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EFFECT OF ESSENTIAL OILS OF *MYRTACEAE* PLANTS ON THE *PENICILLIUM COMMUNE*

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ABSTRACT

The aim of this research was to determine the inhibitory effect of vapor phase of five essential oils (EOs) on the growth of seven strains of *Penicillium commune* isolated from moldy milk products. Another objective was to determine the minimum inhibitory doses (*in vitro* and probit analyses) of EOs, which at concentration 625 µL.L^{-1} of air completely inhibited the growth of all strains. The antifungal activity was evaluated by the micro-atmosphere method. The essential oils used in this study were extract of plants from family *Myrtaceae*. Only one essential oil – clove (from *Syzygium aromaticum* L.; leaves) completely inhibited the growth of all strains during cultivation at 25 °C and 5 °C. Eucalyptus essential oil (from *Eucaliptus globulus*; leaves), tea tree essential oil (from *Melaleuca alternifolia* Cheel; leaves), cajeput essential oil (from *Melaleuca leucadendra* L.; leaves and twigs), niaouli essential oil (from *Melaleuca quinquenervia* (Cav.) S. T. Blake; leaves) have different effects on the growth of *P. commune* strains. The order of tested essential oils according to the inhibition effect on the growth of the strains of *P. commune* (from the strongest to the weakest effect) was: clove > tea tree > cajeput > niaouli > eucalyptus. Clove EO that completely inhibited the growth of all strains was used to determine minimum inhibitory doses (MIDs). The MIDs were 125 µL.L^{-1} of air for two strains of *P. commune* and 250 µL.L^{-1} of air for five strains of *P. commune* on the 7th and 14th day of cultivation, also. Using probit analysis, predicted MIDs90 and MIDs50 were calculated. The MIDs90 were determined from 104.93 to 301.37 µL.L^{-1} of air.

Keywords: *Penicillium commune*; essential oils; antifungal activity; vapor phase

INTRODUCTION

Today's consumers demand food that is minimally technologically processed and without synthetic preservatives or additives, because of the possible adverse health effects. Therefore, the food industry is now focused on finding solutions that fully satisfy the criteria of consumers while retaining the food safety. The application of natural antimicrobial agents such as extracts, essential oils, components of spices, and other aromatic plants could be significant in resolving these problems. These agents may be useful as additives in limiting or preventing the development of harmful fungi in food, as food surface protectants, or in modified atmosphere packaging of food **(Kocic-Tanackov et al., 2014)**. Since the middle ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural, and food industries. Because of the mode of extraction, mostly by distillation from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components, and aliphatic components **(Bakkali et al., 2008)**. Natural preservatives could also constitute a viable alternative to address the critical problem of microbial resistance, and to hamper the negative side effects of some synthetic compounds, while meeting the requirements for food safety, and exerting no negative impact on nutritional and sensory attributes of foodstuffs **(Pisoschi et al., 2018)**.

Molds are the most common cheese spoilage organisms which can lead to economic loss as well as raising public health concerns due to the production of mycotoxins **(Cheong et al., 2014)**. *Penicillium commune* is a microscopic filamentous fungus that very often causes molding of cheeses **(Lund, Filtenborg and Frisvad, 1995; Kure and Skaar, 2000; Kure et al., 2001; Lund, Nielsen and Skouboe, 2003; Garnier et al., 2017)**.

The aim of the present research was to determine the inhibitory effect of vapor phase of five essential oils on the growth of seven different strains of *Penicillium commune* isolated from moldy milk products. Another objective was to determine the minimum inhibitory doses of essential oils, which at a concentration 625 µL.L^{-1} of air completely inhibited the growth of all the strains.

Scientific hypothesis

Growth of *Penicillium commune* is affected by essential oils of family *Myrthaceae*.

MATERIAL AND METHODOLOGY

Plant essential oils

The essential oils used in this study were extracts of plants from family *Myrtaceae*. Specifically, we used clove essential oil (from *Syzygium aromaticum* L.; leaves), eucalyptus essential oil (from *Eucaliptus globulus* leaves), Tea Tree essential oil (from *Melaleuca alternifolia* Cheel; leaves) cajeput essential oil (from *Melaleuca leucadendra* L.; leaves and twigs), niaouli essential oil (from *Melaleuca quinquenervia* (Cav.) S. T. Blake; leaves). Essential oils were commercially produced.

Chemical composition of essential oils

Semi-quantitative composition of the essential oil samples was determined by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890B oven coupled with Agilent 5977A mass detector (Agilent Technologies Inc., Palo Alto, CA, USA) and CombiPal autosampler 120 (CTC Analytics AG, Zwingen, Switzerland). Prior to the analysis, essential oil samples were diluted in hexan (HPLC ≥97%, Sigma Aldrich GmbH, Germany) to a concentration of 10 $\mu L.mL^{-1}$. One microliter of diluted sample was injected in inlet operated in split mode (1:10; 250 °C). Separation was achieved using a ZB-WAXplusTM capillary column (10 m × 0.1 mm × 0.10 µm) (Phenomenex Inc., Torrance, CA, USA) and the following oven temperature programme: 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. Helium was used as carrier gas at the constant flow (1.2 $mL.min^{-1}$ – constant flow). The mass detector parameters were as follows: ionization energy of filament: 70 eV, transfer line temperature: 250 °C, MS source temperature: 230 °C, quadrupole temperature: 150 °C. The mass spectrometer was programmed under electron impact (EI) in a full scan mode at m/z 40 – 400. The identification of compounds was carried out by comparing of mass spectra (over 80% match) with a commercial database NIST® 2014 and retention times of reference standards (nerol, linalool, geraniol, citral, α-pinene, and β-pinene). The semi-quantitative content of determined compounds was calculated by dividing individual peak area (excluded by solvent peak area) by total area of all peaks. Peaks under 0.10% were not counted.

