JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IN VITRO ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS (FAMILY LAMIACEAE) AGAINST *CLADOSPORIUM* SP. STRAINS – POSTHARVEST PATHOGENS OF FRUITS

Dana Tančinová[1], Zuzana Barboráková[1], Zuzana Mašková[1], Monika Mrvová[1], Juraj Medo[1], Marcel Golian[2], Jana Štefániková[3], Július Árvay[4]*

Address(es): prof. Ing. Dana Tančinová, PhD.
[1] Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, phone number: +421 37641 4433.
[2] Slovak University of Agriculture, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[3] Slovak University of Agriculture, AgroBioTech Research Centre, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[4] Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

*Corresponding author: dana.tancinova@uniag.sk

https://doi.org/10.55251/jmbfs.9921

ARTICLE INFO

Received 24. 2. 2023
Revised 6. 7. 2023
Accepted 31. 7. 2023
Published 1. 10. 2023

Regular article

OPEN ACCESS

ABSTRACT

Essential oils (EOs) are a suitable alternative for extending the shelf life of foods. The aim of this research was to test the effect of fifteen EOs extracted from plants of the Lamiaceae family on five strains of *Cladosporium cladosporioides*. *Cladosporium cladosporioides* is frequently found on fruit and may be involved in fruit spoilage. The strains used in the study were isolated directly from lesions on berries. The growth of the fungi on the fruit was the reason for its rejection from sale. The antifungal activity of EOs against *Cladosporium cladosporioides* strains was determined by the microatmospheric method (625 µl EO/L air) during 14 days of cultivation. Thirteen EOs: thyme and red thyme (from *Thymus vulgaris* L.), mitcham mint (*Mentha x piperita* L. var. Mitcham), peppermint (*Mentha x piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), spearmint (*Mentha spicata* L. var. *crispa*), lavender (*Lavandula angustifolia* Mill.), marjoram (*Origanum majorana* L.), bergamot-mint (*Mentha citrata* Erh.), wild thyme (*Thymus serpyllum* L.), hyssop (*Hyssopus officinalis* L.), and oregano (*Origanum vulgare* L.) were shown to inhibit growth of all strains to 100%. Basil (*Ocinum basilicum* L.) and rosemary (*Rosmarinus officinalis* L.) EOs did not have a 100% inhibitory effect on all strains throughout the cultivation period. Subsequently, minimum inhibitory concentrations (MIDs) were determined using the microatmospheric method. LD_{90} and LD_{50} values were estimated by probit analysis. Based on the MID, LD_{90}, and LD_{50} results, the essential oils can be divided into three groups. The first group (most effective): thyme, red thyme, spearmint, wild thyme, and oregano EO; the second group: peppermint, lavender, mitcham mint and savory EO; the third group: sage, marjoram, bergamot-mint and hyssop EO. The most effective EOs can be used to suppress the growth of *Cladosporium cladosporioides* in the vapour phase.

Keywords: essential oils, *Cladosporium cladosporioides*, antifungal activity, vapour phase, Lamiaceae

INTRODUCTION

Fruits and vegetables are rich in essential vitamins, minerals, fiber and have health benefits. Their consumption has increased in recent years. Consumers have a right to quality product that is safe to consume and are therefore increasingly concerned about the nutritional value, safety, and taste of the fruit and vegetables they consume. The term 'quality', according to consumers, can be defined as fruit with perfect shape, size, colour, aroma, and without defects such as cuts, bruises, or signs of spoilage. The presence of signs of spoilage indicates that the commodity is not safe for consumption **(Sivakumar *et* Bautista-Baños, 2014).** Nearly 40% of fruits and vegetables are wasted annually due to improper handling, improper storage, packaging, and during transportation **(Singh *et al.*, 2014).** Fresh fruit is susceptible to contamination by microscopic fungi during growth, harvesting, transport, sale, and at the consumer. It is important to identify these contaminants in fresh fruit because they can grow on commodities and some species can produce mycotoxins on them, while some can cause infections or allergies in consumers **(Tournas *et* Katsoudas, 2005).** These authors report *Botrytis cinerea*, *Rhizopus* (in strawberries), *Alternaria*, *Penicillium*, *Cladosporium*, and *Fusarium* as the most common fungi isolated from berries. Fungi belonging to the genus *Cladosporium* are cosmopolitan and occur in a variety of substrates or hosts. *Cladosporium* spp. are responsible for the economic losses of many agricultural crops. They cause leaf spots, leaf scab, postharvest rots and other crop diseases. The etiology of many diseases associated with this genus is still unclear **(Rosado *et al.*, 2019**). **Watanabe *et* al. (2011)** report the genus *Cladosporium* as the most commonly occurring genus on various fruits (including strawberries). Traditionally, sprays or dipping in fungicides have been used to control post-harvest diseases of fruit and vegetables. Growing consumer concerns about food safety due to the presence of fungicide residues on these products have resulted in a search for natural, organic and alternative disease control strategies. The use of essential oil (EO) vapours is a new approach to combat postharvest diseases of fruits and vegetables **(Sivakumar *et* Romanazii, 2019).** Conversely, the frequent use of synthetic fungicides is undesirable and can be equally problematic. In addition, the increasing fungal resistance to commercial synthetic fungicides justifies growing efforts to find new effective, yet environmentally friendly alternatives. Preparations based on EOs undoubtedly represent such an alternative **(Zabka *et al.*, 2014, Sakkas *et al.*, 2017**). EOs due to their antimicrobial potential and low toxicity represent a sustainable and safe alternative in controlling microbial growth **(Macedo-Arantes, *et al.*, 2021)**. EOs are volatile natural substances of plant origin that are used in medicine, for flavoring and preserving food **(Khorshidian *et* al., 2018**). The application of EOs as antimicrobial agents is currently a subject of research and a promising approach in terms of natural preservation **(Thielmann *et al.*, 2019).** These diverse compounds present a considerable potential due to their antioxidant, antibacterial and antifungal activities through a variety of mechanisms. Plants of the Lamiaceae family are an important source of EOs **(Karpiński, 2020).**
The presented research was focused on the determination of antifungal activity of fifteen EOs in air vapour phase against five strains of *Cladosporium cladosporioides*.

MATERIAL AND METHODS

Fungal cultures

The strains of *Cladosporium* sp. were obtained from mouldy fruits from the stores in the Nitra region. Microscopic fungi were isolated directly from lesions on the fruit. Characteristic of isolates is as follows:

Cladosporium cladosporioides KMi-1034 – from raspberries (Morocco)
Cladosporium cladosporioides KMi-1035 – from blackberries (Spain)
Cladosporium cladosporioides KMi-1036 – from strawberries (Slovakia)

Cladosporium cladosporioides KMi-1037 – from raspberries (Poland)
Cladosporium cladosporioides KMi-1038 – from blueberries (Peru)

Identification of the strains was made according to **Samson *et al.* (2019)**. The strains are deposited in the Collection of Microorganisms of the Institute of Biotechnology, Faculty of Biotechnology and Food Sciences of the Slovak University of Agriculture in Nitra.
For growth-inhibition assays, the strains were grown on potato-dextrose agar (PDA; HIMEDIA India) at 25 ± 1 ^{O}C for 7 days. Spores were collected by rinsing the colony with physiological saline solution supplemented with Tween 80 (0.5%). Conidial suspension with a concentration of 10^6 spores/mL (0.5 McFarland units) was prepared for each *Cladosporium* strain. Spore concentration was determined using a densitometer (Densilameter II, Erba Lachema s.r.o. Brno). Calibration was made using Thom's chamber for each isolate.

