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Effect of essential oils on fungi isolated from apples and vegetables

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The aim was to investigate the effects of volatile fraction of essential oils from *Picea abies*, *Eucalyptus globulus*, *Rosmarinus officinalis* and volatile fraction of *Abies sibirica* oil on fungi isolated from apple, leek, carrot, onion. Researches were made in 2008–2009. Tested fungi were as follows: *Penicillium roqueforti*, *Aspergillus flavus*, *Aspergillus flavus* var. *oryzae*, *Mortierella hyalina* var. *hyaline*, *Sclerotinia sclerotiorum*, *Sporotrichum aurantiacum*, *Phoma exiqua*, *Clonostachys rosea* f. *catenulata*. All parts of the plant from which fungi were isolated were injured by rot. Fungi of the tested species grew on the potato dextrose agar medium in different colonies, which grew at different speed. The oils were dripped on the covers of Petri dishes. There were three different variations taking the different portions of oil: 0.005, 0.01 and 0.015 ml. It was calculated the inhibitory activeness (R). Volatile fractions of all tested oils inhibited the growth of mycelium of all 8 species fungi. The inhibiting effect depended on: 1) the amount of oil, 2) the species of the plant, from which the oil was isolated, 3) the species of the tested fungi and 4) the incubation period.

Key words: essential oil, fungi, inhibitory activeness.

Introduction. Essential oils – volatile substance, which consists of many components and not all of them composition is known so long. 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. Related plants (of the same family or genera) produce different fractions. Plants of some families are distinguished for especial richness of oils. One of these families is labiates (*Labiatae* Lindl.), also large amount of essential oils is isolated by conifers and eucalyptuses.

Plants produce volatile fractions, which have been used for various purposes for almost 4000 years (Hansel et al., 1999). Antimicrobial features and amounts of essential oils isolated from the different plants vary; therefore, laboratory researches *in vitro* are constantly carried out. It is important to test the effect of essential oils of many plants

on different microorganisms. 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 microorganisms is tested.

The essential oils of some plants are used for drugs, also as flavouring and for food (Holeman et al., 1984; Šarkinas, Šipailienė, 2003; Туманова, 2005), in perfumery, medicine, especially in aromatherapy (Motiejūnaitė, Pečiulytė, 2004; Kühne, Friedrich, 2007). The effect of essential oils was tested on fungi isolated from indoor environment (Motiejūnaitė, Kalėdienė, 2003; Мотеюнайте, Пячюлите, 2004; Mickienė et al., 2007). The results suggest that the essential oils inhibit the growth of most of the microorganisms and the effect on some fungi is fungicidal.

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. Researches of other authors confirm this (Simoni et al., 1993; Klimach et al., 1996; Antonov et al., 1997; Bartynska, 1999; Snieškienė et al., 2008). Nevertheless, so far the possibilities of essential oils usage in plant protection have been less explored compared with other areas.

One of the most important causes, which interrupts the usage of the essential oils for plant protection, is a quick fragmentation of volatile fraction of oils, dispersion in the environment. These features are not such an impediment if oils are used in a room as fruit and vegetable storage. It is important to preserve the agricultural production using as far as possible less chemicals harmful to human health.

Antimicrobial features characterize not only essential oils but also common oils of some plants. One of these oils is oil from Siberian fir (*Abies sibirica*).

The aim of work was to define the effect of volatile fraction of essential oils of *Picea abies*, *Eucalyptus globulus*, *Rosmarinus officinalis* and volatile fraction of *Abies sibirica* oil on fungi (8 species) isolated from apple, leek, carrot and onion.