Fungal strains using in research

Seven strains from different moldy milk products were used. Specifically: *Penicillium commune* KMi 177 (from moldy cheese flavored with pepper); *P. commune* KMi 270 (smoked cheese – block); *P. commune* KMi 276 (smoked cheese – slices); *P. commune* KMi 277 (smoked cheese – slices); *P. commune* KMi 370 (sour cream); *P. commune* KMi 402 (sour cream); *P. commune* KMi 403 (parenica – pasta filata). These strains belong to the Collection of Fungi of Department of Microbiology; Faculty of Biotechnology and Food Sciences SUA in Nitra, Slovakia. Five-day old cultures cultivated on Czapek yeast extract agar (CYA) at 25 ±1 °C were used for each experiment (CYA) **(Pitt and Hocking, 2009)**.

Antifungal activity of essential oils

The antifungal activity of selected essential oils was evaluated by the micro-atmosphere method. The test was performed in sterile plastic Petri dishes (Ø 90 mm) containing 15 mL of CYA. The evaluation by filter paper was made by the adapted method from **Guynot et al. (2003)**. Essential oils were tested in concentration 625 $\mu L.L^{-1}$ of air. A round sterile filter paper (Ø 40 mm) was placed in the lid of Petri dish, and 50 µL of essential oil was pipetted by micropipette on the paper. Dishes were kept in inverted position. Filter paper discs impregnated with sterilized distilled water were used as a control. Each strain was inoculated in the center of Petri dishes with sterilized needle. Dishes were tightly sealed with parafilm and incubated for 14 days at 25 ±1 °C and for 35 days at 5 ±1 °C (four replicates were used for each treatment). The diameters (Ø mm) of the growing colonies (from the reverse side) were measured with a digital caliper on the 3rd, 7th, 11th, and 14th day – the strains cultivated at 25 ±1 °C and on 3rd, 7th, 11th, 14th, 21st, 28th, and 35th day – the strains cultivated at 5 ±1 °C.

Inhibition of mycelial growth

According to **Cakir et al. (2005)** and **Kordali et al. (2008)**, growth inhibition of treated samples (T) against control (C) was calculated by the percentage of growth inhibition using the following equation:

$$\% \text{ of inhibition} = (C - T)/C \times 100$$

where, C is the mean of eight replicates of the hyphal extension (mm) of controls and T is the mean of eight replicates of the hyphal extension (mm) of plates treated with either essential oil.

Minimum inhibitory doses (MIDs)

Essential oils that completely inhibit the growth of all strains at both temperatures were used to determine their minimum inhibitory doses (MIDs). EOs dissolved in dimethyl lsulfoxid (DMSO) were prepared at different concentrations (625; 500; 250; 125; 63; 31.25; and 15.63 $\mu L.L^{-1}$ of air). For each fungal strain, a conidial spore suspension of 10^6 spore's ml^{-1} was prepared. The EVE™ Automatic cell counter (NanoEnTek, Korea) was used to determine the number of spores. Petri dishes (Ø 90 mm, two-sector, three replicates) containing 15 mL of CYA were inoculated by 5 µl spores suspension. Cultivation was carried out at the 25 ±1 °C and measured after 7 and 14 days. The MID (expressed as microliters of EOs per volume unit of atmosphere above the organism growing on the agar surface) was defined as the lowest concentration of the oil which did not permit any visible growth after 7 or 14 days in comparison with control sets.

Probit analyses

The ability of the strains to grow in the presence of EO was coded to binomial scale (1 – growth observed, 0 – without growth). Such data were processed by probit analysis in Statgraphics Centurion XV (Statgraphics) software. Doses that inhibit the growth in 50% respectively 90% of cases (MID50 and MID90) were reversely predicted from regression equation.

Statistic analysis

The statistical data evaluation was performed using SAS 9.3 package. The glimmix was used to test the effect of strain, essential oil, day, and the interaction of the strain, essential oil, and day on the mold growth with repeating random-residual-option and unstructured covariate structure. Ranked data were analysed. Holm-Tukey post hoc test was applied to performe for multiple comparison. Results were considered as significant at p value ≤0.05.

RESULTS AND DISCUSSION

In this study, the antifungal properties of five essential oils (clove, eucalyptus, Tea Tree, cajeput, niaouli) from family *Myrtaceae* were evaluated. Essential oils are complex mixtures of low molecular weight compounds extracted from plants by steam distillation and various solvents. Terpenoids and phenylpropanoids are the major constituents which provide characteristic aroma and biological properties to essential oils **(Raut and Karuppayil, 2014)**. According to authors **(Ben Farhat, et al., 2016; Méndez-Tovar et al., 2016; Dušková et al., 2016)** the effect of the growing seasons, different growth stage of plants, and climatic conditions of each year in terms of the essential oil content and composition were proven. Based on the above, we also focused on the composition of the essential oils used. Essential oils are very complex natural mixtures which can contain about 20 – 60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20 – 70 %) compared to other components present in trace amounts **(Bakkali et al., 2008)**. The GC-MS analyses of the essential oils led to identification of 78 compounds, 31 from them are presented in amount of ≥1 percentage minimally in one essential oil. The identified compounds (31) are listed in Table 1. The major components according to the specific essential oil were: clove – Eugenol (73.3%); eucaliptus – Eucalyptol (78.7%); Tee tree – 4-terpinenol (39.6%), gamma – Terpinene (20.0%) and (+)-4-Carene (9.38%); niaouli Eugenol (50.2%), alfa-pinene (9.78%), Viridiflorol (8%).

