m non-polar HP 1MS column (0.25 mm diameter and 0.25 µm steady state film thickness). The carrier gas flow (helium) was 1 mL /min. chamber temperature was 250°C, furnace – 100°C (increase 10°C/min). The chromatograph was coupled with an MS 5973 Network mass spectrometer from Agilent Technology with a quadrupole mass analyzer.

The antibacterial activity of the essential oils will be evaluated using the disk-diffusion method by Kirby-Bauer [Bauer et al. 1966]. Six commercial essential oils – from *Eucalyptus globulus*, *Pinus silvestris*, *Lavandula angustifolia* (produced by Etja, Elbląg, Poland), *Juniperus virginiana*, *Rosmarinus officinalis* product of (Sabana Optima Sp. z. o.o., Płosnica, Poland) and *Citrus paradise* (produced by Zakład

Produkcji Farmaceutyczno-Kosmetycznej Pharma Tech, Zukowo, Poland) – and a streptomycin sulphate solution (200 ppm) were prepared. Sterile Petri dishes (10 cm diameter) were filled with a Nutrient Agar (NA) medium. A 0.1 ml suspension of the bacteria tested (24-hour-old cultures; 10^7 CFU/ml) was placed on each Petri dish. A cork borer was used to cut three wells (7 mm diameter) in the medium. Two wells were filled with eucalyptus, pine, true lavender, juniper, rosemary or grapefruit essential oils (200 μ l each), and the third well was filled with 200 μ l of streptomycin sulphate solution. After the plates were then incubated at room temperature for 24 h, the diameter of the inhibition zones was measured. The measurements were performed for four strains of bacteria: *P. carotovorum* subsp. *carotovorum*, *P. atrosepticum*, *Pectobacterium parmentieri*, *Dickeya solani*, six essential oils (eucalyptus, pine, true lavender, juniper, rosemary, grapefruit) and a streptomycin sulphate solution in three replications for each combination which constituted a factorial design.

One-way analysis of variance (ANOVA) was performed to compare the influence of each of the six essential oils and a streptomycin sulphate control on the inhibition of growth of each of the four tested bacteria 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 Statistica 13.

RESULTS AND DISCUSSION

The substances identified in the essential oils isolated from *Eucalyptus globulus*, *Pinus silvestris*, *Lavandula angustifolia*, *Juniperus virginiana*, *Rosmarinus officinalis* and *Citrus paradisi* by using GC-MS analyses are presented in Table 1.

The components identified in the six essential oils constituted from 79.20% to 95.50% of the peak surface in ion current of chromatogram. Some substances were present in more than one essential oil (α - and β -pinene, camphene, eucalyptol, limonene, carene, linalool, borneol, terpinen 4-ol, α -terpineol, carvone, linalyl anthranilate, bornyl acetate, bornylene and geranyl acetate and caryophyllene), while others were specific to one plant essential oil. The majority of the aforementioned components belong to the

terpene group, with linalyl anthranilate and borneol being esters. In the essential oil isolated from *Eucalyptus globulus*, three substances were detected. The major component was eucalyptol (66.21%); α -pinene accounted for 27.42% and α -terpineol for 1.82%. In the essential oil obtained from *Citrus paradise*, 14 different substances were identified, the main components being limonene (32.46%) and borneol (10.94%). In the essential oil isolated from *Rosmarinus officinalis* 10 substances were detected, the major components being eucalyptol and camphor representing 34% and 23%, respectively. The sample of essential oil obtained from *Lavandula angustifolia* EO contained 18 substances. The main components were linalyl anthranilate (23.71%), linalool (15.22%) and borneol (15.04%). The sample of essential oil isolated from *Juniperus virginiana* showed the presence of 7 substances, among which the main component was beta himachalene (48.02%), with the percentage of cedrol and cuparene being two and four times lower, respectively. The sample of the *Pinus silvestris* essential oil contained 16 substances. The major components were carene (23.51%) and bornyl acetate (20.31%) (Tab. 1). The number of the substances identified in the six essential oils was lower than the number detected in the research conducted by Smigielski et al. [2009], Verma et al. [2009], Damjanović-Vratnica et al. [2011], Kamal et al. [2011], Tarakemeh et al. [2012], Okunowo et al. [2013], Adaszyńska-Skwirzyńska and Swarczewicz [2014], Badawy et al. [2014], Mehrsororosh et al. [2014], Prusinowska and Smigielski [2014], Bachheti [2015], or Tomescu et al. [2015]. Some of the major components of the essential oils obtained from *Eucalyptus globulus*, *Citrus paradise*, *Rosmarinus officinalis*, *Lavandula angustifolia* and *Pinus silvestris* were similar to those reported for plants growing in Algeria [Fekih et al. 2014], Egypt [Badawy et al. 2014] Ethiopia [Kamal et al. 2011] Montenegro [Damjanović-Vratnica et al. 2011], India [Verma et al. 2009], Iran [Tarakemeh et al. 2012, Mehrsororosh et al. 2014], Romania [Tomescu et al. 2015], as well as in Poland [Smigielski et al. 2009]. However, the composition percentages differed. The main substances of the essential oil obtained from *Juniperus virginiana* tested in this paper were different from those reported by Hădărugă et al. [2011] and Stewart et al. [2014]. On the other hand, the major components of the *Juni-*

