

# Antifungal activity of essential oils against selected terverticillate penicillia

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## Abstract

The aim of this study was to screen 15 essential oils of selected plant species, viz. *Lavandula angustifolia*, *Carum carvi*, *Pinus mungo* var. *pulmilio*, *Mentha piperita*, *Chamomilla recutita* L., *Pinus sylvestris*, *Satureia hortensis* L., *Origanum vulgare* L., *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abietis albia etheroleum*, *Chamomilla recutita* L. Rausch, *Thymus vulgaris* L., *Origanum vulgare* L. for antifungal activity against five *Penicillium* species: *Penicillium brevicompactum*, *Penicillium citrinum*, *Penicillium crustosum*, *Penicillium expansum* and *Penicillium griseofulvum*. The method used for screening included the disc diffusion method. The study points out the wide spectrum of antifungal activity of essential oils against *Penicillium* fungi. There were five essential oils of the 15 mentioned above which showed a hopeful antifungal activity: *Pimpinella anisum*, *Chamomilla recutita* L., *Thymus vulgaris*, *Origanum vulgare* L. The most hopeful antifungal activity and killing effect against all tested penicillia was found to be *Origanum vulgare* L. and *Pimpinella anisum*. The lowest level of antifungal activity was demonstrated by the oils *Pinus mungo* var. *pulmilio*, *Salvia officinalis* L., *Abietis albia etheroleum*, *Chamomilla recutita* L. Rausch, *Rosmarinus officinalis*.

## Key words

Antifungal activity, essential oils, disc diffusion method, *Penicillium* species

## INTRODUCTION

The terverticillate penicillia are among the most frequently encountered filamentous fungi and they influence the everyday life of many people. These penicillia have given us penicillin, mycophenolic acid, compactin, fungal steroid transformations, white and blue mould cheeses, fermented sausages and extra cellular enzymes, but they have also been cause of severe mycotoxicoses, allergy and indoor air problems [1]. The species in *Penicillium* subgenus *Penicillium* have terverticillate penicilli and are related to the ascomycete genus *Eupenicillium* series *Crustacea*. Many of its species are very common and associated with stored foods of human beings and animals, but also with animal dung and building materials, indoor air, and several types of habitat [2].

Most of the species produce mycotoxins which can often have some pharmacologically interesting characteristics. The important antibiotic penicillin, which has made *Penicillium* famous, occurs only in several species of *Penicillium* [2].

The essential oils are aromatic oily liquids obtained from some plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction, but the method of steam distillation is most commonly used for the commercial production of essential oils. There are about 3,000 essential oils and about 300 of them are commercially important – destined chiefly for the flavours and fragrances market in medicine to be beneficial in the treatment of fungal problems in humans [3].

Many studies have documented the antifungal properties of plant essential oils. Most essential oil extracts completely inhibit the growth of all tested mould at the concentration of 1% w/v on nutrient medium [4]. The essential oils of spices and herbs (thyme, origanum, mint, cinnamon, salvia and clove) were found to possess the strongest antimicrobial properties among the many tested microscopic fungi [5]. The natural plant extracts can provide an alternative way to protect foods or feeds from fungal contamination [6].

However, there is only sparse knowledge about the antifungal activity of essential oils against terverticillate penicillia. Because of this, the aim of this study was to examine the inhibitory effect of Slovak essential oils against fungi of the genus *Penicillium*.

## MATERIALS AND METHOD

**Plant essential oils.** The samples of plant essential oils were obtained from Calendula a.s., Nova Lubovna, Slovakia (*Lavandula angustifolia*, *Carum carvi*, *Pinus mungo* var. *pulmilio*, *Mentha piperita*, *Chamomilla recutita* L., *Pinus sylvestris*, *Satureia hortensis* L., *Origanum vulgare* L., *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abietis albia etheroleum*, *Chamomilla recutita* L. Rausch, *Thymus vulgaris* L., *Origanum vulgare* L.). All tested samples were stored at 4 °C in a dark glass flask until analyzed. An aliquot of dimethylsulfoxide (DMSO, Sigma–Aldrich) was added to the essential oils in order to obtain a 0.09375–0.75 µl/ml of concentration range.