Antifungal activity of essential oils

All strains of *P. commune* without essential oil in the atmosphere – controls (without essential oil at the atmosphere), were grown on the first measurement (3rd day of incubation) at 25 ±1 °C. The intensity of the growth of the strains at 25 ±1 °C is shown in Figure 1. Dairy products are stored at low temperatures, so an additional cultivation temperature of 5 ±1 °C was used. At 5 ±1 °C (controls), the growth of two strains (KMi 270 and KMi 276) was recorded on the 3rd day and of other five strains on the 7th day. The intensity of the growth of the strains at 5 ±1 °C is shown in Figure 2.

Plant oils obtained from plants of three genera of family *Myrthaceae* were used in the research: *Syzygium*, *Eucaliptus*, and *Melaleuca*. The growth of the strains of *P. commune* was affected by all the essential oils used (Table 2 and Table 3). Only clove (from *Syzygium aromaticum* L.) essential oil, as the only one, completely inhibited the growth of the strains of *P. commune* at 5 ±1 °C, respectively 25 ±1 °C, throughout the experiment. **Guynot et al. (2003)** demonstrated the potential of clove essential oil aganist species belonging to *Eurotium, Aspergillus* and *Penicillium* genera. According to **Císarová, Tančinová and Medo (2016)** clove essential oil (500 µL.L^{-1} of air) completely inhibited growth of isolates of *Aspergillus flavus* and *Aspergillus parasiticus*.

Eucaliptus (from *Eucaliptus globulus*) essential oil only partially inhibited the growth of strains of *P. commune* at 25 ±1 °C and 5 ±1 °C. Even the growth stimulation of the strain *P. commune* KMi 277 was observed (at 25 ±1 °C). The influence of the eucalitptus esseniatial oil is shown in Figure 3 and Figure 4. Unlike our results using another type of volatile essential oil, **Ramezani et al. (2002)** showed a strong fungicidal activity of the volatile oil of *Eucaliptus citriodora*.

Three essential oils were used from genus *Melaleuca:* tea tree, cajeput, and niaouli. Tea tree (from *Melaleuca alternifolia* Cheel) essential oil showed a strong inhibitory effect on the strains of *P. commune*. The growth of six strains at 25 ±1 °C, respectively five at 5 ±1 °C was completely inhibited throughout all cultivations. Only one strain (*P. commune* KMi 403) in the presence of tea tree oil grew at 25 ±1 °C, but its growth was small (84.8 – 99.5% of inhibition). The influence of tea tree essential oil on the growth of the strains *P. commune* at 25 ±1 ºC is shown in Figure 5. Two strains of *P. commune* (KMi 276 and KMi 403) were able to grow in the presence of tea tree essentail oil at 5 ±1 °C from the 28th day of the experiment, but growth inhibition was significant (Figure 6). A weaker inhibitory effect was found in cajeput (from *Melaleuca leucadendra*) essential oil (Figure 7 and Figure 8) and the weakest in niaouli (from *Melaleuca quinquenervia* (Cav.) S. T. Blake) essential oil (Figure 9 and Figure 10). **Stević et al. (2014)** also reported excellent antifungal activity of tea tree essential oil against the most of tested fungi (species of genera *Fusarium, Aspergillus, Alternaria, Penicillium* and others).

Minimum inhibitory doses (MIDs)

In this study clove essential oil (625 µL.L^{-1} of air) was able to inhibit the growth of all strains at all days of cultivation on the Czapek yeast extract agar at 5 ±1 °C and 25 ±1 °C also. Clove essential oil was used for the determination of MIDs. The results are shown in Table 4. The MIDs were 125 µL.L^{-1} of air for the strains *P. commune* KMi 177 and KMi 277 and 250 µL.L^{-1} of air for the strains *P. commune* KMi 270, KMi 276, KMi 370, KMi 402 and KMi 403 on the 7th day of cultivation. On the 14th day of incubation, the same values of MIDs were determined. In some strains, the number of growing colonies increased, but this did not affect the MIDs. **Císarová et al. (2016)** determined lower MIDs of clove essential oil in vapor phase against the tested aspergilly strain 62.5 µL.L^{-1}.

Using probit analysis, predicted MIDs90 and MIDs50 were calculated. The results are shown in Table 5. The highest MIDs90 were determined for strain KMi 403 – 301.37 µL.L^{-1} of air on 7th day and 292.56 µL.L^{-1} of air on 14th day of cultivation. The lowest MIDs90 were determined for the strains KMi 177 and KMi 277 – 104.93 µL.L^{-1} of air on the 7th day and on the 14th day of cultivation.

Table 1 Essential oils tested for the fungicidal effect and their compounds (%)* determined by gas chromatography coupled with mass spectrometry (GC-MS).