Table 1. Chemical composition of eucalyptus, pine, true lavender, juniper, rosemary and grapefruit essential oils

Chemical compound	Retention time (min)	Content of peak surface in ion current of chromatogram (%)					
		Essential oils					
		1	2	3	4	5	6
santene	1.94	0	0	0	0	0	0.58
α -pinene	2.17	1.17	7.19	2.81	27.42	0	9.25
camphene	2.23	0	2.51	1.22	0	0	5.04
β -pinene	2.37	0	8.85	0.36	0	0	7.07
eucalyptol	2.81	0	28.40	8.05	66.21	0	0
limonene	2.64	32.46	0	0	0	0	8.43
carene	3.00	1.50	1.07	5.85	0	0	23.51
linalool	3.07	3.25	0	15.22	0	0	0
thujone	3.18	0	0	0	0	0	1.00
menthone	3.46	0	0	0	0	0	0.71
camphor	3.49	0	19.18	0	0	0	0
isoborneol	3.60	0	2.83	0	0	0	0
borneol	3.70	10.94	0	15.04	0	0	0
terpinen-4-ol	3.75	7.68	0	0	0	0	8.72
α -terpineol	3.84	1.86	2.34	4.35	1.82	0	2.35
cis-carveol	4.08	5.40	0	0	0	0	0
fenchyl acetate	4.10	0	0	2.38	0	0	0
carvone	4.28	8.13	0	0	0	0	6.38
linalyl anthranilate	4.36	0.76	0	23.71	0	0	0
citral	4.45	1.82	0	0	0	0	0
limonene dioxide	4.56	1.17	0	0	0	0	0
bornyl acetate	4.83	0	8.81	0.52	0	0	20.31
bornylene	5.36	0	1.88	5.392	0	0	0.70
geranyl acetate	5.62	0.80	0	5.654	0	0	0
coumarin	6.04	0	0	0.67	0	0	0
α -urjunene	6.26	0	0	1.64	0	0	0
caryophyllene	6.35	2.26	0	0.84	0	0	0.74
β -himachalene	6.58	0	0	0	0	48.02	0
α -caryophyllene	6.68	0	0	0	0	0	0.41
curcumene	7.02	0	0	0	0	1.05	0
β -chamigrene	7.10	0	0	0	0	1.87	0
delta-cadinene	7.36	0	0	0	0	0	0.30
cuparene	7.40	0	0	0	0	12.73	0
cedrol	8.55	0	0	0	0	22.55	0
α -longipinene	8.66	0	0	0	0	0.64	0
bisabolol	9.14	0	0	0	0	1.25	0
benzyl benzoate	9.70	0	0	1.60	0	0	0
elaidyl alcohol	12.83	0	0	4.67	0	0	0
Total (%)		79.20	83.06	99.98	95.45	88.11	95.50

1 – *Citrus paradisi* essential oil, 2 – *Rosmarinus officinalis* essential oil, 3 – *Lavandula angustifolia*, 4 – *Eucalyptus globulus* essential oil, 5 – *Juniperus virginiana* essential oil, 6 – *Pinus silvestris* essential oil

perus virginiana essential oil presented in this study were similar to those detected in German commercial essential oils isolated from *Juniperus virginiana* and in Virginian cedarwood oil produced by companies in the United States [Adams 1991]. Moreover, differences in the percentage of the major substances of the Polish, German and American *Juniperus virginiana* essential oils were observed. The differences in the essential oil composition could be caused by several factors, such as plant genetic variations, geographical location, season, environmental conditions, nutritional status of the plants, age of the plants and method of isolation [Viturro 2003, Smigielski et al. 2009, Badaway et al. 2014, Mehrosorosh et al. 2014].

