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# THE EFFECT OF THE ESSENTIAL OILS ON MICROMYCETES ISOLATED FROM PLANTS

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## Abstract

The influence of volatile fractions of essential oils of *Lavandula angustifolia*, *Salvia officinalis,* and *Picea abies* on micromycetes of 9 species was investigated at Kaunas Botanical Garden of Vytautas Magnus University during 2005–2007. The inhibitory effect of each kind of essential oil depended on the plant species from which essential oil was isolated, also on oil content, micromycete species and incubatory period. Of all oils used in the research the fungistatical effect of volatile fractions of essential oils of *Salvia officinalis* on the micromycetes tested was the strongest, and the effect of this oil on *Mortierella hyalina* and *Monilia* sp. was fungicidical.

Key words: essential oil, volatile fractions, micromycetes, fungistatical and fungicidical effect.

## Introduction

Plants produce volatile fractions which have been used for various purposes for almost 4.000 years /Hansel et al., 1999/. These multiplex fractions have been isolated and purified. Essential oils are volatile, mostly liquid substances present in many plants. Chemically, essential oils are the mixture of various substances and their combinations /Йорданов et al., 1976; Ragažinskienė et al., 2005/. These fractions are responsible for the distinctive smell of each plant. The interest in essential oils as antimicrobial material is still growing. In Lithuania and also abroad new researches are made, the effect of essential oils of various plants on different micromycetes is tested.

Different plants produce different amounts of essential oil. Plants of some families are distinguished for especially high content of oils, labiates (*Labiatae* Lindl.) being one of them. Many plants of this family are used for drugs, also as flavouring and for food /Holeman et al., 1984; Šarkinas, Šipailienė, 2003; Туманова, 2005/. From some plants of this family essential oils have been isolated for many years and lately new areas of use of essential oils have been explored. The effect of essential oils on micromycetes isolated from indoor environment /Motiejūnaitė, Kalėdienė, 2003; Мотеюнайте, Пячюлите, 2004; Mickienė et al., 2007/ and food has been investigated /Šarkinas, Šipailienė, 2003/. The results suggest that the essential oils of this family inhibit the growth of most of the micromycetes and the effect on some micromycetes is fungicidical.

Volatile substances produced by conifers possess antibacterial characteristics. Initially, the places around growing pines, cades or other conifers were used for setting up sanatoriums or other health centres. Later, isolated essential oils were used in perfumery, medicine, especially in aromatherapy /Motiejūnaitė, Pečiulytė, 2004; Kühne, Friedrich, 2007/.

Pathogenic fungi do much harm to plants. Phytopathogenic fungi and bacteria decrease the yield, plants lose their decorativeness, and the quality of green raw material deteriorates. Along with chemical plant protection agents, research is done on other environment and human-friendly means which could reduce the negative effect of pathogens and inhibit their occurrence. Also one of the areas where essential oils can be applied is plant protection. It is considered that among other functions essential oils are helpful to protect plants from disease agents and pests. Research of other authors confirms this /Simoni et al., 1993; Klimach et al., 1996; Antonov et al., 1997; Bartynska, 1999/. But so far the possibilities of essential oils' application in plant protection have been less explored compared with other areas.

Essential oils are volatile substances that consist of many components, the make-up of some of which is still not known. Related plants (of the same family or genera) produce different fractions. Antimicrobical features and amounts of essential oils isolated from the different plants vary. As a result, laboratory research *in vitro* is being continuously done, since it is important to test the effects of essential oils of many plants on different micro-organisms.

The aim of our work was to determine the effect of volatile fraction of essential oils of *Lavandula angustifolia*, *Salvia officinalis* and *Picea abies* on micromycetes of 9 species isolated from various injured parts of the plant.

## Materials and Methods

Research was done in Kaunas Botanical Garden of Vytautas Magnus University during 2005–2007. The following micromycetes were tested: *Verticillium catenulatum* (Kamyschko ex G. L. Barron et Onions) W. Gams, *Fusarium culmorum* (Wm. G. Sm.) and *Monilia* sp. isolated from the berries of large cranberry (*Oxyccocus macrocarpus* (Aiton) Pursh; *Fusarium moniliforme* Scheldon – from the stem of torch thistle (*Cereus* sp.) *Fusarium oxysporum* Schltdl. – from the roots of peony (*Paeonia lactiflora* Pall.) and *Fusarium* sp. – from the apple (*Malus domestica* Bernh.). All parts of the plants from which micromycetes were isolated were injured by rot.

Essential oils used in the study were isolated from three plant species, one of them – spine of common spruce (*Picea abies* Karst.) (oil manufacturer Sensient Ess. Oils GmgH, Germany). This oil is recommended as antiseptic in medicine and perfumery. Essential oils of the other two species were isolated in Medicinal Plant Science Section in Kaunas Botanical Garden of Vytautas Magnus University. These were essential oils of *Lamiaceae* family plants: true lavender (*Lavandula angustifolia* Mill.) and common sage (*Salvia officinalis* L.).