	Compaund	Essential oils				
		Clove	**Eucaliptus**	**Tea Tree**	**Cajeput**	**Niaouli**
1	alfa-pinene		2.71	3.63	1.81	9.78
2	beta-pinene		0.19		1.14	
3	p-Cymene		6.37	5.95		
4	beta.-M+E5:G25yrcene				1.54	
5	gamma.-Terpinene		2.36	20.0		
6	gamma-terpineol			6.74		
7	D-Limonene	0.08	8.03	0.92	5.17	7.11
8	Eucalyptol	0.35	78.7		60.76	50.2
9	gamma-Terpinene				1.21	0.56
10	Caryophyllene	9.23		0.54	1.52	1.56
11	o-Cymene				1.22	1.66
12	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	2.68				
13	alpha-Terpinyl acetate					1.44
14	Linalool				3.49	
15	Nerolidol					3.59
16	Caryophyllene oxide	2.19				0.55
17	Humulene				1.09	0.36
18	Eugenol	73.3				
19	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	10.1				
20	alfa-selinene				0.87	
21	beta-selinene				1.04	
22	Aromandendrene			1.02		
23	alpha-guaiol				1.00	
24	beta-gurjunene			1.06		
25	(+)-4-Carene			9.38	0.33	
26	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-			0.71		2.27
27	4-terpinenol			39.6		
28	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene			1.35		
29	Cerd-8-ene			0.23		6.88
30	alpha-Terpineol			3.24	12.9	
31	Viridiflorol			0.12		8.00

Note: *listed are the components that represented min. 1% in at least one essential oil.

Table 2 The effect of essential oil on the growth of different strains during cultivation at 25 ±1 °C.

Strain of *P. commune*	Essential oil	Day of cultivation			
		3rd	7th	11th	14th
KMi 177	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	5.13 ± 1.17^{aB}	20.75 ± 1.09^{bB}	34.63 ± 0.99^{aB}	43.00 ± 0.71^{dB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{aA}	0^{aA}	0^{aA}	4.63 ± 1.49^{bC}
	Niaouli	0^{aA}	2.75 ± 0.66^{bC}	6.88 ± 0.78^{cC}	9.75 ± 1.20^{dD}
	Control	16.00 ± 1.80^{aC}	30.63 ± 0.70^{bD}	41.63 ± 1.49^{cD}	45.88 ± 3.62^{cB}
KMi 270	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	1.16 ± 1.80^{aB}	21.25 ± 1.39^{bB}	29.63 ± 1.11^{cB}	35.00 ± 0.71^{dB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	2.13 ± 1.62^{aC}	5.25 ± 2.63^{bC}	12.00 ± 3.00^{cC}	23.50 ± 2.78^{dC}
	Niaouli	3.13 ± 0.93^{aD}	7.75 ± 0.97^{bD}	15.50 ± 1.22^{cC}	25.25 ± 1.79^{dC}
	Control	20.00 ± 1.50^{aE}	27.25 ± 0.66^{bE}	32.88 ± 1.62^{cB}	39.00 ± 1.58^{dB}
KMi 276	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	8.50 ± 0.71^{aB}	22.63 ± 0.86^{bB}	27.38 ± 0.99^{cB}	35.63 ± 1.49^{dB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{A}	0^{A}	0^{A}	0^{A}
	Niaouli	0^{aA}	1.88 ± 0.60^{bC}	4.63 ± 0.48^{cC}	9.88 ± 0.60^{dC}
	Control	16.38 ± 0.48^{aC}	25.00 ± 1.12^{bB}	31.25 ± 1.09^{cB}	38.38 ± 0.86^{dB}
KMi 277	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	7.38 ± 0.86^{aB}	31.38 ± 0.86^{bB}	36.75 ± 0.97^{cB}	41.25 ± 3.80^{dB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{aA}	0^{aA}	0.38 ± 0.70^{bC}	3.88 ± 1.54^{cC}
	Niaouli	0^{aA}	4.38 ± 0.99^{bC}	9.38 ± 3.94^{cD}	14.88 ± 5.01^{dD}
	Control	14.75 ± 1.09^{aC}	31.88 ± 2.52^{bB}	36.13 ± 3.10^{cB}	39.25 ± 1.09^{dB}
KMi 370	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	3.00 ± 0.00^{aB}	16.50 ± 0.50^{bB}	27.00 ± 1.00^{cB}	35.00 ± 0.50^{dB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{aA}	0^{aA}	2.50 ± 1.73^{bC}	4.88 ± 2.20^{cC}
	Niaouli	0^{aA}	3.50 ± 1.00^{bC}	9.25 ± 2.38^{cD}	13.75 ± 2.86^{dD}
	Control	10.63 ± 0.70^{aC}	25.75 ± 0.83^{bD}	35.13 ± 0.60^{cE}	40.25 ± 0.92^{dE}
KMi 402	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	1.00 ± 0.00^{aB}	14.63 ± 0.86^{bB}	27.50 ± 1.32^{cB}	34.38 ± 1.58^{dB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{aA}	0^{aA}	0.50 ± 0.5^{bC}	2.37 ± 0.99^{cC}
	Niaouli	0^{aA}	4.75 ± 0.83^{bC}	12.25 ± 1.79^{cD}	17.50 ± 1.50^{dD}
	Control	9.50 ± 0.50^{aC}	24.63 ± 0.48^{bD}	36.25 ± 1.20^{cE}	44.13 ± 3.10^{dE}
KMi 403	Clove	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	5.88 ± 1.69^{aB}	34.25 ± 1.71^{bB}	57.00 ± 1.22^{cBE}	66.13 ± 0.78^{cBE}
	Tea Tree	0^{aA}	0.25 ± 0.43^{bC}	4.88 ± 0.78^{cC}	11.12 ± 1.17^{dC}
	Cajeput	0.50 ± 0.87^{aC}	4.63 ± 1.31^{bD}	21.13 ± 2.37^{cD}	31.50 ± 1.50^{dB}
	Niaouli	2.75 ± 0.43^{aD}	21.75 ± 1.09^{bF}	40.25 ± 1.85^{cB}	59.88 ± 1.17^{dE}
	Control	19.75 ± 0.43^{aE}	46.25 ± 0.83^{bF}	67.13 ± 0.78^{bcE}	73.13 ± 0.78^{cBF}

Note: The numbers represent mean of colony size. a, b, c, d shows significant differences within row. A, B, C, D, E, F shows significant differences within column and within strain. Data presented as mean ± stdev in mm.