Object, methods and conditions. Research was done at the Lithuanian Institute of Horticulture and Kaunas Botanical Garden of Vytautas Magnus University during 2008–2009. The following fungi were tested: *Penicillium roqueforti* Thom was isolated from apple; *Aspergillus flavus* Link. – from leek and onion; *A. flavus* var. *oryzae* (Ahlb.) Kurzman, M. J. Smiley, Robnett Wicklow (sin. *A. oryzae* (Ahlb. E. Cohn) – from carrot; *Mortierella hyalina* var. *hyalina* (Harz) W. Gams (sin. *Mortierella hygrophila* Linnem., *M. hyalina* (Harr) W. Gams) – from apple; *Sclerotinia sclerotiorum* (Lib.) de Bary – from carrot; *Sporotrichum aurantiacum* (Bull.) Fr. – carrot; *Phoma exiqua* Sacc – from apple; *Clonostachys rosea* f. *catenulata* (J. C. Gilman, E. V. Abbott) Schroers (sin. *Gliocladium catenulatum* J. C. Gilman, E. V. Abbott) – from carrot. Fungi were identified according to Klich (2007); Gams, (1971); Lugauskas et al. (2002); Studies …, (1981); Samson, Pitt (2000), currant names are given according to Index Fungorum (2004).

Essential oils used in the study were isolated from three plant species: *Picea abies* Karst., *Eucalyptus globulus* Labill. and *Rosmarinus officinalis* L. (oils manufacturer Sensient Ess. Oils GmgH, Germany). Also there was tested the volatile fraction of Siberian fir (*Abies sibirica*) oil. The oil is extracted from thorns of young sprouts (producer "ALMEDA", Russia). This oil is recommended as antiseptic in medicine and perfumery.

Fungi were grown in Petri dishes on the potato dextrose agar medium (Билай, 1982). The oils were not added into the medium but dripped on the covers of Petri dishes. There were three different variations taking the different portions of oil: 0.005, 0.01 and 0.015 ml. After sowing the fungi and dripping oil, the dishes were sealed using the adhesive tape, turned over and put into a thermostat (temperature 26 ± 2 °C). The tests on the effect of the oil were started by measuring the diameter of fungi colonies after four days of incubation and by comparing them to the control sample (dishes with fungi but without oil). Fungi of the tested species produce different colonies, which grow at different speed. For instance the average growth rate of mycelium after four days of incubation in the control dishes were: *Penicillium roqueforti* – 8.3 cm, *Aspergillus flavus* – 50.3 cm, *Aspergillus flavus* var. *oryzae* – 32.8 cm, *Mortierella hyalina* var. *hyaline* – 80.3 cm, *Sclerotinia sclerotiorum* – 85.0 cm, *Sporotrichum aurantiacum* – 35.0 cm, *Phoma exiqu*a – 25.0 cm, *Clonostachys rosea* f. *catenulate* – 21.0 cm. Therefore, to compare the effect of the oils on the growth of various fungi mycelium the inhibitory activeness (%) was calculated using the formula (Билай, 1982):

$R = (Do - D) / Do \times 100$

R – inhibitory activeness (%)

Do – diameter of a control colony (cm),

D – diameter of a tested colony (cm).

Results. Volatile fractions of all tested essential oils and Siberian fir oil inhibited the growth of mycelium of all eight species of fungi.

The bigger concentration (amount of the oil) of volatile oil was in the sealed dishes the weaker was the radial growth of fungi mycelium. The growth of fungi of all species was the least in the dishes with the biggest oil amount used in a research (0.015 ml) (Table, Fig. 1, 2).

Table. Effect of essential oils of *Picea abies*, *Eucalyptus globulus* and *Rosmarinus officinalis* L. on fungi isolated from vegetables and apples
Lentelė. *Picea abies*, *Eucalyptus globulus* ir *Rosmarinus officinalis* eterinių aliejų poveikis grybams, išskirtiems iš daržovių ir obuolių

Fungi Grybai	Amount of essential oil Eterinio aliejaus kiekis (ml)	*Eucalyptus globulus*			*Rosmarinus officinalis*			*Picea abies*		
		Inhibitory effect after days Inhibicinis aktyvumas po dienų (%)								
		4	7	14	4	7	14	4	7	14
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Aspergillus flavus	0.005	75.1 c	72.4 d	22.0	81.8	31.8	60.1	12.9	55.5	0
	0.01	89.5 d	74.4 d	33.8	100	100	18.5	18.5	81.8	3.2
	0.015	90.5 d	90.8 e	41.2	100	100	100	80.1	91.2	47.1
A. flavus var. *oryzae*	0.005	41.2 a	26.2 a	0	54.9	47.5	0	4.6	3.3	0
	0.01	100 e	83.7 e	3.5	80.8	59.6	13.2	36.0	9.2	0
	0.015	100 e	91.9 e	13.8	100	100	86.1	52.7	25.9	0