**Fungal strains and media.** The strains *Penicillium brevicompactum*, *Penicillium citrinum*, *Penicillium crustosum*,

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*Penicillium expansum* and *Penicillium griseofulvum* were included in this study. These strains were isolated from different honey samples kept in the fungal culture collection bank of the Department of Microbiology at the University of Agriculture in Nitra, Slovakia. Fungal isolates were maintained on Czapek yeast extract agar (CYA, HiMedia, Bombay, India) and the cultures were stored at refrigerator temperature (4°C). Genus *Penicillium* identified to species level based on morphological characteristics according to the manuals of Pitt and Hocking [7], Samson et al. [8].

**Disc diffusion method.** The disc diffusion test was performed using Sabouraud dextrose agar (SDA, Hi Media, Bombay, India). The inoculum was prepared using the fungi from seven days culture on CYA and the suspension was made in a sterile saline solution. The turbidity of the suspension was adjusted with a spectrophotometer – densilameter II (Erba-Lachema, Brno, Czech Republic) at 530 nm to obtain a final concentration to match that of the 0.5 McFarland standard. Briefly, 100 µL of spore suspension (0.5 McF) was spread thoroughly all over the surface onto Sabouraud dextrose agar plates. The plates were dried in an air-dry stiller at 60°C until evaporation of residual water. Blank disk 6 mm in diameter (Oxoid, Cambridge, UK) impregnated with 20 µL of pure essential oil suspensions, resulting in final concentrations of 0.75, 0.375, 0.1875 and 0.09375 µl/ml/disk. The quadruplicate plates were cultivated for each isolate. The plates were incubated at 25 ± 1°C in a thermostat. The zones of inhibition of the growth of the fungi were measured as semi-diameter (in millimeters) after 24 h of incubation. Pure DMSO was used as a control for each tested fungi.

**Statistical analysis.** The basic variation – statistical values including means and standard deviation from the obtained data were calculated using statistical programme SAS (one-factorial variance analysis ANOVA) at  $p < 0.05$ ;  $p < 0.01$ ;  $p < 0.001$  level of significance.

## RESULTS AND DISCUSSION

The antifungal activity of various essential oils on the growth of *Penicillium brevicompactum* on SDA is presented in Table 1. The essential oils of *Chamomilla recutita* L. and *Pimpinella anisum* showed the highest antifungal activity of all observed concentrations after incubation of 24 h as compared to control. These oils had the great inhibitory effect, but the zone around the entire surface of the dish could not be measured. It was found that *Origanum vulgare* L. and *Thymus vulgaris* L. showed a strong and wide spectrum of activity against *Penicillium brevicompactum* with a zone of inhibition ranging from 3.00 ± 1.41 – 10.75 ± 1.75 mm. However, the essential oils of *Pinus mungo* var. *pulmilio* showed the least antifungal activity from 0.25 ± 0.50 – 2.75 ± 0.96 mm against all 15 tested oils.

Statistical differences were found ( $p < 0.05$ ) between *Pimpinella anisum* and other essential oils, and *Thymus vulgaris* and others essential oils at *Penicillium brevicompactum*

The essential oils of *Syzygium aromaticum*, *Citrus limon* and *Mentha piperita*, according to Verma et al. [9], exhibited the highest antifungal activity on the growth of *Aspergillus niger* and *Geotrichum candidum*, which are commonly

**Table 1.** Inhibition zones in mm of essential oils at different concentration (mean ± SD) against *Penicillium brevicompactum*

