THE EFFECT OF BACTERIA AND ESSENTIAL OILS ON MYCOTOXIN PRODUCERS ISOLATED FROM FEED OF PLANT ORIGIN

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Abstract. The aim of the investigation was to test the antifungal activity of *Pantoea, Streptomyces* and *Sphingomonas* bacterial strains and essential oils from *Abies sibirica* (siberian fir), *Thymus pulegioides* (broad-leaved thyme), *Carum carvi* (caraway), *Pimpinella anisum* (anise), *Eucalyptus globulus* (tasmanian blue gum), *Syzygium aromaticum* (clove), *Lavandula hybrida* (lavender) and *Melaleuca alternifolia* (tea-tree) – a pilot study. The antifungal activity was tested against mycotoxin producing fungi from *Aspergillus, Penicillium, Fusarium* and *Alternaria* genera. The killer activity of the bacterial isolates was evaluated by the ability of the tested strains to form lysis zones on fungal lawns. The antifungal activity of the essential oils was assessed by the agar diffusion method. It was revealed that the isolated strains of *Pantoea citrea*, *Streptomyces* sp. and *Sphingomonas* sp. showed a wide fungicidal spectrum against *Aspergillus flavus, A. terreus, A. versicolor, A. fumigatus, Penicillium verrucosum, P. cyclopium, P. chrysogenum, Fusarium poae, F. avenaceum, F. culmorum, F. solani* and *Alternaria alternata*. The most efficient antifungal activity was characteristic of essential oils from *Syzygium aromaticum* and *Pimpinella anisum*.

Keywords: bacteria; fungi; essential oils; antifungal activity.

Introduction. Microscopic fungi are known to considerably deteriorate feed of plant origin as well as produce harmful secondary metabolites – mycotoxins. It was established that fungi from *Penicillium, Aspergillus, Fusarium* and *Alternaria,* etc. genera produce particularly harmful toxins such as aflatoxins, ochratoxins, zearalenons, deoxinivalenol, T-2 toxin, fumonizines, aflatrems, patulins, rugulosins, emodines and other toxic compounds (Yiannikouris and Jouany, 2002; Santin, 2005). The presence of mycotoxins in feed can cause significant economic losses. The most important damages are: decreased resistance to diseases, reduced productivity and worsened production quality (Brake et al., 2000; Denli and Perez, 2010).

Recently producers of feed have been applying various means in order to prevent growth of microscopic fungi on feed and consequent mycotoxin contamination. Protection against toxic fungi starts already under field conditions. Contamination risk can be reduced by crop rotation, an efficient use of fungicides and proper fertilization (Suproniene et al., 2010).

Nowadays many scientists have been engaged in research on the ability of plants to eliminate harmful mycotoxin effect by themselves. Among such innovative investigations the following trends can be pointed out: genetic selection aiming at strengthening of plant immunity against fungi; development of transgenic plants resistant towards attacks of fungi and eventually excretion of mycotoxins; production of seeds with endophytic bacteria preventing growth of toxic fungi; and early contamination of plants with microorganisms competing with toxin-producing fungi. The latter means are described as biological fight against harmful microorganisms (Wang et al., 2007; Rajasekaran et al., 2009).

Researchers in various countries have been searching for biological substances with a fungicidal effect against mycotoxin producers. It has been indicated that fungicidal substances can be produced by bacteria *Bacillus, Enterobacter, Streptomyces* and *Pseudomonas* (Munimbazi and Bulerman, 1998; Li and Rinaldi, 1999, Kaleli et al., 2006, Etcheverry et al., 2009). Many bacterial metabolites belong to polyketide compounds and behave as antibiotics (Levenfors et al., 2004). Strains of *Pseudomonas aeruginosa* produce pyrolnitrin and pseudomonic acid with fungicidal activity against the yeast *Candida* (Kaleli et al., 2006). Proteins syringomycin E, syringotoxin B and syringostatin A from *Pseudomonas syringae* pv. *syringae* showed fungicidal effect against *Cryptococcus*, *Candida* and *Aspergillus in vitro* (Sorensen et al., 1996). The protein nikkomycin Z from *Streptomyces tendae* is effective against many fungi and yeasts (Li and Rinaldi, 1999). Lactic acid bacteria have a broad antifungal spectrum against *Aspergillus, Penicillium, Fusarium, Alternaria* and other micromycetes (Lavermicocca et al., 2000; Schnürer and Magnusson, 2005).

Investigation of natural substances as fungal growth inhibitors ensuring a high quality of feed has been of a great interest because feed quality determines not only productivity but also health of livestock. It has been reported that essential oils show antifungal properties (Bluma and Etcheverry 2008; Choudhary and Kumari 2010, Alpsoy 2010). Plants of *Juniperus*, *Pimpinella, Thymus* and *Achillea* genera were shown to have a significant fungicidal effect (Cavaleiro et al., 2006,

Kosalec at al., 2005, Karaman et al., 2001, Bezić et al., 2003). Antimicrobial activity of essential oils has been investigated in Lithuania, too. It has been demonstrated that essential oils from *Melaleuca, Picea, Citrus, Carum, Cuminum, Eucalyptus*, *Mentha* and *Thymus, etc.* have a broad spectrum against microorganisms including deteriorators of food products (Šipailienė ir kt., 2005, Damašius ir kt. 2007) and airborne micromycetes (Mickienė et al., 2008, Mickienė, 2009).