The antibacterial activity of the essential oils isolated from *Acorus calamus*, *Achiollea filipendula*, *A. cartilaginea*, *Artemisia kermanensis*, *Boswellia serrata*, *Citrus* spp., *Echinacea purpurea*, *Lavandula angustifolia*, *Origanum vulgare*, *Thymus vulgaris*, *Rosmarinus officinalis*, *Cinnaminum cassia*, *Cassia angustifolia*, *Coriandrum sativum*, *Cuminum cuminum*, *Eucalyptus globulus*, *E. caesia*, *Thuja occidentalis*, *Apium graveolens*, *Pinus roxburghii*, *Pinus sylvestris*, and *Rosmarinum officinalis* against soft-rot bacteria was reported by many authors [Vasinauskiene et al. 2006, Almashahi et al. 2010, Neshad et al. 2012, Badawy and Abdelgaleil, 2014, Fernández et al. 2014, Mehrosorosh et al. 2014, Jamshidi and Adargani 2016,

Elansary et al. 2018, Popović et al. 2018, Salem et al. 2018, Okla et al. 2019].

No significant statistical differences were detected in the effect of the streptomycin sulphate solution on the inhibition of growth of the four tested bacteria strains (Tab. 2). The antibacterial effect of the streptomycin sulphate solution against *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum* and *Pectobacterium parmentieri* was weaker than that of the essential oil obtained from *Pinus sylvestris* and its activity against *Pectobacterium parmentieri* was weaker than that of the *Citrus paradisi* essential oil (Fig. 1). The effect of the essential oils and their components is known to sometimes be stronger than antibiotics, e.g. the activity of the *Thymus vulgaris* and *Coriandrum sativum* essential oils against *Pectobacterium carotovorum* subsp. *carotovorum* [Almashahi et al. 2010, Nezhad et al. 2012, Mehrosorosh et al. 2014, Rojas et al. 2014].

There are also cases of essential oils showing lower activity than antibiotics. According to Jamshidi and Adargani [2016], the inhibition zones of *Pectobacterium atrosepticum* growth were considerably smaller after the use of the essential oil isolated from *Echinacea purpurea* than after the application of gentamycin. Salem et al. [2018] have detected weaker activity of the essential oils obtained from *Cupressus macrocarpa* and *Corymbia citriodora* against *Dickeya solani*

Table 2. Influence of the essential oils and streptomycin sulphate on the bacteria growth inhibition

Essential oils	Inhibition of the growth zone (mm)			
	<i>Dickeya solani</i>	<i>Pectobacterium atrosepticum</i>	<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	<i>Pectobacterium parmentieri</i>
<i>Juniperus virginiana</i>	4.3a*	12.2b	5.3a	4.9a
<i>Eucalyptus globulus</i>	7.3b	7.7b	5.4a	9.5c
<i>Citrus paradisi</i>	4.4a	9.8b	16.8c	16.8c
<i>Lavandula angustifolia</i>	5.5a	7.2b	12.4d	10.0c
<i>Rosmarinus officinalis</i>	3.0a	6.1b	10.8c	9.0c
<i>Pinus sylvestris</i>	8.8a	19.2b	26.6c	31.7d
<i>Streptomycin sulphate</i>	14.6a	15.2a	14.8a	13.1a

* homogenous group according to Newman–Keuls test. Values marked with the same letter in the row do not differ statistically at the significance level $p = 0.05$