Essential oils

Fifteen commercially available oils were used in the experiment. According to the producers, they were prepared by steam distillation. All EOs were prepared from plants of the Lamiaceae family. EOs used in the research: basil (from *Ocimum basilicum* L.; country of origin Slovakia), thyme and red thyme (*Thymus vulgaris* L.; thyme – Balkan and red thyme – Spain), rosemary (*Rosmarinus officinalis* L.; Slovakia), mitcham mint (*Mentha x piperita* L. var. Mitcham; Italia), peppermint (*Mentha x piperita* L.; India), savory (*Satureja hortensis* L.; Eastern Europe), sage (*Salvia officinalis* L.; Balkan), spearmint (*Mentha spicata* L. var. *crispa*; China), lavender (*Lavandula angustifolia* Mill.; France), marjoram (*Origanum majorana* L.; Egypt), bergamot-mint (*Mentha citrata* Erh.; America), wild thyme (*Thymus serpyllum* L.; Europe), hyssop (*Hyssopus officinalis* L.; Balkan), and oregano (*Origanum vulgare* L.; Eastern Europe).

Determination of chemical composition of essential oils

The essential oil was diluted in hexane (HPLC ≥97%, Sigma Aldrich GmbH, Germany) in a ratio 1:99. One microliter of diluted sample was injected via CombiPal autosampler 120 (CTC Analytics AG, Zwingen, Switzerland) in gas chromatography inlet operated in split mode (1:10; 250 °C) (GC-MS Agilent 7890B-5977A, Agilent Technologies Inc., Palo Alto, CA, USA). Separation of volatile organic compounds was achieved using a HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies, Palo Alto, CA, USA) and the following oven temperature programme started at 50 °C for the first 5 minutes, increased to 240 °C at the rate of 3 °C min^{-1}, where it was kept constant for 2 minutes. The constant flow of helium 1.2 mL.min^{-1} was used. The ionization energy of the filament was 70 eV, the transfer line temperature was 250 °C, the MS source temperature was 230 °C, and the quadrupole temperature was 150 °C. The mass spectrometer was programmed under electron impact (EI) in a full scan mode at 40 – 400 m/z. The individual compounds were identified based on the comparison of the mass spectra with a commercial database National Institute of Standards and Technology (NIST® 2017, Gaithersburg, MD, USA) and WILEY library (over 80% match) and on the assessment of retention times of reference standards (linalool, geraniol, α-pinene, and β-pinene). The relative content (expressed in percentage) of determined compounds was calculated by dividing individual peak area by the total area of all peaks. Peaks under 1% were not counted. Each sample was measured in triplicate.

Antifungal activity of essential oils

A modified methodology previously reported by **Guynot *et al.* (2003)** was used to determine the antifungal activity of plant essential oils. The vapour phase diffusion method was used to determine the inhibitory effect of EOs on the growth of *Cladosporium cladosporioides* strains. A volume of 15 ml of PDA medium was added into the Petri dishes with a diameter of 9 cm. Five microlitres of spore's suspension prepared according to the above methodology was inoculated into the centre of the medium. A small piece (1.5 cm × 1.5 cm) of Whatman No. 1 filter paper was placed in the centre of the Petri dish and saturated with 50 µl of concentrated EO. The Petri dishes were sealed with parafilm. The dishes were cultivated in the upside-down position. The evaporated EO had the concentration of 625 µL of EO in one litre of air. A volume of 50 µl of sterile distilled water was used in place of EO in the control treatment. The experiment was performed in three replications. The strains were cultivated on PDA for 14 days at 25±1 °C. The growth of colonies was observed on the 2nd, 3rd, 4th, 7th, 9th, 11th, and 14th day of cultivation. Colonies size was measured on the reverse side of colonies using a digital calliper. Colony diameters were calculated from two right-angle measurements on three colonies (six measurements for each strain and treatment). The antifungal activity of EO was expressed as relative inhibition calculated using equation, where RI is relative inhibition in %, c is the colony diameter in the control, and t is the mean of the EO-treated colony.

$$RI = [(c - t)/c] \times 100$$

Determination of inhibitory concetrations

Minimum inhibitory concentrations (MIDs) were estimated only for EOs that completely inhibited the growth of all strains tested in the previous step with a concentration of 625 µL/L. The EOs were diluted in DMSO to a concentration that provided 500 µL/L in the vapour phase when the oil was applied to the paper in 50 µL. This concentration was successively diluted in DMSO to give vapour phase concentrations of 250, 125, 62.5, 31.25, and 15.625 µL/L. Six replicates were performed for each concentration. The presence of fungal growth was assessed on the 7th and 14th day of cultivation. The results were also used for probit analysis to estimate inhibitory doses when 50% (LD_{50}) or 90% (LD_{90}) of the colonies were unable to grow.

Statistical analysis

Data from fungal inhibition analysis were evaluated using one-way variance analysis (ANOVA) followed by a post hoc Tukey HSD test at α=0.05 significance level. Inhibitory concentrations of LD_{50} and LD_{90} were estimated using probit analysis. All statistical analyses were done in R version 4.1.2 (R core team 2022).

RESULTS

Results of the chemical composition of the essential oils used

The main determined components of EOs are listed in table 1.

Table 1 Components* of essential oils determined by gas chromatography with mass spectrometry

Essential oil	Plant	Main components	%	RT (min)	Formula	Score with NIST17/Wiley libraries
Basil	*Ocimum basilicum* L.	Estragole	84.89	27.46	C10H12O	99.21
		Eucalyptol	4.1	11.13	C10H18O	99.26
		Cis-α-Bergamotene	2.76	21.27	C15H24	97.49
		Linalool	1.84	22.11	C10H18O	98.58
Thyme	*Thymus vulgaris* L.	Cymene	43.85	12.53	C10H14	97.71
		Thymol	33.65	41.12	C10H14O	97.12
		Linalool	7.12	22.12	C10H18O	99.33
		α-Pinene	3.49	4.42	C10H16	98.7
		α-Terpineol	1.59	28.04	C10H18O	95.35
		Caryophyllene	1.43	23.43	C15H24	98.56
		β-Myrcene	1.20	7.70	C10H16	97.11
		D-Limonene	1.03	8.47	C10H16	98.42
		endo-Borneol	1.02	28.31	C10H18O	97.08
Rosemary	*Rosmarinus officinalis* L.	Eucalyptol	43.17	11.96	C10H18O	98.95
		(+)-2-Bornanone	12.80	25.58	C10H16O	99.05
		α-Pinene	10.74	4.42	C10H16	99.19
		β-Pinene	7.43	6.34	C10H16	98.41
		Camphene	4.66	5.49	C10H16	99.19
		endo-Borneol	3.83	28.33	C10H18O	98.42
		Caryophyllene	3.78	23.44	C15H24	99.18
		D-Limonene	2.82	8.48	C10H16	99.21
		Cymene	2.75	12.45	C10H14	97.51