Table continued
Lentelės tęsinys

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Clonostachys rosea f. *catenulata*	0.005	66.7 b	43.5 b	51.3	100	100	77.6	60.5	51.1	40.1
	0.01	100 e	100 e	100	100	100	100	100	70.1	47.9
	0.015	100 e	100 e	100	100	100	100	100	85.6	57.8
Mortierella hyalina var. *hyalina*	0.005	75.3 c	38.2 b	0	89.4	82.0	37.1	40.8	0	0
	0.01	100 e	61.8 c	0	100	90.8	51.2	58.5	0	0
	0.015	100 e	100 e	39.6	100	95.5	60.6	71.6	0	0
Penicillium roquefortii	0.005	47.9 a	58.8 c	2.7	81.8	31.8	60.1	100	55.3	36.2
	0.01	75.8 c	41.2 b	29.3	100	100	100	100	72.9	50.5
	0.015	100 e	100 e	60.1	100	100	100	100	82.4	62.8
Phoma exiqua	0.005	65.5 b	49.4 b	30.1	70.0	52.0	45.0	40.7	26.9	14.0
	0.01	100 e	93.3 e	61.2	80.0	56.0	40.0	49.1	33.1	24.0
	0.015	100 e	100 e	64	90.0	60.0	32.0	67.3	46.9	30.1
Sclerotinia sclerotiorum	0.005	83.5 d	39.6 b	0	100	61.8	25.5	0	0	0
	0.01	100 e	91.2 e	21.2	100	100	87.9	25.3	0	0
	0.015	100 e	96.1 e	33.8	100	100	100	35.3	0	0
Sporotrichum aurantiacum	0.005	100 e	83.1 e	52.9	100	100	100	27.7	21.2	19.6
	0.01	100 e	100 e	100	100	100	100	44.9	33.9	23.5
	0.015	100 e	100 e	100	100	100	100	53.4	48.3	26.1

Essential oils are volatile and fissile fractions and their concentration in the environment after some time increased, ant the inhibitory effect increased also. The effect of all three species of essential oils was the greatest after four days incubation (R from 100 %) and later decreased. After seven days *M. hyalina* var. *hyalina* and *S. sclerotiorum* and after fourteen days *A. flavus* var. *oryzae* (in all test treatments) the space of the mycelium in the dishes with spruce (*Picea abies*) essential oil and *S. sclerotiorum* and *A. flavus* var. *oryzae* with Siberian fir oil have matched the mycelium area in control dishes.

Volatile fraction of *Rosmarinus officinalis* essential oil had the strongest fungistatic and fungicidal effect on all of the investigated micromycetes. *Especially sensitive* to these fractions was *C. rosea* f. *catenulata* and *S. aurantiacum. P. exiqua* (R up to 32–45 %) was the most resistant to the effect of *Rosmarinus officinalis* essential oil.

Volatile fraction of *Picea abies* essential oil had the least fungistatic effect on all of the investigated fungi (Table).

Fungi of different species have different reaction on different essential oils. From the investigated 8 fungi species the most resistant for all essential oil were *M. hyalina* var. *hyaline*, and sensitive for the influence of volatile fractions of all essential oils were *C. rosea* f. *catenulate*, *P. roquefortii* and *S. aurantiacum*.

Volatile fractions of Siberian fir oil affected investigated fungi not very evenly. Mycelium of *P. roquefortii* responded firmly to the volatile fraction of this oil (Fig. 1).