Table 3 The effect of essential oil on the growth of different strains during cultivation at 5 ±1 °C.

Strain of *P. commune*	Essential oil	Day of cultivation						
		3rd	7th	11th	14th	21st	28th	35th
KMi 177	Clove	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{a}	0^{aA}	2.00 ± 0.72^{bB}	3.88 ± 0.78^{cB}	8.25 ± 1.20^{dB}	13.25 ± 1.20^{eB}	17.75 ± 1.39^{fB}
	Tea Tree	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Niaouli	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	1.88 ± 0.60^{bC}	3.63 ± 0.99^{cC}
	Control	0^{a}	5.88 ± 1.05^{bB}	13.38 ± 0.70^{cC}	17.13 ± 1.45^{cC}	27.5 ± 1.22^{dD}	34.25 ± 1.79^{eD}	43.38 ± 2.96^{fD}
KMi 270	Clove	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{aA}	0^{aA}	1.88 ± 1.77^{bB}	2.25 ± 1.48^{bB}	6.88 ± 1.90^{cB}	9.50 ± 1.50^{dB}	15.63 ± 2.06^{eB}
	Tea Tree	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{aA}	0^{aA}	0^{aA}	0^{aA}	1.88 ± 0.60^{bC}	2.00 ± 0.71^{bC}	5.00 ± 1.00^{cC}
	Niaouli	0^{aA}	0^{aA}	1.13 ± 1.17^{bC}	2.13 ± 0.33^{cC}	2.50 ± 0.71^{cD}	4.13 ± 0.60^{dD}	6.88 ± 1.27^{eD}
	Control	1 ± 1^{aB}	2.38 ± 1.49^{bB}	7.38 ± 2.45^{cD}	9.88 ± 3.02^{cD}	24.50 ± 1.58^{dE}	34.38 ± 1.32^{eE}	43.00 ± 2.00^{fE}
KMi 276	Clove	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{aA}	0^{aA}	0^{aA}	1.88 ± 0.60^{bB}	5.75 ± 1.09^{cB}	12.25 ± 1.39^{dD}	18.00 ± 2.18^{eB}
	Tea Tree	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	1.50 ± 0.50^{bC}	2.88 ± 0.60^{cC}
	Cajeput	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	2.75 ± 0.66^{bD}	4.38 ± 0.70^{cD}
	Niaouli	0^{aA}	0^{aA}	0^{aA}	0^{aA}	2.13 ± 0.60^{bC}	3.75 ± 0.43^{cE}	6.63 ± 1.32^{dE}
	Control	1.88 ± 0.33^{aB}	6.38 ± 1.22^{bB}	10.75 ± 0.66^{bB}	18.75 ± 0.97^{cC}	24.63 ± 0.70^{D}	36.00 ± 0.71^{dF}	41.50 ± 1.80^{dF}
KMi 277	Clove	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{a}	0^{aA}	1.38 ± 0.86^{bB}	2.50 ± 0.87^{cB}	6.00 ± 2.43^{dB}	10.13 ± 3.18^{eB}	16.88 ± 1.62^{gB}
	Tea Tree	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	1.38 ± 0.48^{bC}	3.63 ± 0.48^{cC}
	Niaouli	0^{a}	0^{aA}	0^{aA}	2.50 ± 0.71^{bB}	4.00 ± 0.87^{cC}	5.50 ± 0.71^{dD}	8.25 ± 1.20^{eD}
	Control	0^{a}	7.00 ± 1.22^{bB}	$11.25^{b} \pm 0.83^{C}$	18.75 ± 1.30^{cC}	32.25 ± 0.97^{dD}	35.00 ± 1.66^{eE}	39.50 ± 1.80^{eF}
KMi 370	Clove	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{a}	0^{aA}	2.00 ± 0.00^{bB}	3.75 ± 0.66^{cB}	8.00 ± 0.71^{dB}	13.00 ± 0.71^{eB}	15.13 ± 0.93^{fB}
	Tea Tree	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Niaouli	0^{a}	0^{aA}	0^{aA}	0^{aA}	1.00 ± 0.00^{bC}	2.13 ± 0.33^{cC}	4.13 ± 0.78^{dC}
	Control	0^{a}	4.63 ± 0.48^{bB}	9.38 ± 0.48^{cC}	12.63 ± 0.48^{cC}	18.00 ± 1.00^{dD}	22.63 ± 0.48^{eD}	28.50 ± 0.71^{fD}
KMi 402	Clove	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{a}	0^{aA}	1.00 ± 0.00^{bB}	2.75 ± 0.83^{cB}	7.75 ± 1.09^{dB}	12.63 ± 1.22^{eB}	14.38 ± 0.70^{fB}
	Tea Tree	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Cajeput	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Niaouli	0^{a}	0^{aA}	0^{aA}	1.13 ± 0.33^{bC}	3.00 ± 0.71^{cC}	4.00 ± 0.71^{eC}	5.38 ± 0.70^{fC}
	Control	0^{a}	3.25 ± 0.43^{bB}	9.25 ± 0.83^{cC}	12.50 ± 0.50^{cD}	20.50 ± 0.50^{dD}	23.75 ± 0.66^{dD}	30.50 ± 0.50^{eD}
KMi 403	Clove	0	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
	Eucaliptus	0^{a}	0^{aA}	3.13 ± 0.78^{bB}	4.88 ± 0.60^{cB}	11.13 ± 0.78^{dB}	15.38 ± 0.70^{eB}	20.13 ± 1.17^{fB}
	Tea Tree	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	1.00 ± 0.00^{bC}	2.75 ± 0.43^{cC}
	Cajeput	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	1.00 ± 0.00^{bC}	3.25 ± 0.43^{cD}
	Niaouli	0^{a}	0^{aA}	0^{aA}	2.50 ± 1.12^{bC}	5.63 ± 0.86^{cC}	8.75 ± 0.66^{dD}	13.38 ± 1.66^{eE}
	Control	0^{a}	4.00 ± 0.71^{bB}	9.75 ± 0.66^{cC}	15.00 ± 0.50^{dD}	28.00 ± 0.71^{eD}	33.00 ± 0.87^{fE}	39.00 ± 0.71^{gF}