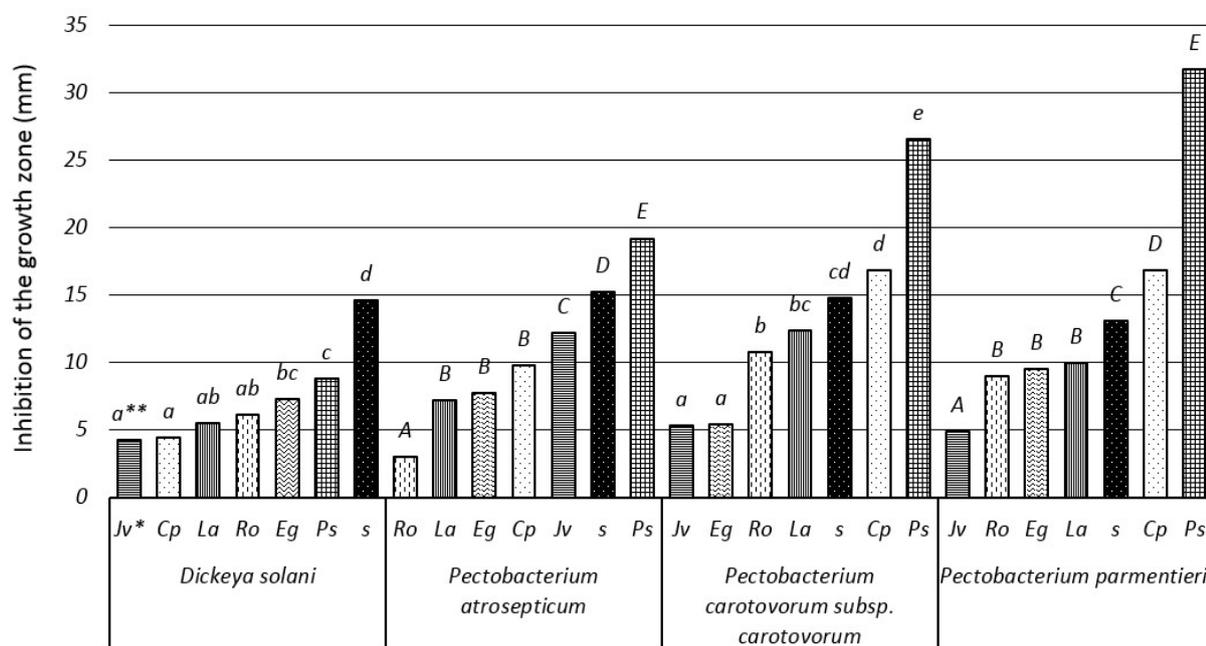


Fig. 1. Impact of essential oils on the inhibition of the bacteria growth zone

Jv – *Juniperus virginiana* essential oil, Cp – *Citrus paradise* essential oil, La – *Lavandula angustifolia* essential oil, Ro – *Rosmarinus officinalis* essential oil, Eg – *Eucalyptus globulus* essential oil, Ps – *Pinus silvestris* essential oil, s – streptomycin sulphate

**homogenous group according to Newman – Keuls test. Values marked with the same letter (capital, italic) do not differ statistically at the significance level $p = 0.05$

than streptomycin. Similar observations were made in this paper after comparing the antibacterial activity against *Dickeya solani* of each of the six essential oils tested (Fig. 1).

Of the six essential oils tested, the *Pinus silvestris* essential oil had the strongest effect against the four bacteria strains. The diameters of the growth inhibition zones of *Pectobacterium parmentieri*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum* and *Dickeya solani* measured 31.7 mm, 26.6 mm, 19.2 mm and 8.8 mm, respectively. (Tab. 2, Fig. 1).

Research conducted by Badway and Abdegaleil [2014] and Fernández et al. [2014] confirmed the sensitivity of *Pectobacterium carotovorum* subsp. *carotovorum* to the essential oils obtained from *Citrus paradisi* as well as other species of the *Citrus* genus: *C. aurantifolia*, *C. lemon*, and *C. sinensis*. Okla et al. [2019] have shown that the *Citrus aurantium* essen-

tial oil has a strong activity against *D. solani*. In the present, grapefruit essential oil exhibited the strongest antibacterial properties against *Pectobacterium carotovorum* subsp. *carotovorum* and *Pectobacterium parmentieri* (Tab. 2, Fig. 1).

The *Juniperus virginiana*, *Eucalyptus globulus*, *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils used in this research demonstrated weak antimicrobial activity against the tested bacteria strains. The eucalyptus essential oil has exhibited a weak antibacterial activity against *Pectobacterium carotovorum* subsp. *carotovorum* [Alamshahi et al. 2010, Nezhad et al. 2012]. Our results indicated that the essential oil isolated from *Eucalyptus globulus* was the most effective in inhibiting the growth of *Pectobacterium parmentieri* and the least effective against *Pectobacterium carotovorum* subsp. *carotovorum* (Tab. 2). Furthermore, no significant statistical differences were detected between the activities of *Eucalypt-*

tus globulus, *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils on the inhibition of growth of *Dickeya solani* and *P. parmentieri* (Fig. 1).