Essential oil	Concentration of essential oil in µl/ml			
	0.75	0.375	0.1875	0.09375
1. <i>Lavandula angustifolia</i>	2.50 ± 1.29	1.25 ± 0.50	0.50 ± 0.58	0.50 ± 0.58
2. <i>Carum carvi</i>	3.00 ± 0.82	2.00 ± 0.00	0.75 ± 0.50	1.00 ± 0.82
3. <i>Pinus mungo</i> var. <i>pulmilio</i>	2.75 ± 0.96	0.75 ± 0.96	0.25 ± 0.50	0.25 ± 0.50
4. <i>Mentha piperita</i>	4.00 ± 0.82	3.00 ± 0.82	2.25 ± 0.96	1.50 ± 0.58
5. <i>Chamomilla recutita</i> L.	SE	SE	SE	SE
6. <i>Pinus sylvestris</i>	3.75 ± 2.50	1.50 ± 1.00	1.25 ± 0.50	1.25 ± 0.50
7. <i>Satureia hortensis</i> L.	5.50 ± 1.29	3.75 ± 0.96	2.25 ± 0.50	2.00 ± 0.82
8. <i>Origanum vulgare</i> L.	10.75 ± 1.50	7.25 ± 2.22	4.50 ± 1.29	3.00 ± 1.41
9. <i>Pimpinella anisum</i>	SE	SE	SE	SE
10. <i>Rosmarinus officinalis</i> L.	2.25 ± 1.26	1.50 ± 0.58	0.75 ± 0.50	0.75 ± 0.96
11. <i>Salvia officinalis</i> L.	2.75 ± 1.26	2.25 ± 1.26	1.25 ± 1.26	0.75 ± 0.50
12. <i>Abietis albi aetheroleum</i>	3.00 ± 1.41	2.75 ± 1.50	2.00 ± 0.82	1.75 ± 0.96
13. <i>Chamomilla recutita</i> L. <i>Rausch</i>	0.25 ± 0.50	1.50 ± 1.29	1.50 ± 1.00	3.00 ± 2.16
14. <i>Thymus vulgaris</i> L.	9.00 ± 4.69	8.75 ± 1.50	7.00 ± 2.94	4.75 ± 2.22
15. <i>Origanum vulgare</i> L.	4.50 ± 5.26	4.00 ± 4.90	7.25 ± 3.86	5.25 ± 4.42
DMSO (negative control)	NE	NE	NE	NE

SE – strong inhibition effect; NE – non inhibitory effect

found in buildings and their indoor environment. In the presented case, *Mentha piperita* showed moderate activity on the growth of *Penicillium brevicompactum*. Many scientists have isolated the main components of essential oils exhibiting antimicrobial activity, and it appears that there is a relationship between the chemical structures of the most abundant compounds in the essential oils and the antifungal effect [10]. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity, with the compounds with phenolic groups usually being the most effective [11]. Chamomile oil at 3,000 ppm exhibited the highest inhibition against *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium moniliforme* [12]. Its major components, chamazulene, α-bisabolol, flavonoids and umbelliferone, displayed antifungal properties against *Trichophyton mentagrophytes*, *T. rubrum* and *Candida albicans* [13, 14]. *Pimpinella anisum* L. fruits, known as aniseed, contain around 1.5–5.0% essential oil, mainly composed of volatile phenylpropanoids, such as *trans*-anethole with around 90% [15]. In addition, the essential oil of the anise fruit also contains a small proportion of estragol, anisaldehyde, γ-himachalene and *cis*-anethole [16]. The fruits, as well as the essential oils, are characterized by antispasmodic, antioxidant, antimicrobial, insecticidal and antifungal effects [17]. Because anise favours warm climatic conditions throughout the growing season, it is cultivated primarily in subtropical regions [18, 19]. Anise (*Pimpinella anisum* L.), which belongs to the family *Apiaceae*, is an important spice and medicinal plant used for pharmaceuticals and the perfumery and food industries.

The study on the plants from Iran indicated that menthanol (36.24%) and menthone (32.42%) were the major compounds of the *Mentha piperita* essential oil [20] with antimicrobial activity. Other studies revealed the higher antimicrobial property of *Mentha piperita* essential oil with menthol concentration as low as 3.6% [21].

The screening results of the 15 essential oils for their activity against the growth of *Penicillium citrinum* are shown in Table 2. The essential oil *Pimpinella anisum* was very active against *Penicillium citrinum*; the inhibition zones were not measurable. Statistical differences were found ( $p < 0.05$ ) between *Pimpinella anisum* and other essential oils, and *Thymus vulgaris* and others essential oils.