Continue Table 1

		α-Terpineol	2.31	28.04	C10H18O	82.21
		Isobornil acetate	1.28	24.85	C12H20O	98.19
Red thyme	*Thymus vulgaris* L	Thymol	40.41	40.41	C10H14O	97.11
		Cymene	19.45	12.47	C10H14	97.85
		Caryophyllene	6.77	23.45	C15H24	99.3
		Linalool	5.80	22.12	C10H18O	99.17
		γ-Terpinene	5.67	10.09	C10H16	98.88
		(+)-2-Bornanone	2.36	25.55	C10H16O	98.66
		endo-Borneol	2.25	28.32	C10H18O	98.03
		Terpinen-4-ol	2.16	25.09	C10H18O	98.65
		α-pinene	1.79	4.42	C10H16	97.55
		Eucalyptol	1.64	11.12	C10H18O	98.45
		Camphene	1.62	5.49	C10H16	98.1
		β-Myrcene	1.48	7.70	C10H16	97.18
		(+)-4-Carene	1.02	8.30	C10H16	97.23
Peppermint (mint)	*Mentha x piperita* L.	Levomenthol	42.43	25.82	C10H20O	98.55
		trans-Menthone	22.51	23.13	C10H18O	98.88
		Eucalyptol	7.01	11.13	C10H18O	99.37
		Menthyl acetate	6.30	23.31	C12H22O2C10H18O	97.61
		cis-Menthone	4.22	24.51	C10H20O	98.78
		Neoisomenthol	3.54	23.89	C15H24	98.96
		Caryophyllene	3.33	23.47	C10H16	98.95
		D-Limonene	2.03	8.46	C10H14O	98.86
		Menthofuran	1.62	20.13		96.11
Savory	*Satureja hortensis* L.	γ-Terpinene	45.09	10.17	C10H16	98.65
		Thymol	20.2	42.13	C10H14O	97.01
		Cymene	19.64	12.47	C10H14	97.85
		(+)-4-Carene	3.76	8.30	C10H16	97.6
		α-Pinene	2.71	4.45	C10H16	98.2
		β-Myrcene	2.49	7.69	C10H16	96.83
Sage	*Salvia officinalis* L.	Thujone	22.37	21.50	C10H16O	98.77
		(+)-2-Bornanone	19.65	25.59	C10H16O	99.03
		Eucalyptol	10.84	11.13	C10H18O	99.26
		Humulene	6.92	26.06	C15H24	98.88
		β-Thujone	6.58	22.03	C10H16O	97.86
		α-pinene	6.08	4.42	C10H16	98.93
		Camphene	5.86	5.49	C10H16	99.33
		Caryophyllene	5.62	23.45	C15H24	99.24
		endo-Borneol	4.45	28.32	C10H18O	98.46
		β-Pinene	2.34	6.34	C10H16	98.02
		Bornyl acetate	2.29	24.85	C12H20O2C10H16	98.68
		D-Limonene	1.88	8.46	C10H14	98.79
		o-Cymene	1.74	12.43		97.49
Spearmint	*Mentha spicata* L. var. *crispa*	(-)-Carvone	72.62	31.84	C10H14O	97.84
		D-Limonene	15.23	8.48	C10H16	99.21
		cis-dihydrocarvone	1.66	28.38	C10H16O	93.09
		Levomenthol	1.62	25.65	C10H20O	98.62
Lavender	*Lavandula angustifolia* Mill	Linalyl acetate	38.60	22.84		99.24
		Linalool	33.15	22.19	C12H20O2C10H18O	99.03
		(-)-Lavandulol	3.43	24.75	C12H20O2C15H24	99.05
		Caryophyllene	3.32	23.47	C15H24	99.07
		(E)-β-Famesene	2.58	24.40	C10H18O	98.3
		(-)-Terpinen-4-ol	2.52	25.09	C10H16	98.21
		trans-beta-Ocymene	1.52	9.99	C10H16	98.71
		1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	1.18	10.66	C10H18O	98.77
		α-Terpineol	1.17	28.03	C10H18O	94.77
		Eucalyptol	1.01	11.11		97.66
Marjoram	*Origanum majorana* L.	Terpinene-4-ol	34.52	25.16	C10H18O	95.54
		γ -Terpinene	16.85	10.09	C10H16	90.36
		cis-Sabinene hydrate	15.09	23.66	C10H18O	92.34
		(+)-4-Carene	9.28	8.30	C10H16	97.71
		Sabinene	6.91	6.92	C10H16	97
		Cymene	6.30	12.43	C10H14	81.66
		D-Limonene	2.30	8.45	C10H16	99.03
		β-Myrcene	2.04	7.69	C10H16	94.95
		α-pinene	1.87	4.45	C10H16	96.66
Wild thyme	*Thymus serpyllum* L.	Benzene, 4-ethyl-1,2-dimethyl-	18.07	12.44	C10H14	97.9
		Thymol	12.13	41.06	C10H14O	97.03
		Geraniol	10.74	31.64	C10H18O	98.82
		γ-Terpinene	10.43	10.07	C10H16	98.85
		Linalool	5.06	22.08	C10H18O	99.18
		Geranyl acetate	4.77	29.26	C12H20O2C10H18O	99.02
		endo-Borneol	2.71	28.28	C10H16	97.8
		α-Pinene	2.61	4.41	C15H24	98.34
		Caryophyllene	2.50	23.39	C10H18O	98.46
		Terpinen-4-ol	2.39	25.06	C10H18O	98.5
		α-Terpineol	1.82	28.01	C15H24	95.09
		Humulene	1.60	25.99	C10H16	94.84

Continue Table 1

		D-Limonene	1.57	8.43	C10H16O	98.74
		(+)-2-Bornanone	1.47	25.51	C10H16	97.71
		Camphene	1.28	5.47	C10H16	97.71
		(+)-4-Carene	1.27	8.28	C10H16	97.65
		β-Myrcene	1.25	7.68	C10H18O	96.29
		Eucalyptol	1.16	11.09		97.31
Oregano	*Origanum vulgare* L.	P-Thymol (o-Cymen-5-ol)	60.37	42.17	C10H14O	97.04
		Cymene	13.14	12.42	C10H14	97.86
		γ-Terpinene	7.91	10.06	C10H16	98.78
		α-Thujene	2.92	4.45	C10H16	97.54
		Caryophyllene	2.28	23.38	C15H24	98.39
		β-Myrcene	2.26	7.67	C10H16	97.17
		Thymol	1.66	41.05	C10H14O	97.38
		(+)-4-Carene	1.15	8.27	C10H16	96.74
		Terpinene-4-ol	1.10	25.06	C10H18O	97.39
		endo-Borneol	1.03	28.28	C10H18O	96.61
Bergamot-mint	*Mentha citrata* Erh.	Linalyl acetate	45.02	22.82	C12H20O2C10H18O C12H20O2C12H20O2C10H20O C15H24 C10H18O C10H16 C10H16	99.26
		Linalool	33.95	22.17		98.98
		Geranyl acetate	5.93	29.25		99.02
		Neryl acetate	2.75	28.43		98.18
		Levomenthol	2.72	25.63		98.99
		Caryophyllene	1.30	23.42		98.07
		α-Terpineol	1.20	28.00		95.19
		D-Limonene	1.07	8.43		97.78
		β -Myrcene	1.00	7.67		97.04
Mitcham mint	*Mentha x piperita* L. var. Mitcham	Levomenthol	41.55	25.78		99.45
		l-menthone	19.59	23.07	C10H20O	98.99
		Eucalyptol	8.25	11.09	C10H18O	99.37
		Menthyl acetate	5.23	23.26	C10H18O	98.23
		Methofuran	4.27	20.09	C12H22O2C10H14O	97.04
		Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	3.72	24.47	C10H18O C10H16	98.14
		D-Limonene	3.40	8.43	C10H20O	98.91
		Neoisomenthol	2.93	23.85	C15H24	98.87
		Caryophyllene	2.02	23.40	C10H16O	97.87
		Pulegone	1.86	28.60	C10H18O	97.93
		Terpinen-4-ol	1.31	25.06		92.01
Hyssop	*Hyssopus officinalis* L.	Campholenone	38.39	26.04	C10H16O	98,21
		trans-3-Pinanone	25.05	24.80	C10H16O	98,58
		β-pinene	11.07	6.31	C10H16	98,52
		Myrtenyl methyl ether	2.87	16.02	C11H18O	97,89
		Caryophyllene	2.16	23.41	C15H24	98,18
		beta-Phellandrene	1.71	6.90	C10H16	97,91
		(-)-β -Bourbonene	1.66	19.26	C15H24	98,26
		(-)-Myrtenol	1.63	30.30	C10H16O	96,47
		Terpinene-4-ol	1.22	25.09	C10H18O	97.53
		Linalool	1.14	22.08	C10H18O	90,48
		D-Limonene	1.10	8.42	C10H16	98,16
		Germacrene D	1.08	26.97	C15H24	96,8