There is no research data concerning the effect of the essential oil obtained from *Juniperus virginiana* against soft rot bacteria. According to Popović et al. [2018] the *Juniperus communis* and *Juniperus virginiana* essential oils have no antimicrobial properties against *Pseudomonas syringae* pv. *syringae* and *Xanthomonas campestris* pv. *campestris*. The essential oil from *Juniperus virginiana* tested in this study showed weak activity against *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri* and *Dickeya solani*, while its inhibitory effect on *P. atrosepticum* was considerably stronger (Tab. 2).

In the research described in this paper, the *Rosmarinum officinalis* essential oil showed weak antibacterial activity against *Dickeya solani* and *P. atrosepticum* as well as stronger activity against *Pectobacterium parmentieri* and *Pectobacterium carotovorum* subsp. *carotovorum*, with the growth inhibition zones having diameters of 3 mm, 6.1 mm, 9.0 mm and 10.8 mm, respectively (Tab. 2.). Elasnary et al. [2018] found that the effect of the essential oil isolated from *Rosmarinum officinalis* against *Dickeya solani* was weak. In the present study, the rosemary essential oil exhibited the weakest activity against *P. atrosepticum* (Fig. 1). Other researchers have established that the size of the growth inhibition zones of *Pectobacterium carotovorum* subsp. *carotovorum* after the use of the essential oil obtained from *Rosmarinum officinalis* range from 6.5 mm to 20 mm [Alamshahi et al. 2010, Nezhad et al. 2012, Badawy and Abdelgaleil 2014, Mehrosorosh et al. 2014, Popović et al. 2018].

The lavender essential oil tested in this study showed weak antibacterial effect against *Dickeya solani* and *P. atrosepticum*, but had a stronger effect against *Pectobacterium parmentieri*, especially against *Pectobacterium carotovorum* subsp. *carotovorum*, the diameters of the growth inhibition zones being 5.5 mm, 7.2 mm, 10.0 mm and 12.4 mm, respectively (Tab. 2). According to Mehrosorosh et al. [2014], the growth inhibition zones of *Pectobacterium carotovorum* subsp. *carotovorum* after use of the *Lavandula officinalis* essential oil was 6.16 mm, while Popović et al. [2018] have reported that the diameter of the growth inhibition zone of *Pectobacterium caro-*

tororum subsp. *carotovorum* after use of the same essential oil ranges from 10 to 15 mm.

The research presented in this paper revealed different levels of activity against the tested bacteria of the essential oils applied. The essential oil from *Pinus silvestris* had the strongest inhibitory effect on the growth of the four bacteria strains studied compared to *Lavandula angustifolia*, *Rosmarinus officinalis*, *Citrus paradise*, and *Juniperus virginiana* essential oils (Fig.1). The essential oils isolated from *Lavandula angustifolia*, *Rosmarinus officinalis* and *Citrus paradisi* showed weak antimicrobial properties against *Dickeya solani* and *Pectobacterium atrosepticum*, whereas tested oils from *Juniperus virginiana* exhibited the weakest activity against *Dickeya solani*, *Pectobacterium parmentieri* and *Pectobacterium carotovorum* subsp. *carotovorum* (Tab. 2).

The therapeutic properties of essential oils are diverse and most commonly related to the effect of the dominant components. However, it should be noted that, depending on its origin, an essential oil may contain many other compounds present in smaller concentrations. Consequently, the biological activity of essential oils results from the effect of the dominant component and/or a synergistic effect of a complex of compounds [Adaszyńska-Skwierzyńska and Swarczewicz 2014, Nurzyńska-Wierdak 2015]. The antimicrobial activity of essential oils depends not only on the differences in the percentage of each component between oils obtained from the same plant species, but also on many other factors, such as the place of origin of the plants, the soil conditions and the climate zone [Król et al. 2013, Fekih et al. 2014, Stewart et al. 2014, Czerwińska and Szparaga 2015].

The results presented in this paper constitute the first report on the activity of the essential oils obtained from *Juniperus virginiana* against soft rot bacteria. They are also the first report on the activity of the essential oils obtained from *Eucalyptus globulus*, *Pinus silvestris*, *Lavandula angustifolia* and *Citrus paradisi* against *Pectobacterium atrosepticum*, *Pectobacterium parmentieri* and *Dickeya solani* as well as on the activity of the *Rosmarinus officinalis* essential oils against *Pectobacterium atrosepticum* and *Pectobacterium parmentieri*.