The main task of the work as a pilot study was to determine the antifungal activity of newly isolated bacteria *Pantoea, Streptomyces* and *Sphingomonas* as well as of essential oils from *Abies*, *Thymus, Carum, Pimpinella, Eucalyptus, Syzygium, Lavandula* and *Melaleuca* plants against microscopic fungi producing mycotoxins.

Materials and methods. Preliminary investigation on fungicidal activity of bacteria *Pantoea, Streptomyces* and *Sphingomonas* as well as on that of essential oils from plants *Abies*, *Thymus, Carum, Pimpinella, Eucalyptus, Syzygium, Lavandula* and *Melaleuca* was carried out. Bacteria *Pantoea citrea* (strains Tx, T1x, T2x, T3x), *Streptomyces* sp. (Ux) and *Sphingomonas* sp. (V8) were isolated from spontaneous fermentation of fruits and different berries. The strain *Streptomyces* sp. (Ux308) was isolated from soil (Kryžkalnis, Lithuania). Microscopic fungi, producers of mycotoxins, were collected in 2003–2006 from feed of plant origin (in Aleksandras Stulginskis university training farm, Kaunas district) during implementation of the program "Regularities of mycotoxins' accumulation in food and development of preventive safety measures" (Nr. C-04/2003) supported by the Lithuanian State Science and Study Foundation.

The fungi have been maintained in the collection of the Institute of Botany, Nature Research Centre. The following fungi were used for the investigation: *Aspergillus flavus* Link (SK-1), *Aspergillus terreus* Thom (S-1), *Aspergillus versicolor* (Vuill.) Tirab. (MI-130), *Aspergillus fumigatus* Fresen. (KO-5), *Penicillium verrucosum* Dierckx (DB-1), *Penicillium cyclopium* Westling (21-AL), *Penicillium chrysogenum* Thom (48-L), *Fusarium poae* (Peck) Wollenw. (RM-2), *Fusarium avenaceum* (Fr.) Sacc. (RM-3), *Fusarium culmorum* (W.G. Sm.) Sacc. (L-2), *Fusarium solani* (Mart.) Appel & Wollenw. (SA-4), *Alternaria alternata* (Fr.) Keissl. (I-4U).

A killer activity of the bacterial isolates was tested according to their ability to form lysis zones on fungal lawns. Since all the killer toxins are active at pH 4.8, all experiments were performed using the agar for testing of a killer phenotype (YPED MB) containing the following components (g/ml): yeast extract – 1, peptone – 2, glucose – 2, agar – 2 and methylene blue – 0.00003. For primary screening of bacteria, the following microorganisms were used: *Saccharomyces cerevisiae* strain ά'l (MATα, leu2-2 [kil- 0]), susceptible to any toxin; killer strains of *Saccharomyces cerevisiae*: K7 (MATα arg9 [kil-K1]); DBY4975(MATα ade2 his3-200 leu2-3-112 lys2-801 ura3-52 gal+[kil-K1]); Romanešti K100 (RomK-100) (wt, HM/HM [kil-K2]); K28 (wt, HM/HM [kil-K28]) and MS300 (MATα leu2 ura3-52 [kil-K28]) (Melvydas et al., 2005). The antifungal preparation nystatin 100 IU (Liofilchem, Italy) served as a control.

The fungi were grown for 5 days on YEPD agar at 27±1°C. A suspension from each culture equal to 0.5 McFarland turbidity standard was prepared and 1 ml of the suspension was mixed with cooled to 45–50°C YEPD MB agar and poured into Petri dishes (90 mm). To test the killer activity of the bacterial strains, the bacteria were inoculated onto the solid surface of the nutritive agar mixed with the fungal cultures. The plates were incubated for 5 days at 27±1°C. The efficiency of bacterial killer activity was evaluated by the size of the lysis zone (mm).

Essential oils from the following plants were used: *Abies sibirica* L. („Naujoji Barmunė", Vilnius, Lithuania), *Thymus pulegioides* L. (Institute of Botany, Vilnius, Lithuania), *Carum carvi* L. (Aleksandras Stulginskis University, Kaunas, Lithuania), *Pimpinella anisum* L. („Naujoji Barmunė", Vilnius, Lithuania), *Eucalyptus globulus* Labill. („Naujoji Barmunė", Vilnius, Lithuania), *Syzygium aromaticum* Thunb. („Naujoji Barmunė", Vilnius, Lithuania), *Lavandula hybrida* Rev. („Naujoji Barmunė", Vilnius, Lithuania), *Melaleuca alternifolia* L. („Naujoji Barmunė", Vilnius, Lithuania). Essential oils of 100% concentration were used.

The activity of the essential oils was assessed applying the agar diffusion method. Sabouraud Agar (Oxoid, England) was used to grow fungal lawns. Suspensions of microscopic fungi and their mixtures with the agar were prepared as described above. Paper discs (6 mm diameter) were placed on the plates with fungi, and then 10 µl of each essential oil was dropped onto the discs. The plates were incubated for 3 days at 27±1°C. The antifungal activity of the essential oils was evaluated based on the lysis zone diameter (mm).