**Table 2.** Inhibition zones in mm of essential oils at different concentration (mean  $\pm$  SD) against *Penicillium citrinum*

Essential oil	Concentration of essential oil in $\mu\text{l/ml}$			
	0.75	0.375	0.1875	0.09375
1. <i>Lavandula angustifolia</i>	1.50 $\pm$ 0.58	0.75 $\pm$ 0.96	2.00 $\pm$ 1.83	0.25 $\pm$ 0.50
2. <i>Carum carvi</i>	2.25 $\pm$ 0.50	1.75 $\pm$ 0.96	0.25 $\pm$ 0.50	0.50 $\pm$ 0.58
3. <i>Pinus mungo</i> var. <i>pulmilio</i>	0.50 $\pm$ 0.58	0.75 $\pm$ 0.50	NE	NE
4. <i>Mentha piperita</i>	2.50 $\pm$ 0.58	1.50 $\pm$ 0.58	1.75 $\pm$ 0.96	1.75 $\pm$ 0.96
5. <i>Chamomilla recutita</i> L.	9.50 $\pm$ 6.40	14.00 $\pm$ 1.41	6.00 $\pm$ 1.41	3.50 $\pm$ 0.58
6. <i>Pinus sylvestris</i>	3.25 $\pm$ 1.26	1.00 $\pm$ 0.82	1.00 $\pm$ 0.82	1.00 $\pm$ 0.00
7. <i>Satureia hortensis</i> L.	7.00 $\pm$ 0.82	4.00 $\pm$ 1.15	2.00 $\pm$ 0.82	1.00 $\pm$ 0.00
8. <i>Origanum vulgare</i> L.	12.0 $\pm$ 1.83	8.25 $\pm$ 3.30	4.75 $\pm$ 1.26	2.75 $\pm$ 0.96
9. <i>Pimpinella anisum</i>	SE	SE	SE	SE
10. <i>Rosmarinus officinalis</i> L.	2.75 $\pm$ 1.26	1.5 $\pm$ 0.58	1.00 $\pm$ 0.00	0.75 $\pm$ 0.50
11. <i>Salvia officinalis</i> L.	1.25 $\pm$ 0.50	0.5 $\pm$ 0.58	NE	0.25 $\pm$ 0.50
12. <i>Abietis albi aetheroleum</i>	3.50 $\pm$ 1.91	1.25 $\pm$ 1.26	NE	2.50 $\pm$ 1.73
13. <i>Chamomilla recutita</i> L. <i>Rausch</i>	0.75 $\pm$ 0.50	0.25 $\pm$ 0.50	0.50 $\pm$ 0.58	1.50 $\pm$ 1.29
14. <i>Thymus vulgaris</i> L.	SE	14.5 $\pm$ 1.00	9.50 $\pm$ 4.04	4.75 $\pm$ 0.96
15. <i>Origanum vulgare</i> L.	12.5 $\pm$ 1.73	7.75 $\pm$ 5.56	4.25 $\pm$ 1.50	5.00 $\pm$ 2.94
DMSO (negative control)	NE	NE	NE	NE

SE – strong inhibition effect; NE – non inhibitory effect

Similar results were obtained by Kosalec et al. [22] who tested *in vitro* on clinical isolates of seven species of yeasts and four species of dermatophytes the antifungal activities of fluid extract and essential oil obtained from anise fruits *Pimpinella anisum* L. (*Apiaceae*). Anise essential oil showed stronger antifungal activities against yeasts and dermatophytes. *Chamomilla recutita* L. and two *Origanum vulgare* L. had a strong and wide spectrum of activity with the zone of inhibition ranging from 2.75  $\pm$  0.96 – 14.00  $\pm$  1.41 mm. On the other hand, *Pinus mungo* var. *pulmilio*, *Salvia officinalis* L. and *Abietis alba etheroleum* had low activity, and indicated no zone of inhibition, compared to the control at concentration 0.1875  $\mu\text{l/ml}$ .

The antifungal activity of various essential oils on the growth of *Penicillium crustosum* on SDA is presented in Table 3. The best antifungal activity in all concentrations against *Penicillium crustosum* was shown by *Pimpinella anisum* and at 0.75  $\mu\text{l/ml}$  *Chamomilla recutita* L. and *Thymus vulgaris* L. Two *Origanum vulgare* L. showed an excellent wide spectrum of antifungal activities at the highest concentration, with a zone of inhibition ranging from 3.00  $\pm$  0.50 – 12.50  $\pm$  1.73 mm against tested fungus.

Statistical differences were found ( $p < 0.05$ ) between *Pimpinella anisum* and other essential oils, *Thymus vulgaris* and other essential oils, and *Chamomilla recutita* L. and other essential oils against *Penicillium crustosum*.