Micromycetes were grown in Petri dishes on the cornmeal agar medium /Билай, 1982/.

Our aim was to investigate the effects of volatile fraction of essential oils; therefore the oils were not added into the medium but dripped on the covers of Petri dishes. Three different rates of oil were tested: 0.005, 0.01 and 0.015 ml. After sowing the micromycetes and dripping essential oil, the dishes were sealed using the adhesive tape, turned over and put into a thermostat at a temperature of 26±2° C. The tests on the effect of the essential oil were started by measuring the diameter of fungi colonies after three days of incubation and by comparing them to the control sample (dishes with fungi but without essential oil). Micromycetes of the tested species produce different colonies which grow at different rate. For instance, the average growth rate of mycelium after three days of incubation in the control dishes was: *Fusarium* sp. 1.6 cm; *F. oxysporum* – 3.8 cm; *Alternaria alternata* 4.6 cm; *Fusarium culmorum* – 5.0 cm; *Verticillium catenulatum* – 5.1 cm and *Fusarium moniliforme* – 5.2 cm, therefore to compare the effect of the essential oils on the growth of various micromycetes mycelium the inhibitory activeness (%) was calculated using the formula /Билай, 1982/:

R = (Do − D) / Do x 100,

R – inhibitory activeness (%),

Do – diameter of a control colony (cm),

D – diameter of a tested colony (cm).

**Results and Discussion**

Volatile fractions of all tested essential oils inhibited the growth of mycelium of all nine micromycetes. The inhibiting effect depended on: 1) the amount of essential oil, 2) the species of the plant from which the essential oil was isolated, 3) the species of the tested micromycete and 4) the incubation period.

The higher the concentration (amount of the essential oil) of volatile oil was in the sealed dishes the weaker the radial growth of micromycete mycelium was. The growth of micromycetes of all species was the least in the dishes with the highest essential oil amount used in the experiment (0.015 ml) (Table).

Essential oils are volatile and fissile fractions and their concentration in the environment after some time increased, also the inhibitory effect increased. The effect of all three species of essential oils was the greatest after three days' incubation (R from 79–39.3% with 0.015 ml oil, 43.5–17.4% with 0.005 ml oil) and decreased later. After ten days *Fusarium culmorum, F. moniliforme* and *Verticillium catenulatum* (in all test treatments) the space of the mycelium in the dishes with spruce essential oil and *Mortierella hyalina* and *Fusarium proliferatum* with lavender essential oil (essential oil amount 0.005 ml) matched the mycelium area in the control dishes.

Volatile fraction of sage essential oils had the strongest fungistatical effect on all of the investigated micromycetes. Especially sensitive to these fractions was *Mortierella hyalina* which was fungicidically affected by the amount of 0.015 ml (R = 100%), strong fungicidical effect was exerted on the mycelium of *Fusarium sambucinum* (R to 93.3%). *Verticillium catenulatum* (R to 53.3%) was the most resistant to the effect of sage essential oil.

Volatile fractions of sage and spruce essential oils had similar effect on all investigated micromycetes. None of the amounts of these essential oils used in tests had fungicidical effect. Volatile fraction of spruce essential oil mostly inhibited the growth of mycelium of *Fusarium* sp. and *F. oxysporum* (R to 75% and 79%). Volatile fractions of this oil had the least effect on *Verticillium catenulatum* (R to 39.3%). Conversely, the lavender essential oil had the strongest effect on *Verticillium catenulatum* (R to 69.2%), and the weakest on *Fusarium sambucinum* (R to 36.5%).

***Table.*** The effect of the essential oils of *Lavandula angustifolia*, *Salvia officinalis* and *Picea abies* on micromycetes isolated from the plants