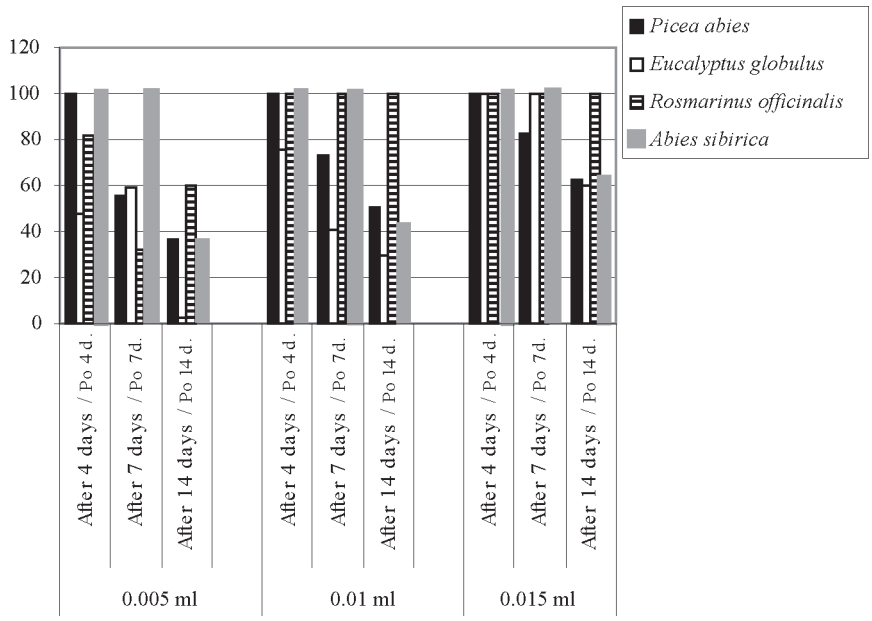

Fig. 1. Effect of essential oils on the growth of *Penicillium roquefortii* mycelium (%)

1 pav. Eterinių aliejų poveikis *Penicillium roquefortii* grybienos augimui, %

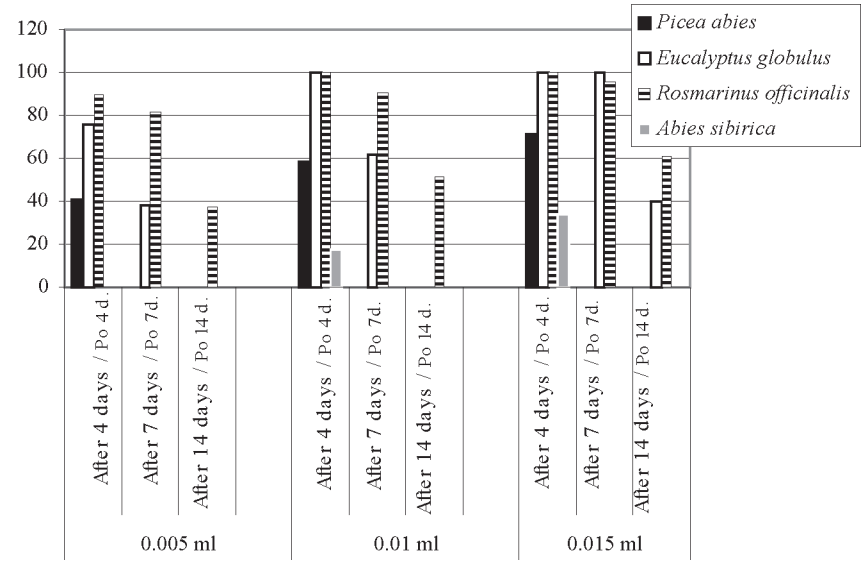

Fig. 2. Effect of essential oils on the growth of *Mortierella hyalina* var. *hyalina* mycelium (%)

2 pav. Eterinių aliejų poveikis *Mortierella hyalina* var. *hyalina* grybienos augimui, %

A. flavus responded a little bit less negatively to the volatile fractions of this oil. The impact persisted for quite a long time: after 14 days of incubation R have increased from 47.1 % (0.005 ml) to 67.6 % (0.015 ml). The oil of Siberian fir had almost no effect on the growth of M. hyalina var hyalina (Fig. 2). Fungus of this species was also resistant to the influence of essential oils of another conifer *Picea abies*. It can be hypothesized that *M. hyalina* var. *hyalina* is resistant to the volatile fractions of conifer essential oils. Investigated oils of other plants (*Rosmarinus officinalis* and *Eucalyptus globulus*) strongly inhibited the growth of this fungus (Fig. 2).