When oregano essential oil was used against *Aspergillus niger* and *A. flavus* by Viuda-Martos et al. [6] the 2  $\mu\text{L}$

**Table 3.** Inhibition zones in mm of essential oils at different concentration (mean  $\pm$  SD) against *Penicillium crustosum*

Essential oil	Concentration of essential oil in $\mu\text{l/ml}$			
	0.75	0.375	0.1875	0.09375
1. <i>Lavandula angustifolia</i>	3.50 $\pm$ 1.29	2.25 $\pm$ 0.50	1.25 $\pm$ 0.50	0.75 $\pm$ 0.96
2. <i>Carum carvi</i>	3.50 $\pm$ 0.58	2.75 $\pm$ 0.96	1.25 $\pm$ 0.50	1.50 $\pm$ 0.58
3. <i>Pinus mungo</i> var. <i>pulmilio</i>	1.75 $\pm$ 0.96	1.25 $\pm$ 0.50	1.25 $\pm$ 0.50	1.00 $\pm$ 0.00
4. <i>Mentha piperita</i>	5.00 $\pm$ 2.45	3.50 $\pm$ 1.29	2.50 $\pm$ 0.58	2.00 $\pm$ 0.82
5. <i>Chamomilla recutita</i> L.	SE	11.75 $\pm$ 3.95	9.25 $\pm$ 6.65	8.75 $\pm$ 7.23
6. <i>Pinus sylvestris</i>	4.00 $\pm$ 1.63	1.50 $\pm$ 1.00	0.75 $\pm$ 0.50	0.50 $\pm$ 0.58
7. <i>Satureia hortensis</i> L.	5.25 $\pm$ 1.26	3.00 $\pm$ 0.82	2.00 $\pm$ 0.82	1.75 $\pm$ 0.50
8. <i>Origanum vulgare</i> L.	11.50 $\pm$ 1.29	5.50 $\pm$ 0.58	4.00 $\pm$ 0.82	3.25 $\pm$ 0.50
9. <i>Pimpinella anisum</i>	SE	SE	SE	SE
10. <i>Rosmarinus officinalis</i> L.	2.25 $\pm$ 0.96	1.25 $\pm$ 0.50	0.75 $\pm$ 0.50	1.25 $\pm$ 0.50
11. <i>Salvia officinalis</i> L.	1.00 $\pm$ 0.00	1.00 $\pm$ 0.82	0.50 $\pm$ 0.58	0.25 $\pm$ 0.50
12. <i>Abietis albi aetheroleum</i>	2.00 $\pm$ 0.82	1.75 $\pm$ 0.50	1.50 $\pm$ 1.00	0.25 $\pm$ 0.50
13. <i>Chamomilla recutita</i> L. <i>Rausch</i>	0.75 $\pm$ 0.50	2.00 $\pm$ 0.82	1.25 $\pm$ 0.50	1.50 $\pm$ 0.58
14. <i>Thymus vulgaris</i> L.	SE	10.00 $\pm$ 3.16	6.50 $\pm$ 2.38	5.00 $\pm$ 0.82
15. <i>Origanum vulgare</i> L.	12.5 $\pm$ 1.73	5.75 $\pm$ 0.96	4.50 $\pm$ 0.58	3.00 $\pm$ 0.82
DMSO (negative control)	NE	NE	NE	NE

SE – strong inhibition effect; NE – non inhibitory effect

concentration reduced mycelium growth (by 57%), total inhibition being achieved with the 4, 6 and 8  $\mu\text{l}/18$  ml culture medium concentrations. Oregano essential oil was a more potential inhibitor of growth than the other essential oils studied – clove and thyme. In the present study, the lowest antifungal activity on the growth of *Penicillium crustosum* was shown by *Salvia officinalis* L., with a zone of inhibition from 0.25  $\pm$  0.50 – 1.00  $\pm$  0.00 mm. Low antifungal effect was also shown by the oil of *Salvia officinalis* against postharvest pathogenic fungi – *Rhizopus stolonifer*, *Penicillium digitatum*, *Aspergillus niger* and *Botrytis cinerea* [22]. The antifungal effect of the 15 tested essential oils against *Penicillium expansum* show Table 4. A high antagonistic effect was found in *Thymus vulgaris* L. and *Origanum vulgare* L. with a zone from 3.50  $\pm$  1.25 – 12.00  $\pm$  1.63 mm. Statistical differences ( $p < 0.05$ ) were found between *Pimpinella anisum* and other essential oils, *Thymus vulgaris* and others essential oils, and *Chamomilla recutita* L. and other essential oils against *Penicillium expansum*.