Note: The numbers represent mean of colony size. a, b, c, d, e, f, g shows significant differences within row. A, B, C, D, E, F shows significant differences within column and within strain. Data presented as mean ± stdev in mm.

Table 4 The inhibitory effect (in %) of clove essential oils on the growth of colonies (n = 6) of *Penicillium commune* on CYA at 25 °C after 7 and, respectively, 14 days of cultivation.

µL of EO.L^{-1} of air	Strain of *Penicillium commune*													
	KMi 177		KMi 270		KMi 276		KMi 277		KMi 370		KMi 402		KMi 403	
	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th
625	100	100	100	100	100	100	100	100	100	100	100	100	100	100
500	100	100	100	100	100	100	100	100	100	100	100	100	100	100
250	100	100	100	100	100	100	100	100	100	100	100	100	100	100
125	100	100	83.33	83.33	50	33.33	100	100	83.33	50	50	50	66.66	66.66
62.50	0	0	0	0	50	50	0	0	33.33	0	33.33	0	0	50
21.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15.625	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Note: EO – essential oil, CYA – Czapek yeast extract agar.

Table 5 Minimal inhibition doses estimated by probit analyses.

Strain	7th day		14th day	
	MID_{50}	MID90	MID50	MID90
KMi 177	94.16	104.93	94.16	104.93
KMi 270	113.34	128.79	113.34	128.79
KMi 276	106.15	173.27	119.56	197.12
KMi 277	94.16	104.93	94.16	104.93
KMi 370	81.45	124.43	125.00	143.92
KMi 402	113.33	176.51	125.00	143.92
KMi 403	200.13	301.37	187.07	292.56
All strains	116.77	182.97	126.27	189.49

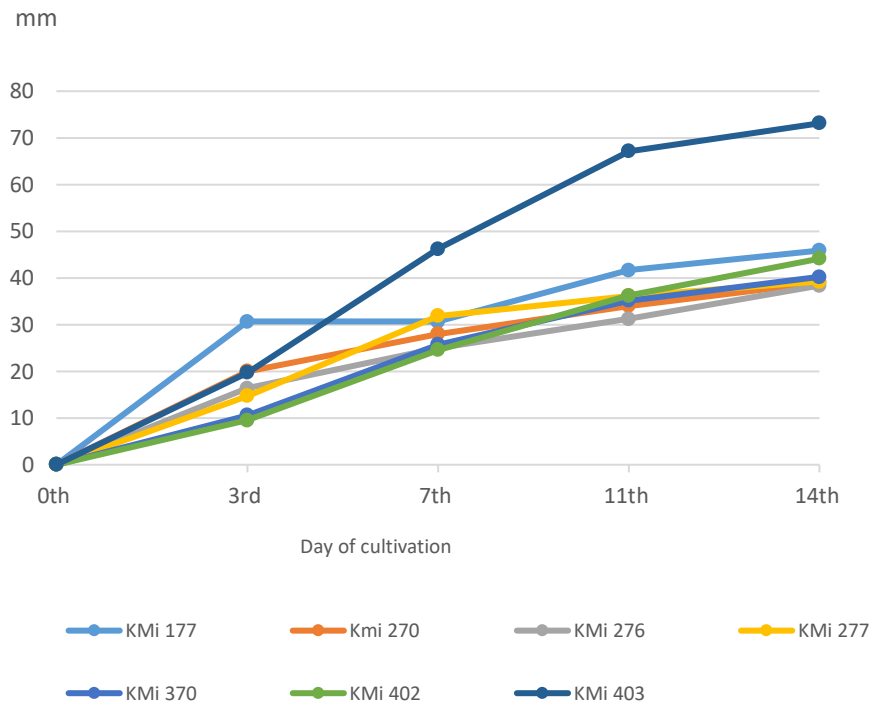

Figure 1 The growth of the strains of *Penicillium commune* on the Czapek yeast extract agar at 25 ±1 °C.