The micromycetes were grouped according to their susceptibility to the bacteria and essential oils. These groups were the following: susceptible – the fungicidal zone reaches 20 mm or more; intermediate – the fungicidal zone of 10–19 mm, and resistant – the zone up to 9 mm or less.

The experiments were carried out in triplicates. The analysis of data was performed with the SPSS program version 11.0. Mean X, and standard deviation S, standard error of the mean Sx, the coefficient of variation CV (%) were calculated.

Results. The results showed that bacterial strains of *Pantoea citrea*, *Streptomyces* spp. and *Sphingomonas* sp. were characterised by different fungicidal activity against fungi – producers of mycotoxins (Table 1).

It was revealed that the standard mycocynogenic strains of the yeast *Saccharomyces cerevisiae* did not exert fungicidal activity on the tested fungi. *Pantoea citrea* strains showed variable fungicidal activity against fungi from *Aspergillus* genus. The strongest activity was recorded against *A. versicolor* MI-130 exerted by the strain *P. citrea* T1x, which caused the lysis zone up to 11.5 mm. The weakest effect was shown against *A. flavus* SK-1 by the strains *P. citrea* T2x and T3x: the lysis zone

was only 7.0 mm. It was found that the strains *Streptomyces* sp. Ux and *Sphingomonas* sp. V8, similarly to the standard killers *Saccharomyces cerevisiae,* had no effect on the *Aspergillus* fungi, whereas the strain *Streptomyces* sp. Ux308-1 had an antifungal effect on *A. terreus* S-1, *A. versicolor* MI-130 and *A. fumigatus* KO-5 with lysis zones of 5.8, 8.7 and 17.0 mm, respectively. Nystatin was shown to have strong activity against fungi from *Aspergillus* genus, their fungicidal zones ranged from 14.0 to 25.5 mm.

Table 1. **Fungicidal activity of bacterial strains against mycotoxin-producing fungi** (lysis zone, mm)

	Control	*Pantoea* sp.				*Streptomyces* sp.		*Sphingomonas* sp.
		Tx	T1x	T2x	T3x	Ux	Ux308	V8
Aspergillus flavus L-1								
X±S	15.0±0	10.0±0	10.0±0	7.0±0	7.0±0	0	0	0
Sx	0	0	0	0	0	0	0	0
CV(%)	0	0	0	0	0	0	0	0
Aspergillus terreus S-10								
X±S	14.0±0	10.3±2.3	10.7±2.5	8.7±2.0	8.7±2.1	0	5.8±0.7	0
Sx	0	0.9	1.0	0.8	0.8	0	0.3	0
CV(%)	0	22.3	23.3	22.9	24.1	0	12.1	0
Aspergillus versicolor MI-130								
X±S	25.5±0.5	8.6±1.5	11.5±3.0	7.8±1.8	9.6±3.3	0	8.7±2.2	0
Sx	0.2	0.6	1.2	0.7	1.5	0	0.9	0
CV(%)	1.9	17.4	26.0	23.1	34.4	0	25.3	0
Aspergillus fumigatus KO-5								
X±S	18.3±0.7	9.0±0	7.5±1.3	7.8±1.3	7.8±1.3	0	17.0±0.9	0
Sx	0.3	0	0.5	0.5	0.5	0	0.3	0
CV(%)	3.8	0	17.3	16.7	16.7	0	5.2	0
Penicillium verrucosum DB-1								
X±S	25.0±0	11.0±1.0	15.0	14.0±0	11.0±0	0	0	0
Sx	0	0.4	0	0	0	0	0	0
CV(%)	0	9.0	0	0	0	0	0	0
Penicillium cyclopium 21-AL								
X±S	29.0±0	0	9.3±0.8	9.0±2.2	11.0±2.9	9.0±1.9	9.1±2.0	13.0±1.5
Sx	0	0	0.3	0.9	1.2	0.8	0.8	0.6
CV(%)	0	0	8.6	24.4	26.4	21.1	21.9	11.5
Penicillium chrysogenum 48-L								
X±S	20.3±0.5	10.1±1.7	8.5±2.0	9.5±0.5	9.5±0.5	4.8±0.9	13.3±2.3	6.0±1.1
Sx	0.2	0.7	0.8	0.2	0.2	0.4	0.9	0.4
CV(%)	2.5	16.8	23.5	5.3	5.3	18.8	17.3	18.3
Fusarium poae RM-2								
X±S	24.3±0.5	10.5±0.5	14.7±0.5	10.3±0.5	11.3±0.5	10.0±0	10.7±0.5	15.0±0
Sx	0.2	0.2	0.2	0.2	0.2	0	0.2	0
CV(%)	2.0	4.7	3.4	4.8	4.4	0	4.7	0
Fusarium avenaceum RM-3								
X±S	23.7±1.2	10.3±0.5	10.7±0.5	9.8±0.4	10.0±0	12.0±0	8.0±0	18.7±0.5
Sx	0.4	0.2	0.2	0.2	0	0	0	0.2
CV(%)	5.1	4.8	4.7	4.1	0	0	0	2.7
Fusarium culmorum L-2								
X±S	28.8±0.7	9.7±1.9	9.7±1.9	10.0±2.6	10.0±2.6	16.7±2.2	23.1±2.3	11.3±1.5
Sx	0.3	0.8	0.8	1.1	1.1	0.9	0.9	0.6
CV(%)	2.4	19.6	19.6	26	26	13.2	9.9	13,3
Fusarium solani SA-4								
X±S	14.0±0	0	0	0	0	9.1±0.7	10.0±0.9	10.0±1.1
Sx	0	0	0	0	0	0.3	0.3	0.4
CV(%)	0	0	0	0	0	7.6	9	11
Alternaria alternata I-4								
X±S	24.0±0	10.0±0.8	8.7±0.8	7.5±1.8	7.5±1.7	9.0±2.1	17.2±2.1	9.8±0.4
Sx	0	0.3	0.3	0.7	0.7	0.8	0.8	0.1
CV(%)	0	8.0	9.1	24	22.7	23.3	12.2	4.1