Akqül and Kivanç [23] also studied the inhibitory effects of oregano essential oil, thymol and carvacrol towards growth of nine foodborne fungi. Concentrations of 0.025% and 0.05% completely inhibited the growth of all fungi. In the presented study, the lowest activity was recorded for oil of *Pinus mungo* var. *pulmilio* and *Rosmarinus officinalis* L. No antagonistic effect was found with 0.75  $\mu\text{l/ml}$  against the test fungus. *Chamomilla recutita* L. and *Pimpinella anisum* were the most efficient against this fungus.

The antifungal activity of various essential oils on the growth of *Penicillium griseofulvum* on SDA is presented in Table 5. In this case, *Pimpinella anisum* showed strong activity against tested fungus. The same strong inhibition effect was recorded for *Thymus vulgaris* L. but only at 0.75  $\mu\text{l/ml}$  and 0.375  $\mu\text{l/ml}$  concentration. Both *Origanum vulgare* L. also had a high and wide spectrum of antifungal activity with

**Table 4.** Inhibition zones in mm of essential oils at different concentration (mean  $\pm$  SD) against *Penicillium expansum*

Essential oil	Concentration of essential oil in $\mu$ l/ml			
	0.75	0.375	0.1875	0.09375
1. <i>Lavandula angustifolia</i>	3.00 $\pm$ 2.31	2.50 $\pm$ 0.58	1.00 $\pm$ 0.82	0.50 $\pm$ 1.00
2. <i>Carum carvi</i>	4.25 $\pm$ 1.26	3.25 $\pm$ 1.26	1.75 $\pm$ 0.50	1.25 $\pm$ 0.50
3. <i>Pinus mungo</i> var. <i>pulmilio</i>	1.50 $\pm$ 0.58	0.75 $\pm$ 0.50	0.75 $\pm$ 0.96	0.25 $\pm$ 0.50
4. <i>Mentha piperita</i>	3.50 $\pm$ 1.00	1.75 $\pm$ 0.50	1.50 $\pm$ 0.58	2.00 $\pm$ 0.82
5. <i>Chamomilla recutita</i> L.	SE	SE	SE	SE
6. <i>Pinus sylvestris</i>	1.75 $\pm$ 0.50	0.75 $\pm$ 0.50	0.50 $\pm$ 0.58	1.00 $\pm$ 0.82
7. <i>Satureia hortensis</i> L.	3.50 $\pm$ 1.29	3.25 $\pm$ 0.50	1.75 $\pm$ 0.96	1.25 $\pm$ 0.50
8. <i>Origanum vulgare</i> L.	11.75 $\pm$ 3.77	7.00 $\pm$ 1.83	5.25 $\pm$ 1.50	3.50 $\pm$ 1.29
9. <i>Pimpinella anisum</i>	SE	SE	SE	SE
10. <i>Rosmarinus officinalis</i> L.	NE	0.75 $\pm$ 0.50	0.20 $\pm$ 0.50	0.25 $\pm$ 0.50
11. <i>Salvia officinalis</i> L.	1.75 $\pm$ 0.96	0.75 $\pm$ 0.50	1.25 $\pm$ 0.50	0.50 $\pm$ 0.58
12. <i>Abietis albi aetheroleum</i>	2.50 $\pm$ 2.38	1.75 $\pm$ 2.06	3.00 $\pm$ 1.41	0.75 $\pm$ 0.50
13. <i>Chamomilla recutita</i> L. Rausch	1.50 $\pm$ 0.58	2.00 $\pm$ 0.82	0.25 $\pm$ 0.50	0.75 $\pm$ 0.50
14. <i>Thymus vulgaris</i> L.	12.0 $\pm$ 1.63	7.50 $\pm$ 2.38	7.00 $\pm$ 2.16	3.50 $\pm$ 1.29
15. <i>Origanum vulgare</i> L.	6.50 $\pm$ 1.29	4.50 $\pm$ 2.38	NE	4.25 $\pm$ 1.71
DMSO (negative control)	NE	NE	NE	NE