Legend: * The table shows the components that have been determined in quantities ≥1%, RT - retention time

Results of testing the antifungal activity of essential oils

Thirteen EOs (thyme, red thyme, peppermint, savory, sage, spearmint, lavender, marjoram, wild thyme, oregano, bergamot-mint, mitcham mint, hyssop) from the fifteen tested, at a vapour phase concentration of 625 µL/L EO, were shown to inhibit the growth of all *Cladosporium cladosporioides* strains during the fourteen days of the experiment. *Cladosporium cladosporioides* strains responded differently to the presence of Eos (Table 2). A partial inhibitory effect was observed for basil and rosemary EOs. But two strains (KMi-1034 and KMI-1038) were also inhibited completely by these EOs. Basil oil completely inhibited the growth of KMi-1034 and 1038 strains. The growth of strain KMi-1037 was recorded on the fourth day of cultivation and the growth of strains KMi-1035 and KMi-1036 on the ninth day. However, relative inhibition was also noted for those strains that grew. In the presence of rosemary EO, growth was recorded for only one strain, KMi-1035. Growth of this strain was observed on the eleventh day of the experiment. However, the colonies in this case were also smaller compared to the control. Based on only a partial inhibitory effect, basil EO and rosemary EO were excluded from further testing.

Table 2 Average growth of fungal strains (diameter in mm; n = 3) and relative inhibition (in %) of *Cladosporium cladosporioides* strains on PDA at 25±1 °C

Day of cultivation	Treatment				
	Control	Basil		Rosemary	
	Average ± sd	Average ± sd	RI	Average ± sd	RI
	***Cladosporium cladosporioides* KMi-1034**				
2nd	10.32±0.47[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
3rd	13.00±0.10[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
4th	16.36±0.59[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
7th	25.53±0.41[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
9th	32.51±0.66[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
11th	37.32±0.80[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
14th	44.23±0.97[b]	0.00±0.00[a]	100	0.00±0.00[a]	100

Continue Table 2

	***Cladosporium cladosporioides* KMi-1035**				
2nd	10.82±0.44[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
3rd	14.44±0.85[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
4th	17.08±0.22[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
7th	25.58±0.55[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
9th	33.28±1.57[b]	1.39±0.37[a]	95.8	0.00±0.00[a]	100
11th	38.27±1.03[b]	6.77±0.81[a]	82.3	5.91±0.47[a]	82.3
14th	44.68±1.30[c]	14.93±0.56[b]	66.6	12.18±0.6[a]	72.7
	***Cladosporium cladosporioides* KMi-1036**				
2nd	11.11±0.25[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
3rd	14.61±0.49[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
4th	16.92±0.60[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
7th	26.41±0.25[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
9th	34.84±1.67[c]	2.03±0.33[b]	94.2	0.00±0.00[a]	100
11th	40.33±0.95[c]	6.02±0.33[b]	85.1	0.00±0.00[a]	100
14th	47.03±0.59[c]	14.25±0.64[b]	69.7	0.00±0.00[a]	100
	***Cladosporium cladosporioides* KMi-1037**				
2nd	11.03±0.23[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
3rd	14.69±0.46[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
4th	15.98±0.49[c]	2.49±0.23[b]	84.4	0.00±0.00[a]	100
7th	20.65±0.49[c]	3.58±0.40[b]	82.7	0.00±0.00[a]	100
9th	34.99±0.46[c]	4.28±0.23[b]	87.8	0.00±0.00[a]	100
11th	39.03±0.62[c]	5.10±0.56[b]	86.9	0.00±0.00[a]	100
14th	43.41±0.51[c]	5.91±0.40[b]	86.4	0.00±0.00[a]	100
	***Cladosporium cladosporioides* KMi-1038**				
2nd	10.84±0.38[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
3rd	13.74±0.71[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
4th	15.10±0.64[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
7th	22.05±0.65[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
9th	28.25±0.63[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
11th	34.78±0.49[b]	0.00±0.00[a]	100	0.00±0.00[a]	100
14th	41.98±0.98[b]	0.00±0.00[a]	100	0.00±0.00[a]	100

Averages accompanied by the same letter (in line) are not significantly different (ANOVA, Tukey test; α=0.05).
Legend: n – number of replications, PDA - potato dextrose agar, RI – relative inhibition, sd – standard deviation.

Results of the determination of inhibitory concentrations of essential oils

In this part of the research, only those essential oils that completely inhibited the growth of all strains tested during the fourteen days in the previous part of the study were used. The results are summarized in Table 3. The lowest MIDs were observed for thyme, red thyme, spearmint, and wild thyme EOs. These EOs completely inhibited the growth of all *Cladosporium cladosporioides* strains on the 7th or 14th day of cultivation at a concentration of 250 µL/L. Oregano EO also appeared to be a very effective EO, which inhibited the four tested strains on both the 7th and 14th day of cultivation when a concentration of 125 µL/L was used. But strain *Cladosporium cladosporioides* KMi-1038 grew at a concentration of 250 µL/L in one of the six repetitions on both the 7th and 14th day. Similarly, savory EO completely inhibited the growth of four strains at concentration of 250 µL/L, only strain *Cladosporium cladosporioides* KMi-1035 grew at this concentration in one repetition out of six. Peppermint and mintcham mint EOs completely inhibited the growth of the strains at 250 µL/L EOs concentration on the 7th day but at 500 µL/L EOs concentration on the 14th day. Savory EO inhibited the growth of four strains at a concentration of 250 µL/L on both day 7 and 14. Only strain *Cladosporium cladosporioides* KMi-1035 grew at this oil concentration in one replicate on the 7th and 14th day, respectively. For the lavender EO, a concentration of 500 µL/L was required for complete inhibition. The weakest EOs were marjoram, bergamot-mint, sage, and hyssop EOs. Concentrations of 500 µL/L or >500 µL/L were required to completely inhibit the colony growth.

Table 3 Minimal inhibition doses (µL/L) of essential oils in vapour phase on the growth of *Cladosporium cladosporioides* colonies (n = 6) on PDA after 7 and 14 days of cultivation at 25±1 ºC

Essential oils	Strain of *Cladosporium cladosporioides*									
	KMi-1034		KMi-1035		KMi-1036		KMi-1037		KMi-1038	
	Day of cultivation									
	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th
	µL essential oil / L of air									
Thyme	125	250	250	250	250	250	125	250	250	250
Red thyme	250	250	62.5	125	125	125	62.5	125	125	125
Peppermint (mint)	250	500	250	250	125	250	250	250	250	500
Savory	250	250	500	500	250	250	125	250	250	250
Sage	>500	>500	>500	>500	>500	>500	500	>500	>500	>500
Spearmint	250	250	125	125	250	250	250	250	250	250
Lavender	250	500	250	250	250	250	500	500	250	500
Marjoram	500	>500	500	500	500	>500	500	>500	500	>500
Wild thyme	250	250	125	125	125	250	125	125	250	250
Oregano	125	125	62.5	62.5	62.5	62.5	125	125	500	500
Bergamot-mint	500	>500	500	500	500	>500	500	>500	500	>500
Mitcham mint	125	500	250	250	125	250	250	250	250	500
Hyssop	500	>500	500	>500	500	500	250	500	500	>500