Strains of *Pantoea citrea* also demonstrated a variable effect against fungi from *Penicillium* genus. Growth of *P. verrucosum* DB-1 and *P. chrysogenum* 48-L was suppressed by all *P. citrea* strains, and their fungicidal zones ranged from 8.5 to 15.0 mm. The fungus *P. cyclopium* 21-AL was not affected by the *P. citrea* Tx strain, whereas *P. verrucosum* DB-1 was not suppressed by *Streptomyces* sp. Ux, *Streptomyces* sp. Ux308-1 and *Sphingomonas* sp. V8. The highest activity against *P. verrucosum* DB-1 was manifested by *P. citrea* T1x (lysis zone – 15.0 mm), while the highest effect against *P. cyclopium* 21-AL and *P. chrysogenum* 48-L was mostly demonstrated by *Sphingomonas* sp., *Streptomyces* sp., Ux308-1 (zones –13.0 mm and 13.3 mm). The fungi from *Penicillium* genus were inhibited by nystatin; the lysis zones ranged from 20.3 to 29.0 mm.

It was determined that all *P. citrea* strains demonstrated a fungicidal activity against *Fusarium poae* RM-2, *F. avenaceum* RM-3 and *F. culmorum* L-2, however they did not affect *F. solani* SA-4. The fungi from *Fusarium* genus were inhibited by *Streptomyces* sp. Ux, *Streptomyces* sp. Ux308-1 and *Sphingomonas* sp. V8. The strongest fungicidal effect was observed in case of *F. culmorum* L-2; it was caused by *Streptomyces* sp. Ux308-1 (lysis zones – 23.1 mm). Nystatin was shown to have strong activity against fungi from *Fusarium* genus; their fungicidal zones ranged from 14.0 to 28.8 mm.

Growth of *Alternaria alternata* was negatively affected by all *Pantoea citrea* strains: the lysis zones from 7.5 to 10.0 mm were formed. The strongest activity on this fungus was exerted by *Streptomyces* sp. Ux308-1 with the fungicidal zone of 17.2 mm, whereas control (nystatin) formed the lysis zone of 24.0 mm.

The investigation showed that growth of micromycetes was also inhibited by essential oils (Fig. 1–3).