SE – strong inhibition effect; NE – non inhibitory effect

**Table 5.** Inhibition zones in mm of essential oils at different concentration (mean  $\pm$  SD) against *Penicillium griseofulvum*

Essential oil	Concentration of essential oil in $\mu$ l/ml			
	0.75	0.375	0.1875	0.09375
1. <i>Lavandula angustifolia</i>	3.25 $\pm$ 0.50	1.75 $\pm$ 0.50	1.00 $\pm$ 0.82	0.75 $\pm$ 0.50
2. <i>Carum carvi</i>	2.75 $\pm$ 1.71	3.75 $\pm$ 0.96	1.25 $\pm$ 0.50	0.75 $\pm$ 0.50
3. <i>Pinus mungo</i> var. <i>pulmilio</i>	0.75 $\pm$ 0.50	0.50 $\pm$ 0.58	0.25 $\pm$ 0.50	NE
4. <i>Mentha piperita</i>	1.75 $\pm$ 0.96	1.50 $\pm$ 0.58	1.75 $\pm$ 0.96	2.00 $\pm$ 1.15
5. <i>Chamomilla recutita</i> L.	9.25 $\pm$ 6.65	8.75 $\pm$ 7.23	8.75 $\pm$ 7.23	8.25 $\pm$ 7.80
6. <i>Pinus sylvestris</i>	2.00 $\pm$ 0.82	1.50 $\pm$ 0.58	0.75 $\pm$ 0.96	0.50 $\pm$ 0.58
7. <i>Satureia hortensis</i> L.	3.25 $\pm$ 0.96	2.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0.50 $\pm$ 0.58
8. <i>Origanum vulgare</i> L.	10.00 $\pm$ 3.56	6.50 $\pm$ 1.29	5.75 $\pm$ 0.96	3.50 $\pm$ 0.58
9. <i>Pimpinella anisum</i>	SE	SE	SE	SE
10. <i>Rosmarinus officinalis</i> L.	2.25 $\pm$ 0.96	1.75 $\pm$ 0.96	1.50 $\pm$ 0.58	0.50 $\pm$ 0.58
11. <i>Salvia officinalis</i> L.	1.75 $\pm$ 0.96	1.50 $\pm$ 0.58	0.75 $\pm$ 0.50	0.50 $\pm$ 0.58
12. <i>Abietis albi aetheroleum</i>	2.25 $\pm$ 0.96	1.75 $\pm$ 0.50	1.25 $\pm$ 1.26	1.25 $\pm$ 0.96
13. <i>Chamomilla recutita</i> L. Rausch	2.00 $\pm$ 1.41	1.75 $\pm$ 0.96	1.75 $\pm$ 0.96	1.00 $\pm$ 0.00
14. <i>Thymus vulgaris</i> L.	SE	SE	5.25 $\pm$ 0.96	3.00 $\pm$ 1.41
15. <i>Origanum vulgare</i> L.	12.25 $\pm$ 3.77	7.50 $\pm$ 8.66	7.25 $\pm$ 2.50	6.25 $\pm$ 4.35
DMSO (negative control)	NE	NE	NE	NE

SE – strong inhibition effect; NE – non inhibitory effect

a zone of inhibition ranging from 3.50  $\pm$  0.58 – 12.25  $\pm$  3.77 mm. *Pinus mungo* var. *pulmilio* was the least active against *Penicillium griseofulvum*. No zone of inhibition was indicated at 0.1875  $\mu$ l/ml concentration. Statistical differences ( $p < 0.05$ ) were found between *Pimpinella anisum* and others essential oils, and *Thymus vulgaris* and other essential oils against *Penicillium griseofulvum*.

## CONCLUSION

The aim of the presented study was to examine the inhibitory effect of 15 essential oils against *Penicillium brevicompactum*, *Penicillium citrinum*, *Penicillium crustosum*, *Penicillium expansum* and *Penicillium griseofulvum*. The inhibitory effects of these oils on five fungi were examined using the disk diffusion method. All tested oils exhibited inhibition over activity relative to controls. However, a number of them exhibited weak inhibition against the penicillia tested: *Pinus mungo* var. *pulmilio*, *Salvia officinalis* L., *Abietis albi aetheroleum*, *Chamomilla recutita* L. Rausch and *Rosmarinus officinalis* L. The inhibitory effect against all tested fungi was shown by *Pimpinella anisum* and *Thymus vulgaris* essential oils. The majority of the essential oils inhibited the growth of all tested mould at the concentration of 0.75  $\mu$ l/ml.