Legend: n - number of repetitions; PDA – potato dextrose agar

Using probit analysis, the doses of EOs that would inhibit the growth of *Cladosporium cladosporioides* in 50% (LD_{50}) or 90% (LD_{90}) of cases were estimated. The results of the probit analysis are presented in Table 4. The LD_{50} and LD_{90} values varied not only depending on the EO used, but also on the tested strain and the day of observation. Based on the results of the probit analysis used to estimate the LD_{50} values, the following EOs were evaluated as the most effective: oregano, red thyme, wild thyme, and peppermint EOs. The next group (less effective) included peppermint and mitcham mint EOs, then lavender EO. Hyssop, bergamot-mint, and marjoram EOs were the next group. The highest LD_{50} values were found for sage EO, which was rated as the least effective. According to the LD_{90}, the cluster of EOs would be a little different, but again thyme, red thyme, spearmint, wild thyme, and oregano EOs would be ranked as the most effective. However, in the case of oregano EO, a significantly higher LD_{90} value was found for strain KMi-1038 (237.9 µL/L) than for the other four strains (ranging from 32.2 to 59.9 µL/L) on the 7th day of cultivation. Peppermint, savory, and mitcham EOs were the next group. Compared to the previous group, lavender EO was less effective. Bergamot-mint, hyssop, and marjoram EOs were rated as less effective. Also, according to LD_{90}, sage EO was rated as the least effective. The strains

responded differently to the presence of different EOs. For example, strain *Cladosporium cladosporioides* KMi-1038 was the most sensitive to the presence of lavender EO and the most resistant to oregano EO on the 7th day of cultivation when compared to the other strains tested.

DISCUSSION

In our study, the effect of fifteen different EOs derived from plants of the Lamiaceae family on five strains of *Cladosporium cladosporioides* was compared. These strains were obtained directly from lesions excised from different berry fruit species. *Cladosporium cladosporioides* is a new pathogenic fungus that causes post-harvest cladosporic rot of table grapes (*Vitis vinifera*) **(Solairaj *et al*., 2020; 2022). Lutz *et al*. (2017)** reported that *Cladosporium* spp. together with *Alternaria* spp. pose a hazard to pear fruit during storage with significant economic losses. The occurrence of blossom blight in some strawberry fruit was reported **(Gubler *et al.*, 1999; Nam *et al*., 2015).** According to the metagenomic analysis completed by **Abdelfattah *et al*. (2016)**, there is a high diversity of microbial organisms on strawberry, but two genera, *Botrytis* and *Cladosporium* were the most abundant and accounted for 70-99% of the relative abundance of all sequences detected.

The chemical composition of plant EOs is influenced by several factors including the species, the part of the plant, the time of harvest, the geographical origin, and the extraction method. These factors affect not only the composition of EOs but also their bioactive properties **(Tilaoui *et al*., 2015; Ben Farhat, *et al*., 2016; Dušková *et al*., 2016; Méndez-Tovar *et al*., 2016).** Commercially available EOs were used in the study. The chemical composition determined corresponds to the data reported by the dealer or in the literature.

In our research, the effect of the vapor phase of EOs was investigated. There is growing evidence that vapour phase of EOs is effective antimicrobial system, and it has advantages over the use of liquid phase of EOs, such as increased activity, use at lower concentrations, ability to be used in a variety of environments **(Nedorostova *et al*., 2008; Nedorostova *et al.*, 2009; Laird *et* Phillips, 2012)**. All EOs used in the study demonstrated inhibitory effects on *Cladosporium cladosporioides* strains to varying degrees of potency. In our study, we evaluated basil (*Ocinum basilicum* L.) and rosemary (*Rosmarinus officinalis* L.) EOs as the weakest EOs. In our experiment these oils inhibited the growth of *Cladosporium cladosporioides* colonies, however, they did not inhibit all tested strains, over the entire period (14 days). These EOs were therefore not used in the next step of the study to determine minimum inhibitory concentrations. However, several authors recommend the use of these oils as antimicrobial agents in food processing. **Abou El-Soud *et al.* (2015)** reported complete growth inhibition of *Aspergillus flavus* at basil EO concentration of 1000 ppm but in culture medium. Similarly, **Al-Maskri *et al*. (2011)** reported a potent antifungal effect of basil EO (measured by inhibition zone assay) against *Aspergillus fumigatus, Aspergillus niger, Penicillium italicum* and *Rhizopus stolonifer*. **Stanojevic *et al.* (2017**) reported the antimicrobial activity of basil EO on *Escherichia coli, Listeria monocytogenes*, *Salmonella enterica, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Providencia stuartii*, coagulase-positive staphylococci, group D streptococci, *Salmonella* spp., and *Candida albicans*. In a previous study (**Tančinová *et al*., 2022**), the effect of the vapour phase of basil EO on the growth of *Rhizopus* strains was observed. When comparing the effect of basil EO on the growth of *Cladosporium cladosporioides* strains, *Cladosporium cladosporioides* was more sensitive to the presence of this essential oil than either *Rhizopus stolonifer* or *Rhizopus oryzae*. **Prakash *et al*. (2015)** based on the significant antifungal (*Aspergillus flavus*), anti-aflatoxigenic, antioxidant activity and efficacy during *in vivo* trials, recommend rosemary EO as a plant-based preservative in protection of the agri-food commodities from moulds infestation and aflatoxin contamination as well as from oxidative deterioration.

Thirteen essential oils were used in the part focusing on the determination of minimum inhibitory concentrations. These EOs at a concentration of 625 µL/L in vapour phase 100% inhibited the growth of *Cladosporium cladosporioides* strains during 14 days of cultivation. In our study, thyme, red thyme (*Thymus vulgaris* L.), spearmint (*Mentha spicata* L. var. *crispa*), wild thyme (*Thymus serpyllum* L.), and oregano (*Origanum vulgare* L.) proved to be the most effective EOs. The second group consisted of essential oils such as peppermint (*Mentha x piperita* L.), lavender (*Lavandula angustifolia* Mill.), mitcham mint (*Mentha x piperita* L. var. Mitcham), and savory (*Satureja hortensis* L.). The highest MID, LD_{90}, and LD_{50} were determined for EOs such as marjoram (*Origanum majorana* L.), bergamot-mint (*Mentha citrata* Erh.), sage (*Salvia officinalis* L.), and hyssop (*Hyssopus officinalis* L). The lowest MID, LD_{90}, and LD_{50} values were found for thyme, red thyme, spearmint, wild thyme, and oregano EOs. **Zabka *et al.* (2014)** tested the effect of EOs on four species of microscopic fungi, including *Cladosporium cladosporioides*. Like our experiment, the highest inhibitory effect was found for the EOs *Origanum vulgare*, *Thymus vulgaris* (100%), less effective were *Lavandula angusifolia* (52.73%), *Salvia officinalis* (29.31%), *Ocimum basilicum* (27.59%), *Rosmarinus officinalis* (27.59%). **Bota *et al.* (2022)** evaluated the effect of the vapour phase of thyme and oregano EOs on selected microscopic fungi. Based on the results, they indicated a selective antifungal effect. Oregano and thyme EOs inhibited *Alternaria*, *Fusarium,* and *Drechslera*, while *Saccharomyces* and *Cladosporium* were found to be the most tolerant fungi. However, both thyme and oregano EOs applied in vapour form at levels ranging from 0.2 to 0.4% were shown to have fungicidal effect on all the microscopic fungi mentioned above. In a previous study **(Tančinova *et al*., 2022)** focusing on the effect of EOs on strains of the genus *Rhizopus*, thyme and red thyme (MID 250 µL/L) were also determined to be the most effective EOs, as in the present study. But for savory, wild thyme and oregano EOs, higher minimum inhibitory concentrations were recorded. Species of the genus *Rhizopus* are also frequently involved in postharvest fruit blight as are representatives of the genus *Cladosporium*. To determine the concentrations needed to inhibit food mould, it is necessary to obtain as much data as possible on the effect of essential oils on potential pathogens. The antifungal activity of *Mentha spicata* (spearmint) EO was pointed out by **Regmi *et* Jha (2017).** In their study, this EO showed the best antifungal effect on *Aspergillus niger* control, which inhibited mycelial growth by 92.93% at a concentration of 40 µL/mL. **Yan *et al*. (2020)** investigated the inhibitory effects of 26 essential oils on the mycelium of *Rhizpus stolonifer*. The inhibitory effects were evaluated using a method based on their volatility. The results showed that all EOs at 150 µL/L exhibited some degree of inhibition on mycelial growth with a wide range of inhibitory potency. Of the EOs tested, eight EOs (including *Rosmarinus officinalis* CT cineol, *Rosmarinus officinalis* CT verbenone) showed little effect on *Rhizopus stolonifer*, with less than 30% inhibition after 72 h of incubation. Other EOs (including *Lavandula angustifolia*, *Thymus vulgaris* CT linalol, *Ocimum basilicum*, *Origanum vulgare*) showed moderate inhibitory effect efficacy against *Rhizous stolonifer* with 30.12-75.15% inhibition after 72 h of incubation. Only *Mentha spicata*, *Mentha piperita*, *Thymus vulgaris* CT carvacrol, *Thymus vulgaris* CT thymol showed sustained and potent inhibition. After 72 hours of incubation, these four essential oils inhibited mycelial growth by 92.41, 98.00, 98.48, and 98.81%, respectively.