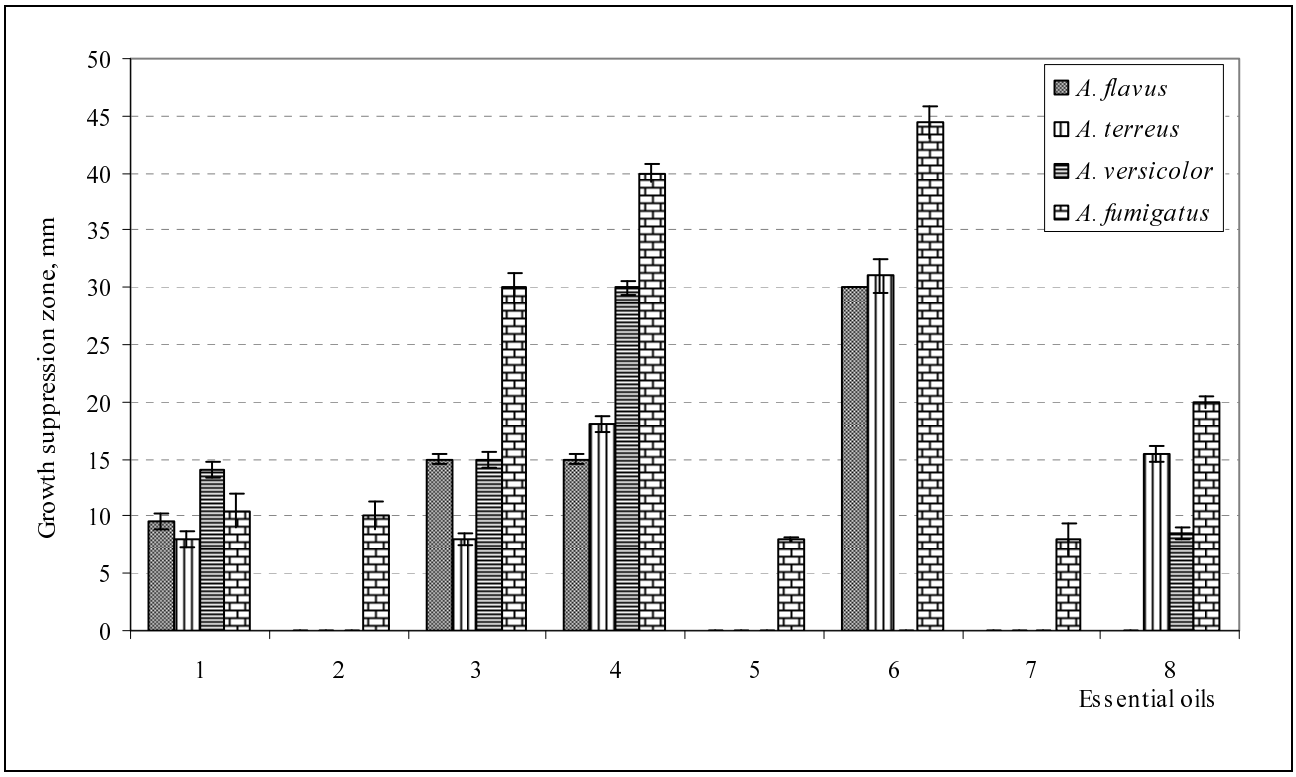

Fig. 1. **Effect of essential oils against *Aspergillus* genera fungi**

Essential oils: 1– *Abies sibirica*; 2– *Thymus pulegioides*; 3– *Carum carvi*; 4– *Pimpinella anisum*; 5– *Eucalyptus globulus*; 6– *Syzygium aromaticum*; 7– *Lavandula hybrida*; 8– *Melaleuca alternifolia*

The essential oil from *Abies sibirica* exhibited a fungicidal activity against fungi of *Aspergillus, Penicillium, Fusarium* and *Alternaria* genera. The strongest fungicidal effect was observed against *P. verrucosum* DB-1, *A. alternata* I-4U and *F. poae* RM-2 with lysis zones of 33.5, 24.1 and 22.0 mm, respectively. The weakest activity of this essential oil was observed against *Aspergillus terreus* S-1, *A. flavus* SK-1 and *P. chrysogenum* 48-L.

Essential oil of *Thymus pulegioides* did not affect *Aspergilus flavus* L-1, *A. terreus* S-1, *A. versicolor* MI-130 or *Penicillium chrysogenum* 48-L, whereas completely inhibited the growth of *P. verrucosum* L-2 and *Fusarium poae* RM-2.

Essential oils of both *Carum carvi* and *Pimpinella anisum* showed antifungal activity against all the studied micromycetes. The essential oil of *Carum carvi* fully inhibited *Penicillium verrucosum* DB-1, *Fusarium poae* RM-2 and *F. avenaceum* RM-3, while the essential oil of *Pimpinella anisum* completely suppressed growth of *Penicillium verrucosum* DB-1 and *Alternaria alternata* I-4.