The authors of this article believe that the present research into antibacterial activity of the essential oils

isolated from six different plants against four economically significant soft-rot bacteria will provide useful information on the antibacterial properties of the essential oils tested, in particular *Pinus silvestris* essential oil. Therefore, further *in vivo* studies are necessary to confirm the safety of these oils as well as their toxicity towards plants. We believe that, in the future, these essential oils should be regarded as potential alternatives to synthetic bactericides or as lead compounds for new classes of natural bactericides.

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REFERENCES

- Adams, R.P. (1991). Cedar wood oil – analyses and properties. In: Essential oils and waxes, Linskens, H.F., Jackson, J.F. (eds). Springer, Berlin, Heidelberg, 159–173.
- Adaszyńska-Skwierzyńska, M., Swarczewicz, M. (2014). Skład chemiczny i aktywność biologiczna lawendy lekarskiej. Wiad. Chem. 68(11–12), 1073–1092.
- Alamshahi, L., Hosseini, M., Nezhad, M.H., Panjehkeh, N., Sabbagh, S.K., Sadri, S. (2010). Antibacterial effects of some essential oils on the growth of *Pectobacterium carotovorum* subsp. *carotovorum*. The 8th International Symposium on Biocontrol and Biotechnology 4th to 6th October 2010. Pattaya, Chonburi, Thailand, 206–212.
- Bachheti, R.K. (2015). Chemical composition and antibacterial activity of the essential oil from the leaves of *Eucalyptus globulus* collected from Haramaya University, Ethiopia. Pharma Chem., 7, 209–214.
- Badawy, M.E.I., Abdelgaleil, S.A.M. (2014). Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Ind. Crops Prod., 52, 776–782.
- Barzic, M.R., Com, E. (2012). Proteins involved in the interaction of potato tubers with *Pectobacterium atrosepticum*: a proteomic approach to understanding partial resistance. J. Phytopathol., 160, 561–575.
- Bauer, A.W, Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45, 493–496.
- Bouhdid, S., Skali, S.N., Idaomar, M., Zhiri, A., Baudoux, D., Amensour, M., Abrini, J. (2008). Antibacterial and antioxidant activities of *Origanum compactum* essential oil. Afr. J. Biotechnol., 7, 1563–1570.
- Churata-Oroya, D.E., Ramos-Perfecto, D., Moromi-Nakata, H., Martínez-Cadillo, E., Castro-Luna, A., Garcia-de-la-Guarda, R. (2016). Efecto antifúngico del Citrus paradisi “toronja” sobre cepas de *Candida albicans* aisladas de pacientes con estomatitis subprotésica. Rev. Estomatol. Hered., 26, 78–84.
- Czerwińska, E., Szparaga, A. (2015). Antibacterial and antifungal activity of plant extracts. Ann. Set Environ. Prot., 17, 209–229.
- Damjanović-Vratnica, B., Đakov, T., Šuković, D., Damjanović, J. (2011). Antimicrobial effect of essential oil isolated from *Eucalyptus globules* Labill. from Montenegro. Czech J. Food Sci., 29, 277–284.
- Duarte, V., Boer, S.H., Ward, L.J. de, Oliveira, M.C. de (2004). Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. J. Appl. Microbiol., 96, 535–545.
- Elansary, H.O., Samir, A.M. Abdelgaleil, S.A.M., Mahmoud, E.A., Kowiyou Yessoufou, K., Khalid Elhindi, K., Salah El-Hendawy, S. (2018). Effective antioxidant, antimicrobial and anticancer activities of essential oils of horticultural aromatic crops in northern Egypt. BMC Complement Altern. Med., 18, 214–218.
- Fekih, N., Allali, H., Merghache, S., Chaïb, F., Merghache, D., El Amine, M., Djabou, N., Muselli, A., Tabti, B., Costa, J. (2014). Chemical composition and antibacterial activity of *Pinus halepensis* Miller growing in west northern of Algeria. Asian Pac. J. Trop. Dis., 4, 97–103.
- Fernández, R.M.M., Corzo, L.M., Sánchez, P.Y., Brito, D., Montes de Oca, R., Martínez, Y. Pino, P.O. (2014). Actividad antibacteriana de aceites esenciales sobre *Pectobacterium carotovorum* subsp. *carotovorum*. Rev. Prot. Veg., 29(3), 197–203.
- Gakuubi, M.M., Wagacha, J.M., Dossaji, S.F., Wanzala, W. (2016). Chemical composition of essential oils of *Tagetes minuta* (Asteraceae) against selected plant pathogenic bacteria. Int. J. Microbiol., 1–9. <https://doi.org/10.1155/2016/7352509>
- Gardan, L., Couy, C., Christen, R., Galan, J.E. (2003). Evaluation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. Inter. J. Syst. Evol. Microbiol., 53, 381–391.
- Goto, M. (1992). Fundamentals of bacterial plant pathology. Acad. Press, New York, 35–40.
- Hădărugă, N.G., Branic, A.G., Hădărugă, D.I., Alexandra Gruia, A., Pleșa, C., Costescu, C., Ardelean, A., Alfa Xenia Lupea, A.X. (2011). Comparative study of *Juniperus communis* and *Juniperus virginiana* essential oils: TLC