Significant antifungal activity of peppermint (*Mentha* × *piperita* L.) EO against *Alternaria alternata* (38.16 ± 0.10 mm), *Fusarium tabacinum* (current name *Plectosphaerella cucumerina*; Mycobank)_(35.24 ± 0.03 mm), *Penicillum* spp. (34.10 ± 0.02 mm), *Fusarium oxysporum* (33.44 ± 0.06 mm), and *Aspergillus fumigatus* (30.08 ± 0.08 mm) was confirmed by the agar disc diffusion method **(Desam *et al*., 2019).**

Nazzaro *et al.* (2017) listed the following basic effects of EO against fungi: disruption of cell membrane, inhibition of cell wall formation, dysfunction of fungal mitochondria, inhibition of efflux pumps. Although the mechanism of effect of several EOs components has been explained, there is still a lack of data on most EOs compounds and their mechanism of action **(Chouhan *et al.*, 2017).** In the present research, the most effective EOs were those in which thymol (thyme, red thyme, wild thyme, and oregano EOs) and carvone (spearmint EO) were the basic components. The antifungal activity (including *Cladosporium* spp.) of thyme EO in the vapour phase is also reported by **Šegvić Klarić *et al.* (2007)**. According to these authors, thyme EO (*Thymus vulgaris* L.) has a broad spectrum of fungicidal activity, and vapour phase EO exhibits long-lasting fungal growth-suppressing activity. Thymol and carvacrol evidently modified the morphology of *Botrytis cinerea* mycelium by disrupting and deforming the hyphae. The membrane permeability of *Botrytis cinerea* hyphae increased with increasing concentrations of these two chemicals, as reflected by an increase in extracellular conductance, release of cellular components, and a decrease in extracellular pH. In addition, thymol and carvacrol induced a significant decrease in total lipid content in *Botrytis cinerea* cells, suggesting that destruction of cell membrane structures occurred **Zhang *et al.* (2019)**. Carvone, which is regarded as a safe (GRAS) natural plant compound, can be considered a potent antifungal and antiaflatoxigenic natural compound against *Aspergillus flavus* **(Lasram *et al.*, 2019)**. Growth of *Penicillium digitatum* and *Geotrichum citri-aurantii* (Ferraris) was completely inhibited by vapour phase application (714 mg/L) of spearmint EO and its main component carvone **(Phala *et al*., 2022).**

The tested EOs may be a potential antifungal agent to suppress cladosporic rot. Plant EOs have multipurpose antimicrobial effects that make them a potent and sustainable antimicrobial agent for postharvest loss control **(Lin *et al*., 2022).**

Table 4 Predicted concentration (µL/L) of essential oils that inhibited the growth of *Cladosporium cladosporioides* strains to 90% or 50% calculated by probit analysis

LD	Essential oils																									
	Thyme		Red thyme		Peppermint		Savory		Sage		Spearmint		Lavender		Marjoram		Wild thyme		Oregano		Bergamot-mint		Mitcham mint		Hyssop	
	Day of cultivation																									
	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th
***Cladosporium cladosporioides* KMi-1034**																										
LD_{50}	67.3	88.39	69.9	88.3	114.3	176.8	119.7	125.0	434.9	515.3	130.4	136.5	176.6	260.9	353.3	491.7	69.9	119.7	32.0	32.0	250	373.3	88.4	158.4	238.7	292.0
LD_{90}	125.9	137.9	128.7	168.4	128.7	275.9	136.1	143.0	459.2	536.2	147.9	153.6	189.1	296.2	378.3	516.7	128.7	136.1	59.9	59.9	285.4	594.6	94.5	270.9	427.8	675.0
***Cladosporium cladosporioides* KMi-1035**																										
LD_{50}	79.2	79.2	44.2	60.0	119.7	119.7	100.4	112.7	507.3	507.3	88.4	88.4	176.6	176.9	273.3	273.3	62.5	62.5	28.6	28.6	273.3	273.3	130.5	136.6	250	479.7
LD_{90}	135.4	135.2	47.3	68.0	136.1	136.7	241.4	244.6	529.6	529.6	94.5	94.5	189.1	189.1	307.5	307.5	71.5	71.5	32.2	32.2	307.5	307.5	148.4	153.5	285.4	506.7
***Cladosporium cladosporioides* KMi-1036**																										
LD_{50}	64.3	64.3	59.9	62.5	88.4	119.7	79.5	79.5	434.9	434.9	125.0	130.5	176.6	250.0	260.9	479.8	65.2	79.2	32.6	34.2	353.3	479.8	65.2	109.6	176.7	250.0
LD_{90}	119.3	119.3	68.0	71.3	94.5	136.1	162.4	162.4	759.2	759.2	143.0	147.9	189.0	285.4	296.3	506.9	74.0	135.4	37.0	38.5	378.3	506.7	74.0	197.6	189.1	285.4
***Cladosporium cladosporioides* KMi-1037**																										
LD_{50}	65.2	79.2	44.2	59.9	114.4	176.7	65.2	114.2	353.3	515.3	119.7	136.6	176.7	273.4	260.9	344.9	65.2	65.2	44.2	59.9	273.4	479.8	121.5	121.5	176.7	353.3
LD_{90}	74.0	135.2	47.4	68.0	128.6	189.1	74.0	128.7	378.3	536.2	136.1	153.6	189.1	307.8	296.3	515.5	74.0	74.0	47.4	68.0	307.8	506.7	225.0	224.5	189.1	378.3
***Cladosporium cladosporioides* KMi-1038**																										
LD_{50}	76.5	119.7	49.3	65.2	136.6	239.6	119.7	130.4	335.2	363.6	130.4	136.6	130.4	273.4	260.9	402.5	71.7	119.7	69.2	90.1	260.9	491.7	130.4	176.7	250.0	500.0
LD_{90}	122.4	136.1	64.4	74.0	153.6	272.0	136.1	148.0	596.9	697.0	148.0	153.6	147.9	307.8	296.3	673.4	153.0	136.1	237.9	262.8	296.3	516.7	147.9	275.6	285.5	523.6