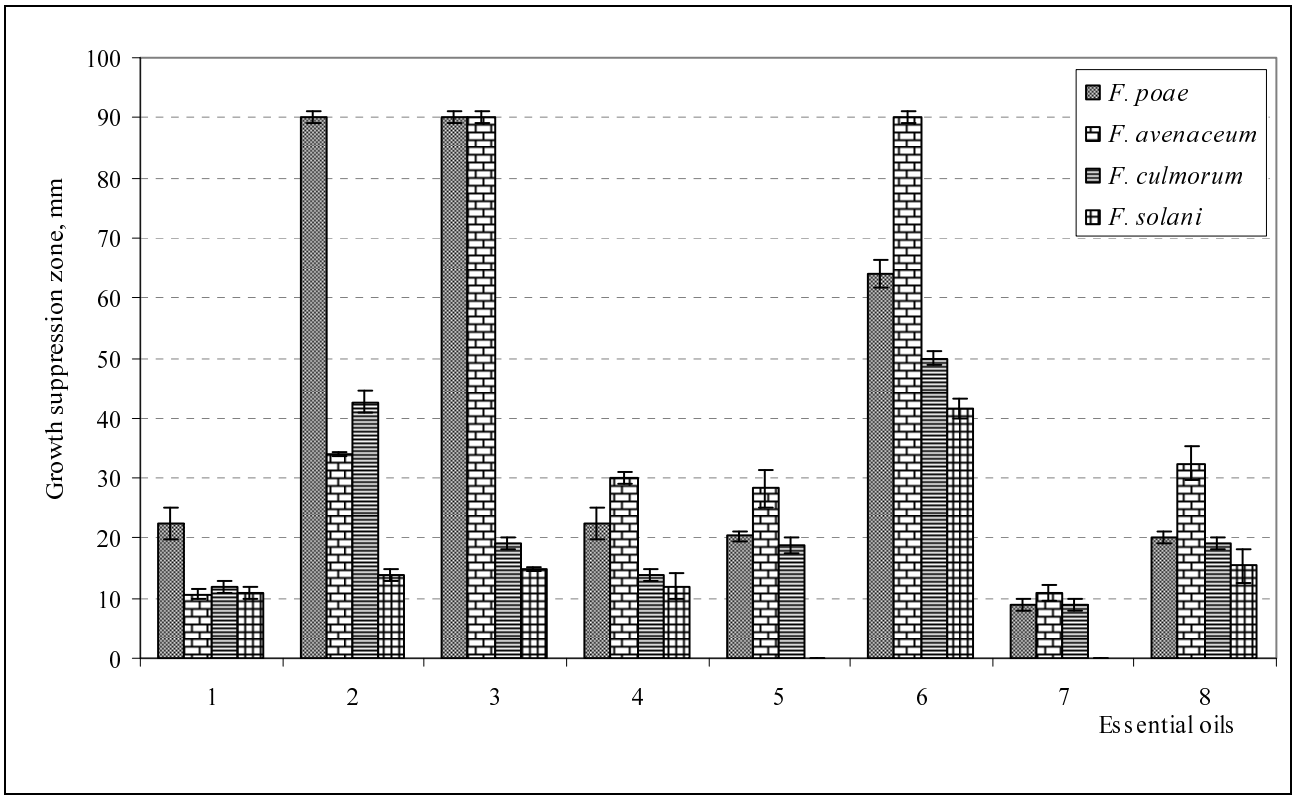

Fig. 2. **Effect of essential oils against *Fusarium* genera fungi**
Essential oils: 1– *Abies sibirica*; 2– *Thymus pulegioides*; 3– *Carum carvi*; 4– *Pimpinella anisum*; 5– *Eucalyptus globulus*; 6– *Syzygium aromaticum*; 7– *Lavandula hybrida*; 8– *Melaleuca alternifolia*

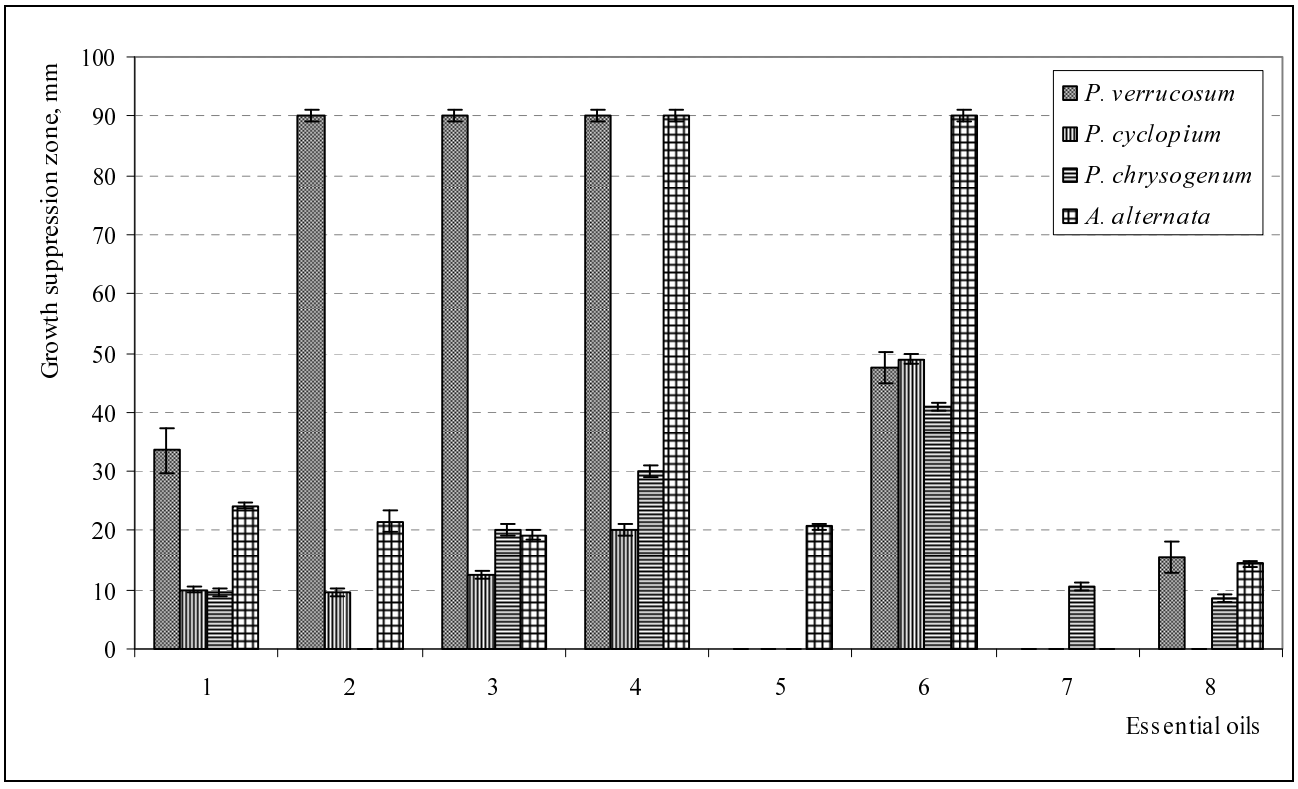

Fig. 3. **Effect of essential oils against *Penicillium and Alternaria* genera fungi**
Essential oils: 1– *Abies sibirica*; 2– *Thymus pulegioides*; 3– *Carum carvi*; 4– *Pimpinella anisum*; 5– *Eucalyptus globulus*; 6– *Syzygium aromaticum*; 7– *Lavandula hybrida*; 8– *Melaleuca alternifolia*

Neither *Eucalyptus globulus* nor *Lavandula hybrida* essential oils showed any effect on growth of *Aspergillus flavus* SK-1, *A. terreus* S-1, *A. versicolor* MI-130, *Penicillium verrucosum* DB-1, *P. cyclopium* 21-AL and *Fusarium solani* SA-4. No activity was demonstrated by essential oil of *Eucalyptus globulus* against *Penicillium chrysogenum* 48-L, and essential oil of *Lavandula hybrida* – against *Alternaria alternata* I-4U. On the other hand, *Eucalyptus globulus* strongly affected *Fusarium avenaceum* RM-3.