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## REFERENCES

- Samson RA, Frisvad JC. *Penicillium* subg. *Penicillium*: new taxonomic schemes and mycotoxins and other extralites. Utrecht Centraalbureau voor Schimmelcultures, 2004.
- Frisvad JC, Samson RA. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycol. 2004; 49: 1–174.
- Van de Braak SAAJ, Leijten GCJJ. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. Rotterdam, CBI, Centre for the Promotion of Imports from Developing Countries, 1999.
- Singh T, Chittenden C. Efficacy of essential oil extract in inhibiting mould growth on panel products. Build Environ. 2010; 45: 2336–2342.
- Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem. 2003; 10: 813–829.
- Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Antifungal activities of thyme, clove and oregano essential oils. J Food Safety. 2007; 27: 91–101.
- Pitt JI, Hocking AD. Fungi and food spoilage. 2nd ed. London Blackie Academic & Professional, 1997.
- Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B. Food and Indoor Fungi. Utrecht CBS – KNAW Fungal Biodiversity Centre, 2010.
- Verma RK, Chaurasia L, Kumar M. Antifungal activity of essential oils against selected building fungi. Ind Nat Prod Resour. 2011; 2: 448–451.
- Farag RS, Daw ZY, Abo-Raya SH. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J Food Sci. 1989; 54: 74–76.
- Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. J Food Microb. 2005; 22: 273–292.
- McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). J Wiley Intersci. 2006; 20: 519–530.
- Kedzia B. Antimicroorganism activity of *Chamomillae* and its components. Herba Polonica. 1991; 37: 29–38.
- Ahmed FH, El Badri AA, Ibrahim MMK, El Shahed AS, El Khalafawy MM. Comparative studies of antifungal potentialities for some natural plant oils against different fungi isolated from poultry. Fats Oils. 1994; 45: 260–264.
- Tabanca N, Demirci B, Kirimer N, Baser KHC, Bedir E, Khan IA, Wedge DE. Gas chromatographic–mass spectrometric analysis of essential oil from *Pimpinella* species gathered from Central and Northern Turkey. J Chromatogr A. 2006; 1117: 194–205.
- Tirapelli CR, Andrade CR, Cassano AO, De Souza FA, Ambrosio SR, Costa FB, Oliveria AM. Antispasmodic and relaxant effects of

- the hydroalcoholic extract of *Pimpinella anisum* (Apiaceae) on rat anococcygeous smooth muscle. *J Ethnopharmacol.* 2007; 110: 23–29.
17. Reineccius G. *Source Book of Flavours*, 2nd ed. New York, Chapman and Hall, 1994.
  18. Hänsel R, Sticher O, Steinegger E. *Pharmakognosie-phytopharmaize*. Heidelberg, 6. Auflage, Springer-Verlag, Berlin, 1999.
  19. Behnam S, Farzaneh M, Ahmadzadeh M, Tehrani AS. Composition and antifungal activity of essential oils of *Mentha piperita* and *Lavendula angustifolia* on post-harvest phytopathogens. *Comm Agric Appl Biol Sci.* 2006; 71: 1321–1326.
  20. Barrera-Necha LL, Garduno-Pizana C, Garcia-Barrera LJ. *In vitro* antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) snyder and hansen. *Pak J Nutr.* 2009, 8, 17–21.
  21. Nabigol A, Farzaneh M. *In vitro* antifungal activity of some plant essential oils on postharvest pathogens of strawberry fruit. In: Herppich WB (ed.). *Proceedings of the IIIrd International Conference Postharvest Unlimited*. Acta Horticult. 2008, 858: 305–311.
  22. Kosalec I, Pepelnjak S, Kuštrak D. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). *Acta Pharm.* 2005; 55: 377–385.
  23. Akqül A, Kivanç M. Inhibitory effects of selected Turkish spices and oregano components on some foodborne fungi. *Int J Food Microbiol.* 1998; 6: 263–268.