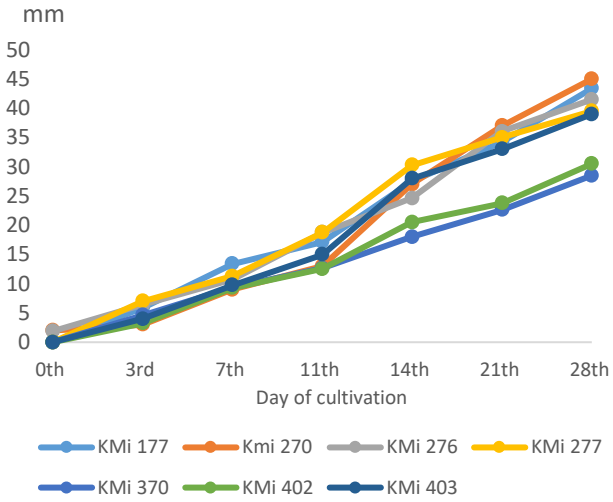

Figure 2 The growth of the strains of *Penicillium commune* on the Czapek yeast extract agar at 5 ±1 °C.

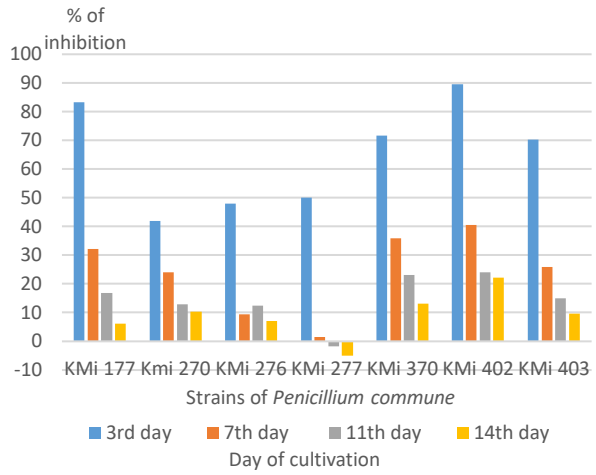

Figure 3 Influence of eucalyptus essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 25 ±1 °C.

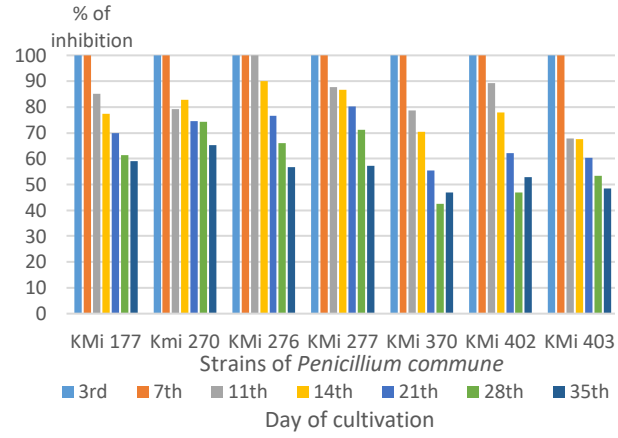

Figure 4 Influence of eucalyptus essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 5 ±1 °C.

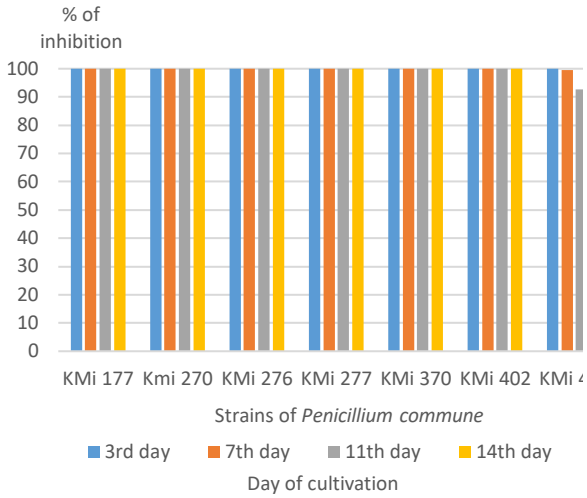

Figure 5 Influence of tee tree essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 25 ±1 °C.

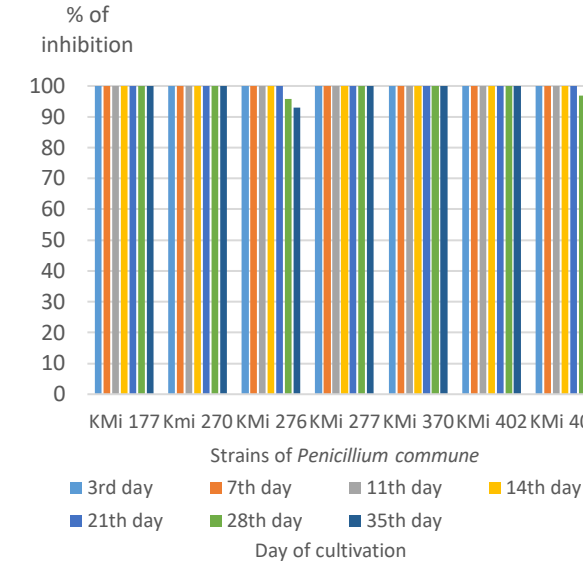

Figure 6 Influence of tee tree essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 5 ±1 °C.

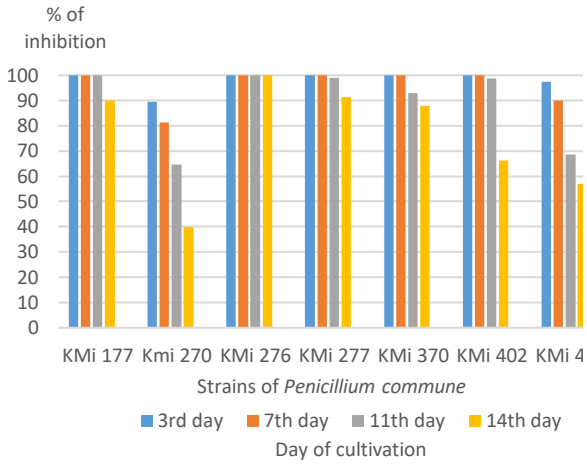

Figure 7 Influence of cajeput essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 25 ±1 °C.