- and GC analysis. J. Planar Chrom., 24, 130–135.
- Holderna-Kędzia, E. (2010). Działanie substancji olejkowych na bakterie i grzyby. Post. Fitoter., 1, 3–8.
- İşcan, G., Kirimer, N.M.K., Fatih Demirci, F. (2002). Antimicrobial screening of *Mentha piperita* essential oils. J. Agric. Food, 50, 3943–3946.
- Jamshidi, S., Adargani, S. (2016). Antibacterial potential of purple coneflower extracts and essential oils against some plant-related bacteria. Agroecol. J., 12 (2), 65–72.
- Kamal, G.M., Anwar, F., Hussain, A.I., Sarri, N., Ashraf, M. (2011). Yield and chemical composition of *Citrus* essential oils as affected by drying pretreatment of peels. Int. Food Res. J., 18, 1275–1282.
- Kędzia, A., Kędzia, A.W. (2009). Działanie *in vitro* olejku sosnowego wobec bakterii beztlenowych wyizolowanych z jamy ustnej i dróg oddechowych. Post. Fitoter., 1, 19–23.
- Kokoskova, B.D., Pouvova, D., Pavela, R. (2011). Effectiveness of plant essential oils against *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae* and associated saprophytic bacteria on/in host plants. J. Plant Pathol., 93, 133–139.
- Lebecka, R. (2017). Screening for potato resistance to blackleg and soft rot. Plant Breed. Seed Sci., 75, 97–104.
- Mehrorosh, H., Gavanji, S., Larki, B., Mohammadi, M.D., Karbasiun, A., Bakhtari, A., Hashemzadeh, F., Mojiri, A. (2014). Essential oil composition and antimicrobial screening of some Iranian herbal plants on *Pectobacterium carotovorum* Global NEST J., 16 (2), 240–250.
- Mikiciński, A., Sobiczewski, P., Berczyński, S. (2012). Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. J. Plant Prot. Res., 52, 467–471.
- Nezhad, M.H., Alamshahi, L., Panjehkeh, N. (2012). Biocontrol efficiency of medicinal plants against *Pectobacterium carotovorum*, *Ralstonia solanacearum* and *Escherichia coli*. The Open Conf. Proc. J., 3 (Suppl 1-M8), 46–51.
- Nurzyńska-Wierdak, R. (2015). Aktywność biologiczna olejków eterycznych roślin z rodziny Pinaceae. Terapeutyczne właściwości olejków eterycznych. Ann. UMCS, sec. EEE Horticultura, 25(3), 19–31.
- Okla, M.K., Alamri, S.A., Salem, M.Z.M., Al, I.H.M., Behiry, S.I., Nasser, R.A., Alaraidh, I.A., Al-Ghtani, S.M., Soufan, W. (2019). Yield, phytochemical constituents, and antibacterial activity of essential oils from the leaves/twigs, branches, branch wood, and branch bark of sour orange (*Citrus aurantium* L.). Processes, 7, 2–15.
- Okunowo, W.O., Oyedeji, O., Afolabi, L.O., Matanmi, E. (2013). Essential oil of grape fruit (*Citrus paradisi*) peels and its antimicrobial activities. Am. J. Plant Sci., 4, 1–9.
- Papadopoulos, Ch.J., Carson, Ch.F., Hammer, K.A., Riley, T.V. (2006). Susceptibility of pseudomonads to *Melaleuca alternifolia* (tea tree) oil and components. J. Antimicrob. Chemother., 58, 449–451.
- Popović, T., Milićević, Z., Orgo, V., Kostić, I., Radović, V., Jelušić, A., Krnjajić, S. (2018). A preliminary study of antibacterial activity of thirty essential oils against several important plant pathogenic bacteria. Pestic. Phytochem. (Belgade), 33(3–4), 185–195.
- Prusinowska, R., Smigielski, K.B. (2014). Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.). A review. Herba Pol., 60(2), 56–66.
- Salem, M.Z.M., Elansary, H.O., Ali, H.M., El-Settawy, A.A., Elshikh, M.S., Abdel-Salam, E.M., Skalicka-Woźniak, K. (2018). Bioactivity of essential oils extracted from *Cupressus macrocarpa* branchlets and *Corymbia citriodora* leaves grown in Egypt. BMC Comp. Altern. Med., 18, 23–30.
- Samson, R., Legendre, J.B., Christen, R., Saux, M.F.L., Achouak, W., Gardan, L. (2005). Transfer of *Pectobacterium chrysanthemi* and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zae* sp. nov. Inter. J. Syst. Evol. Microbiol., 55, 1415–1427. <https://doi.org/10.1099/ijs.0.02791-0>
- Sledz, W., Jafra, S., Waleron, M., Toth, I.K., Hyman, L.J., Perombelon, M.C.M., Łojkowska, E. (1998). Identification of pectolytic erwinias isolated from infected potato plants in Poland. Proceedings 7th International Conference of Plant Pathology, Edinburgh, 9-16th August 1998, 10-12.
- Sławiak, M., Łojkowska, E., Wolf, J. M. van der (2009). First report of bacterial soft rot on potato caused by *Dickeya* sp. (syn. *Erwinia chrysanthemi*) in Poland. Plant Path., 58, 794.
- Smigielski, K., Raj, A., Krosowiak, K., Gruska, R. (2009.) Chemical composition of the essential oil of *Lavandula angustifolia* cultivated in Poland. J. Ess. Oil Res., 12, 338–347.
- Stewart, Ch.D., Jones, Ch.D., William, N. Setzer, W.N. (2014). Essential oil compositions of *Juniperus virginiana* and *Pinus virginiana*, two important trees in Cherokee traditional medicine. Am. J. Ess. Oils Nat. Prod., 2, 17–24.
- Tarakemeh, A., Abutalebi, A. (2012). Effect of drying method on the essential oil quantity of basil (*Ocimum basilicum* L.). J. Ess. Oil Bear. Plants, 15, 503–505.