| Micromycetes | Amount of the essential oil ml | *Lavandula angusifolia* | | | *Salvia officinalis* | | | *Picea abies* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Inhibitory effect (%) after days: | | | | | | | | |
| | | 3 | 6 | 10 | 3 | 6 | 10 | 3 | 6 | 10 |
| *Alternaria alternata* | 0.005 | 25.0 | 20.0 | 17.5 | 75.0 | 30.0 | 21.1 | 17.4 | 15.5 | 26.2 |
| | 0.01 | 45.0 | 20.0 | 21.1 | 80.0 | 36.0 | 35.1 | 41.2 | 48.3 | 43.1 |
| | 0.015 | 50.0 | 30.0 | 21.1 | 90.0 | 40.0 | 36.8 | 56.5 | 68.7 | 50.8 |
| *Fusarium culmorum* | 0.005 | 24.0 | 19.0 | 15.0 | 76.0 | 31.0 | 20.0 | 30.0 | 24.6 | 0 |
| | 0.01 | 43.0 | 20.0 | 19.0 | 82.0 | 35.0 | 33.0 | 52.0 | 21.3 | 0 |
| | 0.015 | 50.0 | 30.0 | 20.0 | 91.0 | 42.0 | 35.0 | 53.8 | 32.8 | 0 |
| *Fusarium oxysporum* | 0.005 | 23.0 | 10.0 | 3.0 | 80.0 | 34.0 | 20.0 | 34.2 | 36.0 | 23.5 |
| | 0.01 | 46.0 | 15.0 | 10.0 | 83.0 | 35.0 | 28.0 | 47.4 | 38.0 | 35.3 |
| | 0.015 | 60.0 | 40.0 | 20.0 | 92.0 | 40.0 | 30.0 | 79.0 | 72.0 | 49.4 |
| *Fusarium proliferatum* | 0.005 | 25.0 | 3.8 | 0 | 80.0 | 35.7 | 20.5 | 40.0 | 25.3 | 0 |
| | 0.01 | 60.0 | 16.7 | 7.1 | 85.0 | 35.7 | 29.5 | 42.3 | 29.3 | 0 |
| | 0.015 | 95.0 | 57.1 | 55.1 | 90.0 | 56.4 | 52.2 | 46.2 | 40.0 | 0 |
| *Fusarium sambucinum* | 0.005 | 33.3 | 15.3 | 3.0 | 66.7 | 61.5 | 32.8 | | | |
| | 0.01 | 33.3 | 32.3 | 25.4 | 80.0 | 78.5 | 42.8 | | | |
| | 0.015 | 36.5 | 33.3 | 22.8 | 93.3 | 78.5 | 70.1 | | | |
| *Fusarium* sp. | 0.005 | 21.0 | 17.0 | 12.0 | 70.0 | 52.0 | 45.0 | 43.5 | 33.3 | 26.2 |
| | 0.01 | 25.0 | 24.0 | 20.0 | 80.0 | 56.0 | 40.0 | 50.0 | 53.3 | 43.1 |
| | 0.015 | 35.0 | 36.0 | 20.0 | 90.0 | 60.0 | 32.0 | 75.0 | 73.3 | 50.8 |
| *Verticillium catenulatum* | 0.005 | 20.0 | 37.0 | 34.5 | 26.7 | 26.1 | 24.0 | 33.3 | 31.4 | 0 |
| | 0.01 | 53.3 | 55.6 | 55.2 | 26.7 | 26.1 | 20.0 | 35.3 | 34.1 | 0 |
| | 0.015 | 96.2 | 90.0 | 86.2 | 53.3 | 47.8 | 40.0 | 39.3 | 35.9 | 0 |
| *Monilia* sp. | 0.005 | 27.3 | 22.1 | 12.3 | 30.0 | 24.7 | 19.1 | | | |
| | 0.01 | 44.0 | 39.0 | 32.0 | 71.4 | 45.0 | 43.0 | | | |
| | 0.015 | 70.3 | 45.3 | 40.1 | 90.0 | 50.0 | 45.6 | | | |
| *Mortierella hyalina* | 0.005 | 13.0 | 4.0 | 0 | 56.0 | 25.0 | 12.0 | 40.0 | 19.2 | 11.0 |
| | 0.01 | 61.0 | 31.0 | 20.0 | 99.0 | 96.0 | 75.0 | 49.1 | 21.0 | 16.2 |
| | 0.015 | 50.0 | 31.0 | 23.0 | 100 | 100 | 100 | 50.3 | 25.0 | 17.8 |

Micromycetes of various species exhibit different responses to different essential oils /Snieškienė et al., 2003, Radušienė et al., 2005/.

**Conclusions**

1. Volatile fractions of essential oils of all investigated plants (*Lavandula angustifolia*, *Salvia officinalis* and *Picea abies*) had the fungistatical effect but not equally strong. The strongest effect on micromycetes was exerted by the volatile fraction of sage essential oils. The amount of 0.015 ml affected the micromycete *Mortierella hyalina* fungicidically (R = 100%).

2. The effect of essential oil of all species was the greatest after three days of incubation (R from 79–39.3% with 0.015 ml of oil, to 43.5–17.4% with 0.005 ml of oil) and decreased later. After ten days, the essential oil of spruce had no negative effect on mycelium growth of *Fusarium culmorum*, *F. moniliforme* and *Verticillium catenulatum* and also essential oil of lavender (0.005 ml) – on mycelium growth of *Mortierella hyalina* and *Fusarium proliferatum.*

3. The strength of the effect of essential oils depended on the amount of volatile fraction in micromycetes area: the greater the concentration the stronger the inhibitory effect was.

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