Legend LD – lethal dose

CONCLUSION

In our research, the effect of fifteen selected EOs derived from Lamiaceae plants was tested on the growth of five strains of *Cladosporium cladosporioides*. At the highest concentration used (625 µL EO/L air), thyme, red thyme, peppermint, savory, sage, spearmint, lavender, marjoram, wild thyme, oregano, bergamot-mint, and hyssop completely inhibited the growth of *Cladosporium cladosporioides* strains during 14 days of cultivation. Basil and rosemary EOs inhibited the growth of only some strains completely and some strains for a shorter period. Based on the results of MID, LD_{90}, and LD_{50}, the other essential oils can be divided into three groups. The first group (most effective): thyme, red thyme, spearmint, wild thyme, and oregano EOs; the second group: peppermint, lavender, mitcham mint, and savory EOs; the third group: sage, marjoram, bergamot-mint, and hyssop EOs. The most effective EOs (thyme, red thyme, spearmint, wild thyme, and oregano) can be used to suppress the growth of *Cladosporium cladosporioides* in the vapour phase. Research has provided new knowledge on the potential to influence the growth of *Cladosporium cladosporioides* with essential oils from plants of the Lamiaceae family. The importance of testing the effect of EOs on more strains of the same species in similar experiments was demonstrated. Testing on only one strain could result in erroneous results.

Acknowledgments: This publication was supported by VEGA 1/0517/21 and the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund. Thanks to technical worker Eva Sádovská for her help with mycological analysis.

REFERENCES

Abdelfattah, A., Wisniewski, M., Li Destri Nicosia, M. G., Cacciola, S. O., & Schena, L. (2016). Metagenomic Analysis of Fungal Diversity on Strawberry Plants and the Effect of Management Practices on the Fungal Community Structure of Aerial Organs. *PLOS ONE*, 11(8), e0160470. https://doi.org/10.1371/journal.pone.0160470

Abou El-Soud, N. H., Deabes, M., Abou El-Kassem, L., & Khalil, M. (2015). Chemical Composition and Antifungal Activity of *Ocimum basilicum* L. Essential Oil. *Macedonian Journal of Medical Sciences*, 3(3), 374–379. https://doi.org/10.3889/oamjms.2015.082

Al-Maskri, A. Y., Hanif, M. A., Al-Maskari, M. Y., Abraham, A. S., Al-Sabahi Jamal Nasser, & Al-Mantheri, O. (2011). Essential oil from *Ocimum basilicum* (omani basil): A Desert Crop. *Natural Product Communications*, 6(10), 1934578X1100601. https://doi.org/10.1177/1934578x1100601020

Ben Farhat, M., Jordán, M. J., Chaouch-Hamada, R., Landoulsi, A., & Sotomayor, J. A. (2016). Phenophase effects on sage (Salvia officinalis L.) yield and composition of essential oil. *Journal of Applied Research on Medicinal and Aromatic Plants*, 3(3), 87–93. https://doi.org/10.1016/j.jarmap.2016.02.001

Bota, V., Sumalan, R. M., Obistioiu, D., Negrea, M., Cocan, I., Popescu, I., & Alexa, E. (2022). Study on the Sustainability Potential of Thyme, Oregano, and Coriander Essential Oils Used as Vapours for Antifungal Protection of Wheat and Wheat Products. *Sustainability*, 14(7), 4298. https://doi.org/10.3390/su14074298

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, *4*(3), 58. https://doi.org/10.3390/medicines4030058

Desam, N. R., Al-Rajab, A. J., Sharma, M., Mylabathula, M. M., Gowkanapalli, R. R., & Albratty, M. (2019). Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha×Piperita* L. (peppermint) essential oils. *Journal of King Saud University - Science*, 31(4), 528–533. https://doi.org/10.1016/j.jksus.2017.07.013

Dušková, E., Dušek, K., Indrák, P., & Smékalová, K. (2016). Postharvest changes in essential oil content and quality of lavender flowers. *Industrial Crops and Products*, 79, 225–231. https://doi.org/10.1016/j.indcrop.2015.11.007

Gubler, W. D., Feliciano, A. J., Bordas, A. C., Civerolo, E. C., Melvin, J. A., & Welch, N. C. (1999). First Report of Blossom Blight of Strawberry Caused by *Xanthomonas fragariae* and *Cladosporium cladosporioides* in California. *Plant Disease*, 83(4), 400–400. https://doi.org/10.1094/pdis.1999.83.4.400a

Guynot, M. E., Ramos, A. J., Seto, L., Purroy, P., Sanchis, V., & Marin, S. (2003). Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. *Journal of Applied Microbiology*, 94(5), 893-899. https://doi.org/10.1046/j.1365-2672.2003.01927.x

Karpiński, T. M. (2020). Essential Oils of Lamiaceae Family Plants as Antifungals. *Biomolecules*, 10(1), 103. https://doi.org/10.3390/biom10010103

Khorshidian, N., Yousefi, M., Khanniri, E., & Mortazavian, A. M. (2018). Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science & Emerging Technologies*, 45, 62–72. https://doi.org/10.1016/j.ifset.2017.09.020

Laird, K., & Phillips, C. (2012). Vapour phase: a potential future use for essential oils as antimicrobials? *Letters in Applied Microbiology*, 54(3), 169–174. https://doi.org/10.1111/j.1472-765x.2011.03190.x

Lasram, S., Zemni, H., Hamdi, Z., Chenenaoui, S., Houissa, H., Tounsi, M. S., & Ghorbel, A. (2019). Antifungal and antiaflatoxinogenic activities of Carum carvi L., Coriandrum sativum L. seed essential oils and their major terpene component against *Aspergillus flavus*. *Industrial crops and products*, *134*, 11-18. https://doi.org/10.1016/j.indcrop.2019.03.037

Lin, H.-J., Lin, Y.-L., Huang, B.-B., Lin, Y.-T., Li, H.-K., Lu, W.-J., Lin, T.-C., Tsui, Y.-C., & Lin, H.-T. V. (2022). Solid- and vapour-phase antifungal activities of six essential oils and their applications in postharvest fungal control of peach (*Prunus persica* L. Batsch). *LWT*, 156, 113031. https://doi.org/10.1016/j.lwt.2021.113031

Lutz, M. C., Sosa, M. C., & Colodner, A. D. (2017). Effect of pre and postharvest application of fungicides on postharvest decay of Bosc pear caused by *Alternaria—Cladosporium* complex in North Patagonia, Argentina. *Scientia Horticulturae*, 225, 810–817. https://doi.org/10.1016/j.scienta.2017.05.007

Macedo-Arantes, S., Piçarra, A., Caldeira, A. T., Candeias, A. E., & Martins, M. R. (2021). Essential oils of Portuguese flavouring plants: potential as green biocides in cultural heritage. *The European Physical Journal Plus*, 136(11). https://doi.org/10.1140/epjp/s13360-021-02018-2

Méndez-Tovar, I., Novak, J., Sponza, S., Herrero, B., & Asensio-S-Manzanera, M. C. (2016). Variability in essential oil composition of wild populations of Labiatae species collected in Spain. *Industrial Crops and Products*, 79, 18–28. https://doi.org/10.1016/j.indcrop.2015.10.009

Nam, M. H., Park, M. S., Kim, H. S., Kim, T. I., & Kim, H. G. (2015). *Cladosporium cladosporioides* and *C. tenuissimum* Cause Blossom Blight in Strawberry in Korea. *Mycobiology*, 43(3), 354–359. https://doi.org/10.5941/myco.2015.43.3.354