- Tomescu, A., Rus, C., Pop, G., Alexa, E., Sumălan, R., Copolovici, D., Negrea, M. (2015). Chemical composition of *Lavandula angustifolia* L. and *Rosmarinus officinalis* L. essential oils cultivated in West Romania. Res. J. Agric. Sci., 47 (3), 246–253.
- Toth, I.K., van der Wolf, J.M., Saddler, G.S., Łojkowska, E., Hélias, V., Pirhonen, M., Tsrer, L., Elphinstone, J.G. (2011). *Dickeya* species: an emerging problem for potato production in Europe. Plant Pathol., 60(3), 385–399.
- Vasinauskiene, M., Radušiene, J., Zittikaitė, I., Survilienė, E. (2006). Antibacterial activities oils from aromatic and medicinal plants against growth of phytopathogenic bacteria. Agron. Res., 4(special issue), 437–440.
- Verma, R.S., Rahman, Lu, Chanotiya, C.S., Verma, R.K., Singh, A., Yadav, A., Chauhan A., Yadav A.K., Singh A.K. (2009). Essential oil composition of *Thymus serpyllum* cultivated in the Kumaon region of western Himalaya, India. Nat. Prod. Commun., 4, 987–988.
- Vituro, I., Molina, A.C., Heit, C.I. (2003). Volatile components of *Eucalyptus globulus* Labill ssp. *bicostata* from Jujuy, Argentina. J. Ess. Oil Res., 15, 206–208.
- Wójtowicz, A., Mrówczyński, M. (2017). Metodyka integrowanej ochrony ziemniaka dla doradców. Instytut Ochrony Roślin – Państwowy Instytut Badawczy, Poznań, 130–136.
- Zimnoch-Guzowska, E., Marczewski, W., Lebecka, R., Flis, B., Schäfer-Pregl, R., Salamini, F., Gebhardt, C. (2000). QTL analysis of new sources of resistance to *Erwinia carotovora* ssp. *atroseptica* in potato done by AFLP, RLFP, and resistance-gene-like markers. Crop Sci., 40, 1156–1167.
- Yanmis, D., Gormez, A., Bozari, S., Orhan, F., Gulluce, M., Agar, G., Sahin, F. (2012). Microbes in applied research. In: Current advances and challenges, Mendez-Vilas, A. (ed.). Word Scientific Publishing, Singapore, 531–535.