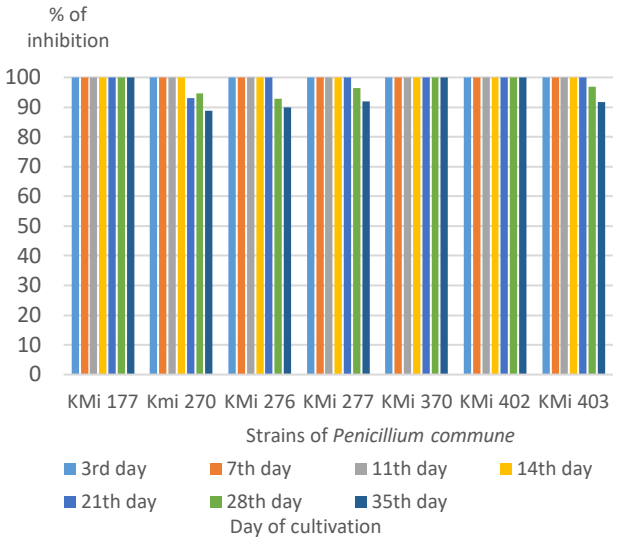

Figure 8 Influence of cajeput essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 5 ±1 °C.

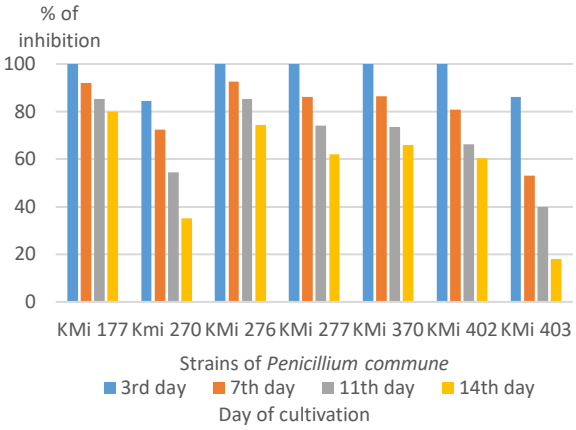

Figure 9 Influence of niaouli essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 25 ±1 °C.

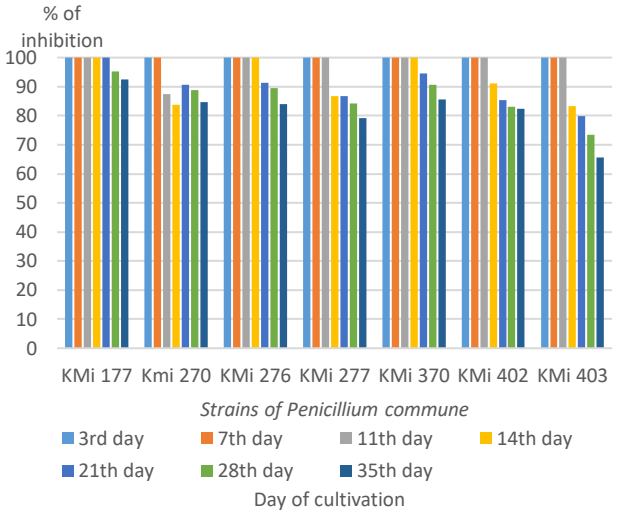

Figure 10 Influence of niaouli essential oil (625 µL.L^{-1} of air) on the growth of *Penicillium commune* on the Czapek yeast extract agar at 5 ±1 °C.

In our research, we have confirmed the ability of five essential oils from plants family *Myrtaceae* to inhibit (partially or completely) the growth of the strains of *P. commune*. Testing should be supplemented by testing the effects of essential oils on the sensory properties of foods. Our tested strains were obtained from the moldy dairy products whose sensory properties could be affected by essential oils.

CONCLUSION

In this study, we evaluated the antifungal properties of clove essential oil (from *Syzygium aromaticum* L.; leaves), eucalyptus essential oil (from *Eucaliptus globulus* leaves), tea tree essential oil (from *Melaleuca alternifolia* Cheel; leaves) cajeput essential oil (from *Melaleuca leucadendra* L.; leaves and twigs), niaouli essential oil (from *Melaleuca quinquenervia* (Cav.) S. T. Blake; leaves). The growth of the strains of *P. commune* was affected by all the essential oils used. But, only clove (from *Syzygium aromaticum* L.) essential oil completely inhibited the growth of the strains of *P. commune* at 5 ±1 °C and 25 ±1 °C respectively, throughout the experiment. The order of tested essential oils according to the inhibition effect on the growth of the strains of *P. commune* was (from the strongest to the weakest effect): clove > tee tree > cajeput > niaouli > eucalyptus. Clove EO that completely inhibit the growth of all strains was used to determine minimum inhibitory doses (MIDs). The MIDs were 125 µL.L^{-1} of air for two strains of *P. commune* and 250 µL.L^{-1} of air for five strains of *P. commune* on the 7th and 14th day of cultivation, also. According to probit analyses, the highest MIDs90 were determined for the strain KMi 403 – 301.37 µL.L^{-1} of air on the 7th day and 292.56 µL.L^{-1} of air on the 14th day of cultivation. The lowest MIDs90 were determined for the strains KMi 177 and KMi277 104.93 µL.L^{-1} of air on the 7th day and on the 14th day of cultivation, also.

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