The inhibiting effect volatile fractions of all the tested essential oils and Siberian fir oil depended on: 1) the amount of oil, 2) the species of the plant, from which the oil was isolated, 3) the species of the tested fungi and 4) the incubation period.

Discussion. Many authors from different countries have described in their works the influence of essential oils on the microorganisms. Essential oils are complex substances; therefore, various effect manners of essential oils are tested (Билай, 1982; Antonov et al., 1997; Мотеюнайте, Пячюлите, 2004). We have tested the effect of essential oils and Siberian fir volatile fraction. Though volatile fractions stay in the environment not for a long time, yet the efforts are being made to use them in practice in Lithuania – for prophylaxis against bird diseases (Mickienė et al., 2007). Further researches are needed to discover the proper essential oils to see their usage possibility in vegetable protection. According to our current and previous works (Snieskienė et al., 2003; Snieškienė et al., 2008), there can be selected effective essential oils against particular fungi species isolated from putrescent vegetables and fruits.

The volatile fraction of *Rosmarinus officinalis* and *Eucalyptus globulus* most strongly inhibited the mycelium growth of all fungi (*Mortierella hyalina* var. *hyalinea*, *Penicillium roquefortii* and *Phoma exiqua*) isolated from apples. Fungi (*Aspergillus flavus* var. *oryzae*, *Clonostachys rosea* f. *catenulate*, *Sclerotinia sclerotiorum* and *Sporotrichum aurantiacum*) isolated from carrots are also strongly affected by the same essential oils. *Aspergillus flavus*, isolated from leeks and onions, is affected by all tested essential oils of a higher concentration (0.01 ml and 0.015 ml).

Conclusions. 1. Fungi of different species have different reaction on different oils.

2. Essential oils of all investigated plants (*Rosmarinus officinalis*, *Eucalyptus globulus* and *Picea abies*) and cedar oil had the fungistatic effect. The strongest effect (fungistaticaly and fungicidicaly) on fungi was exerted by the volatile fraction of *Rosmarinus officinalis* essential oils. The mentioned oil most strongly affected *Sporotrichum aurantiacum Penicillium roquefortii* and *Clonostachys rosea* f. *catenulate*.

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

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Eterinių aliejų poveikis grybams išskirtiems iš obuolių ir daržovių

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Santrauka

Darbo tikslas buvo ištirti *Picea abies*, *Eucalyptus globulus*, *Rosmarinus officinalis* eterinių aliejų ir *Abies sibirica* aliejaus lakiųjų frakcijų poveikį grybams, išskirtiems iš obuolių, porų, morkų ir svogūnų. Tyrimai atlikti 2008–2009 m. Buvo tiriami šie grybai: *Penicillium roqueforti*, *Aspergillus flavus*, *Aspergillus flavus* var. *oryzae*, *Mortierella hyalina* var. *hyaline*, *Sclerotinia sclerotiorum*, *Sporotrichum aurantiacum*, *Phoma exiqua*, *Clonostachys rosea* f. *catenulata*. Grybai buvo išskirti iš pūvančių augalų dalių. Tiriamų rūšių grybai buvo auginami ant bulvių dekstrozės agarizuotos terpės. Skirtingų rūšių grybų kolonijos augo nevienodu greičiu. Ant Petri lėkštelių dangtelių buvo lašinamos trys normos aliejų: po 0,005, 0,01 ir 0,015 ml. Buvo apskaičiuojamas kiekvieno tirto aliejaus inhibicinis aktyvumas (R). Visų tirtų aliejų lakiosios frakcijos stabdė visų 8 tirtų rūšių grybų grybienos augimą. Inhibicinis aktyvumas priklausė nuo: 1) aliejaus kiekio, 2) augalo, iš kurio buvo išskirtas aliejus, rūšies, 3) tiriamų grybų rūšies ir 4) inkubacijos laiko.

Reikšminiai žodžiai: eterinis aliejus, grybai, inhibicinis aktyvumas.