The essential oils of *Syzygium aromaticum* and *Melaleuca alternifolia* showed different effect on the fungi. Essential oil of *Syzygium aromaticum* did not affect *Aspergillus versicolor* MI-130 and that of *Melaleuca alternifolia* had no effect on *Penicillium cyclopium* 21-AL. Nevertheless, the essential oil of *Syzygium aromaticum* completely inhibited the growth of *Fusarium avenaceum* RM-3 and *Alternaria alternata* I-4.

The investigation showed that the effect of *Lavandula hybrida* essential oil was the weakest of all tested essential oils, even 83.3% of the fungi were resistant to this substance. It was revealed that the antifungal effect of *Syzygium aromaticum* essential oil was the strongest among all tested essential oils; even 92% of the fungi were susceptible towards its activity.

Discussion. The pilot study of screening bacteria with fungicidal activity showed that the bacteria from *Pantoea, Streptomyces* and *Sphingomonas* genera had a wide fungicidal activity against *Aspergillus flavus*, *A. terreus*, *A. versicolor*, *A. fumigatus*, *Penicillium verrucosum*, *P. cyclopium*, *P. chrysogenum*, *Fusarium poae, F. avenaceum, F. culmorum*, *F. solani*, and *A. alternata*.

The obtained results revealed that growth of *Aspergillus flavus* SK-1 was most efficiently inhibited by *Pantoea citrea* T1x and T3x strains, while *Streptomyces* sp. Ux and Ux308-1 as well as *Sphingomonas* V8 did not manifest fungicidal activity.

Some authors (Bueno et al., 2006; Palumbo et al., 2007; Zhang et al., 2008) have indicated that *Bacillus, Lactobacillus, Pseudomonas, Ralstonia* and *Burkholderia* can intensively suppress the growth of *Aspergillus* fungi. Reddy et al. (2009) reported that *B. subtilis, P. fluorescens* and *R. erythropolis* strongly inhibited the growth of *A. flavus.* Other studies show that lactic acid bacteria fungicidally affect *Aspergillus, Penicillium, Fusarium* and *Alternaria,* etc. (Lavermicocca et al., 2000; Schnürer and Magnusson, 2005). It has been indicated that *Bacillus pumilus* can also inhibit the growth of *Aspergillus flavus* (Sinha and Choudhary, 2008). S Kumar and K. Kannabiran (2010) demonstrated a fungicidal activity of *Streptomyces* spp. against *Aspergillus* strains resistant to pharmaceuticals.

Trichotecenes, zearalenones and other mycotoxins (nivalenole, HT-2 toxin, fumonisin) are produced by species from *Fusarium* genus: *F. graminearum, F. culmorum, F. poae* and *F. sporotrichoides,* etc. Fumonisin B_1 is one of most urgent worldwide problems encompassing agroeconomic as well as food and feed safety issues. Many researchers associate the solution of this problem with application of bacteria possessing fungicidal properties (Palumbo et al., 2007; Etcheverry et al., 2009).

Our investigation shows that all investigated strains of *Pantoea, Streptomyces* and *Sphingomonas* were characterized by fungicidal activity against *Fusarium poae* RM-2*, F. avenaceum* RM-3 and *F. culmorum* L-2, and formed lysis zones from 8.0 to 23.1 mm. It was established that *Pantoea citrea* strains had no influence on the growth of *F. solani* SA-4; whereas the strains of *Streptomyces* spp. and *Sphingomonas* sp. exerted antifungal activity with lysis zones of 9.1–10.0 mm.

Other studies have demonstrated that isolates of bacteria *B. amyloliquefaciens* and *Microbacterium oleovorans* slowed down the growth of *F. verticillioides* (Pereira et al., 2007). Khamna et al. (2009) indicated that *Streptomyces* sp. isolated from Thai medicinal plants had a strong activity against *Fusarium oxysporum, Penicillium digitatum, Alternaria brassicicola* and *A. porri*.

Our study showed that all *Pantoea citrea* strains demonstrated a fungicidal effect against *Aspergillus* and *Penicillium* fungi, with the exception of the strain *Pantoea citrea* Tx, which did not affect the growth of *P. cyclopium* 21-AL. The strains *Streptomyces* sp. Ux and *Sphingomonas* sp. V8 had no effect against *Aspergillus flavus* fungi and against *P. verrucosum* DB-1, while they negatively affected the growth of *P. cyclopium* 21-AL and *P. chrysogenum* 48-L with lysis zones ranging from 4.8 to 13.3 mm.