Mycobank. https://www.mycobank.org/Basic%20names%20search

Nazzaro, F., Fratianni, F., Coppola, R., & De Feo, V. (2017). Essential oils and antifungal activity. *Pharmaceuticals*, 10(4), 86. https://doi.org/10.3390/ph10040086

Nedorostova, L., Kloucek, P., Kokoska, L., & Stolcova, M. (2008). Comparison of antimicrobial properties of essential oils in vapour and liquid phase against foodborne pathogens. *Planta Medica*, 74(09). https://doi.org/10.1055/s-0028-1084914

Nedorostova, L., Kloucek, P., Kokoska, L., Stolcova, M., & Pulkrabek, J. (2009). Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food Control*, 20(2), 157–160. https://doi.org/10.1016/j.foodcont.2008.03.007

Phala, K., Augustyn, W., Combrinck, S., Botha, B., Regnier, T., & Du Plooy, W. (2022). Inhibition of kumquat postharvest fungi through vapor contact with spearmint essential oil and carvone. *ACS Agricultural Science & Technology*, *2*(2), 330-339. https://doi.org/10.1021/acsagscitech.1c00232

Prakash, B., Kedia, A., Mishra, P. K., Dwivedy, A. K., & Dubey, N. K. (2015). Assessment of chemically characterised *Rosmarinus officinalis* L. essential oil and its major compounds as plant-based preservative in food system based on their efficacy against food-borne moulds and aflatoxin secretion and as antioxidant. *International Journal of Food Science & Technology*, 50(8), 1792–1798. https://doi.org/10.1111/ijfs.12822

R Core Team. *R: A language and environment for statistical computing.*; R Foundation for Statistical Computing: Vienna, Austria., 2022.

Regmi, S., & Jha, S. K. (2017). Antifungal activity of plant essential oils against *Fusarium oxysporum* schlecht. and *Aspergillus niger* van tiegh. from papaya. *Inter. J. Curr. Trends Sci. Tech*, *8*, 20196-20204. https://doi.org/10.11648/j.ijbc.20180301.11

Rosado, A. W., Custodio, F. A., Pinho, D. B., Ferreira, A. P. S., & Pereira, O. L. (2019). *Cladosporium* species associated with disease symptoms on *Passiflora edulis* and other crops in Brazil, with descriptions of two new species. *Phytotaxa*, *409*(5), 239-260. https://doi.org/10.11646/phytotaxa.409.5.1

Sakkas, H., & Papadopoulou, C. (2017). Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. *Journal of Microbiology and Biotechnology*, 27(3), 429–438. https://doi.org/10.4014/jmb.1608.08024

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2019). *Food and indoor fungi*. Utrecht: Westerdijk Fungal Biodiversity Institute. 481 p. ISBN 978-94-91751-18-9.

Šegvić Klarić, M., Kosalec, I., Mastelić, J., Piecková, E., & Pepeljnak, S. (2007). Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. *Letters in applied microbiology*, *44*(1), 36-42. https://doi.org/10.1111/j.1472-765X.2006.02032.x

Singh, V., Hedayetullah, M., Zaman, P., & Meher, J. (2014). Postharvest technology of fruits and vegetables: an overview. *Journal of Postharvest Technology*, *2*(2), 124-135. http://jpht.in/MenuscriptFile/01a7790a-4a7b-4e3a-b009-25441b8c1177.pdf

Sivakumar, D., & Bautista-Baños, S. (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop protection*, *64*, 27-37. https://doi.org/10.1016/j.cropro.2014.05.012

Sivakumar, D., & Romanazzi, G. (2019). Use of Essential Oils to Improve Postharvest Quality and Control Postharvest Decay of Tropical, Subtropical, and Temperate Fruits. *Postharvest Pathology of Fresh Horticultural Produce*, 659–676. https://doi.org/10.1201/9781315209180-20

Solairaj, D., Guillaume Legrand, N. N., Yang, Q., & Zhang, H. (2020). Isolation of pathogenic fungi causing postharvest decay in table grapes and *in vivo* biocontrol activity of selected yeasts against them. *Physiological and Molecular Plant Pathology*, 110, 101478. https://doi.org/10.1016/j.pmpp.2020.101478

Solairaj, D., Legrand, N. N. G., Yang, Q., Liu, J., & Zhang, H. (2022). Microclimatic parameters affect *Cladosporium* rot development and berry quality in table grapes. *Horticultural Plant Journal*, 8(2), 171–183. https://doi.org/10.1016/j.hpj.2021.07.002

Stanojevic, L. P., Marjanovic-Balaban, Z. R., Kalaba, V. D., Stanojevic, J. S., Cvetkovic, D. J., & Cakic, M. D. (2017). Chemical composition, antioxidant and antimicrobial activity of basil (*Ocimum basilicum* L.) essential oil. *Journal of Essential Oil Bearing Plants*, 20(6), 1557-1569. https://doi.org/10.1080/0972060x.2017.1401963

Tančinová, D., Medo, J., Maskova, Z., Barborakova, Z., & Hlebova, M. (2022). Selected plant essential oils of the Lamiaceae and Apiacea family as the antifungal agents in the vapour phase against *Rhizopus stolonifer* and *Rhizopus oryzae* isolated from moulding breads. *Journal of Microbiology, Biotechnology and Food Sciences*, 12(3), e9113. https://doi.org/10.55251/jmbfs.9113

Thielmann, J., Muranyi, P., & Kazman, P. (2019). Screening essential oils for their antimicrobial activities against the foodborne pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. *Heliyon*, 5(6), e01860. https://doi.org/10.1016/j.heliyon.2019.e01860

Tilaoui, M., Ait Mouse, H., Jaafari, A., & Zyad, A. (2015). Comparative phytochemical analysis of essential oils from different biological parts of Artemisia herba alba and their cytotoxic effect on cancer cells. *PloS one*, 10(7), e0131799. https://doi.org/10.1371/journal.pone.0131799

Tournas, V. H., & Katsoudas, E. (2005). Mould and yeast flora in fresh berries, grapes and citrus fruits. *International journal of food microbiology*, *105*(1), 11-17. https://doi.org/10.1016/j.ijfoodmicro.2005.05.002

Watanabe, M., Tsutsumi, F., Konuma, R., Lee, K.-I., Kawarada, K., Sugita-Konishi, Y., Kumagai, S., Takatori, K., Konuma, H., & Hara-Kudo, Y. (2011). Quantitative Analysis of Mycoflora on Commercial Domestic Fruits in Japan. *Journal of Food Protection*, 74(9), 1488–1499. https://doi.org/10.4315/0362-028x.jfp-10-485

Yan, J., Wu, H., Shi, F., Wang, H., Chen, K., Feng, J., & Jia, W. (2020). Antifungal activity screening for mint and thyme essential oils against *Rhizopus stolonifer* and their application in postharvest preservation of strawberry and peach fruits. *Journal of Applied Microbiology*, 130(6), 1993–2007. Portico. https://doi.org/10.1111/jam.14932

Zabka, M., Pavela, R., & Prokinova, E. (2014). Antifungal activity and chemical composition of twenty essential oils against significant indoor and outdoor toxigenic and aeroallergenic fungi. *Chemosphere*, 112, 443–448. https://doi.org/10.1016/j.chemosphere.2014.05.014

Zhang, J., Ma, S., Du, S., Chen, S., & Sun, H. (2019). Antifungal activity of thymol and carvacrol against postharvest pathogens *Botrytis cinerea*. *Journal of food science and technology*, *56*, 2611-2620. https://doi.org/10.1007/s13197-019-03747-0