Recently, the essential oils as a measure against mycotoxin producers have drawn a great attention. About 3 000 essential oils are currently known, and 300 of them are applied in various fields (pharmacy, food industry, cosmetics and perfumery). Moreover, properties of plant essential oils have been widely investigated (Giordani et al., 2004; Pozzatti et al., 2010).

Our study revealed that *Aspergillus flavus* L-1*, A. fumigatus* KO-5 and *A. terreus* S-1 were most efficiently inhibited by essential oil of *Syzygium aromaticum,* whereas *A. versicolor* MI-130 – by essential oil of *Pimpinella anisum.* It was found that the essential oils of *Thymus pulegioides, Eucalyptus globulus* and *Lavandula hybrida* had no effect against *Aspergillus flavus* L-1*, A. versicolor* MI-130 and *A. terreus* S-1, and exerted weak activity against *A. fumigatus* KO-5. The essential oils produced diverse effect on *Penicillium* fungi. *P. verrucosum* L-2 was most strongly inhibited by essential oils of *Carum carvi* and *Pimpinella anisum*, while *P. cyclopium* 21-AL and *P. chrysogenum* 48-L – of *Syzygium aromaticum. Fusarium* fungi were most efficiently suppressed by the essential oils of *Thymus pulegioides, Carum carvi* and *Syzygium aromaticum,* whereas *Alternaria alternata* I-4U – by those of *Pimpinella anisum* and *Syzygium aromaticum.*

Research data indicates that essential oils inhibit the growth of mycotoxin-producing fungi (Soliman and Badeaa, 2002; Marin et al. 2004; Choudhary and Kumari, 2010). It was reported that essential oils of *Origanum vulgare, Aloysia triphylla, A. polystachya* and *Mentha piperita* were effective against *Fusarium verticillioides* (Lopez et al., 2004), essential oils of *Pimpinella anisum,*

Hedeoma multiflora, Syzygium aromaticum and *Lippia turbinate* var. *integrifolia* inhibited *Aspergillus* fungi from the section *Flavi* (Bluma and Etcheverry, 2008). Alpsoy (2010) indicated that essential oils of *Punica granatum* and *Zingiber officinale* suppressed the growth of *Aspergillus flavus,* a producer of aflatoxin, and its mycotoxin production.

Our present study revealed that *Aspergillus flavus* L-1, *A. fumigatus* KO-5 and *A. terreus* S-1 were most efficiently inhibited by essential oil of *Syzygium aromaticum,* whereas *A. versicolor* MI-130 – by essential oil of *Pimpinella anisum.* It was found that the essential oils of *Thymus pulegioides, Eucalyptus globulus* and *Lavandula hybrida* had no effect against *Aspergillus flavus* L-1, *A. versicolor* MI-130 and *A. terreus* S-1, and exerted weak activity against *A. fumigatus* KO-5. The essential oils produced diverse effect on *Penicillium* fungi. *P. verrucosum* L-2 was most strongly inhibited by essential oils of *Carum carvi* and *Pimpinella anisum*, while *P. cyclopium* 21-AL and *P. chrysogenum* 48-L – of *Syzygium aromaticum. Fusarium* fungi were most efficiently suppressed by the essential oils of *Thymus pulegioides, Carum carvi* and *Syzygium aromaticum,* whereas *Alternaria alternata* I-4U – by those of *Pimpinella anisum* and *Syzygium aromaticum.*

Valuable data on fungicidal activity of essential oils were presented by Mickienė et al. (2008), who indicated that *Thymus vulgaris* and *Mentha piperita* had a significant effect against micromycetes *Paecilomyces, Cladosporium, Fusarium* and *Aspergillus* spread in the air of a poultry farm. The study conducted by O. Motiejūnaitė and D. Pečiulytė (2004) demonstrated that according to their resistance towards *Pinus sylvestris* essential oil, microorganisms ranged as follows: micromycetes > yeast-like-fungi and yeasts > bacteria. The authors indicated that the lowest concentration of the oil inhibiting fungal growth was 2.5% in a medium. It was found out that *Thymus vulgaris* and *Melaleuca alternifolia* had a strong effect against *Trichophyton* dermatophytes (Shin and Lim, 2004). It was also shown that the minimal inhibiting concentration of *Eucalyptus globulus* was 0.25 mg ml^{-1} and that of *Melaleuca alternifolia* and *Thymus vulgaris* – 1 mg ml^{-1}.

Conclusion. The results revealed that the newly isolated bacterial strains of *Pantoea citrea*, *Streptomyces* sp. and *Sphingomonas* sp. demonstrated an intermediate fungicidal activity against *Aspergillus, Penicillium, Fusarium* and *Alternaria* fungi. The most significant fungicidal effect was characteristic of *Streptomyces* sp. Ux308-1 under which 8.33% of micromycetes were susceptible and 58.31% were of intermediate susceptibility.

The essential oils were characterized by a variable activity against the tested fungi, and the most efficient were the oils of *Syzygium aromaticum* and *Pimpinella anisum,* whereas the weakest effect was shown by *Lavandula hybrida* eesential oil. The essential oil of *Syzygium aromaticum* demonstrated a broad spectrum of the activity – even 91.63% of the micromycetes were susceptible.

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