

ENVIRONMENT PROTECTION

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APPLICATION OF PLANT ESSENTIAL OILS FOR IMPROVING AIR QUALITY IN THE LECTURE-HALLS OF UNIVERSITIES

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Abstract

Purpose: The general goal of the article is to analyze air contamination of lecturer-halls by microorganisms and to investigate antimicrobial activity of plant essential oils for improvement of ecological state of indoor microflora. **Methods:** For determination of the total number of microorganisms and assessment of air microflora contamination, Sedimentation method (Koch's Pour Plate method) was used. The colonies of microorganisms were calculated according to the V. Omelianskii rule. Statistical processing of data made on the Microsoft Office Excel (with adequacy 95%). Indicators of standard deviations were calculated by conventional procedures. The morphology of microorganisms was studied on microscope "Mikmed 2" (with zoom $\times 1500$). The impact of plant essential oils on chosen strains of microorganisms (*Staphylococcus aureus* UCM B-904, *Pseudomonas aeruginosa* UCM B-907, *Escherichia coli* UCM B-906) was estimated with the help of disc-diffusion method. **Results:** The round, white, yellow with a matte or shiny surface and with a smooth edge of the colonies of microorganisms were identified during the investigation. By the morphological features, microorganisms were gram-negative bacilli or gram-positive cocci. It has been established that the efficiency of plant essential oils appears in small concentrations and during of its increasing it changes insignificantly. In the research of antimicrobial activity of essential oils with the help of disco-diffusion method, it has been determined that the essential oils of lavender and eucalyptus have antimicrobial activity against to all the strains investigated. **Conclusions:** It has been investigated that investigated essential oils (lavender, eucalyptus, and juniper) inhibit the microorganisms to increase in the concentrations 5-15 mcl/m³ and can be used as the effective method of air treatment. Based on the research, the essential oils of lavender and eucalyptus can be recommended to use after classes in the concentrations of 5 mcl/m³.

Keywords: air microflora; antimicrobial activity; aromatherapy; biological activity; essential oils; phytoncide

1. Introduction

Nowadays, with the increase of population, there has been a number of important issues and problems such as providing comfortable conditions for education, work, recreation and other living conditions. Increasing of population result the spreading of infection and using of antibiotics – mutation of pathogenic microorganisms. People spend more than half of their lives indoors, staying in which can affect health very much. The buildings similar to artificial ecosystems have tendency to

accumulate pathogenic microorganisms, due to their insufficient ventilation, lighting, hygienic activities etc.

Air is the environment, which may contain a considerable number of microorganisms. However, it is less favorable place for them (in comparison with soil and water), in which they die because of influence of such factors: low level of humidity; action of solar radiation and temperature; lack of nutrients. There are many bacteria in public place, which crowded by people (train stations, cinemas,

educational institutions). First of all, it connects with fact that a person is an owner of a specific microflora. It is believed that each third person of our planet is the carrier of *Staphylococcus aureus*, which he can infect others [1-3].

The creation of health environment is the foundation for improving working ability, productivity of labor and studying. For these reasons, it is important to investigate and apply practically methods of reduction of air microflora contamination with the help of natural substances, which affect positively human overall health, immunity and well-being.

2. Analysis of the research and publication

One of the main discovery, which important for understanding bactericidal, fungicidal and others properties of plant essential oils and plant, was happen in 1928, when P.B. Tokin acknowledged bactericidal properties of plant selections. These vegetable selections was called phytoncids. Phytoncids are well known as biologically active substances released by plants and they have bactericidal, fungicidal and anti-protist properties [4]. Phytoncids cause various changes in microbial cells such as inhibit breathing, dissolve and destroy the surface layers and components of protoplasm (enzymes etc.).

Others important researches for our investigation related to study of microbiological contamination of indoor were happen in the 1940's and 1950's. Great interest to the problem of dissemination of nosocomial infections and development of modern instruments for air sampling led to systematic studies of the microbiological composition of hospital air, and subsequently to discovery of allergenic varieties of mold contained in the air of streets, residential and public buildings [4]. The study of occupational respiratory diseases among farmers, and workers in contact with malt and cotton was carried out in the 1950's and 1960's.

In 1959, it was first described a flash of wet fever similar to influenza, but more than a decade passed before another similar case was documented [5].

The first recognized cases of Legionnaires' disease occurred in Philadelphia, Pennsylvania (1976). Among more than 2000 attendees of an American Legion convention held at the Bellevue-Stratford Hotel, 182 attendees contracted the disease and 29 of them died [5].

In 1986 A. Grodzinsky and others published a monograph "Phytoncids in ergonomics", which

described the effects of various compositions of plant essential oils on the air environment in order to improve labor productivity [6]. Principles of research described in this monograph were taken as a basis for our studies.

3. The purpose and tasks of the work

The general goal of the article is to analyze air contamination of lecturer-halls by microorganisms and to investigate antimicrobial activity of plant essential oils for improvement of ecological state of indoor microflora.

The main tasks of our investigation were:

- to investigate the influence of different concentrations of plant essential oils (lavender, eucalyptus and juniper) on air microflora of lecturer-halls;
- to compare the influence of lavender, eucalyptus and juniper essential oils and their combination on the total amount of microorganisms;
- to determine antimicrobial activity of plant essential oils in accordance to define strains of microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, representatives of the most common cause of inflammatory diseases.

4. Materials and methods of the investigation

A. Essential oils

Three types of essential oils were used in this research such as: lavender, juniper and eucalyptus. All these oils were purchased at "Aromatika" firm.

Lavender essential oil is made from inflorescence lavender – *Lavandula angustifolia* Mill. in Latin (family – *Lamiacea*) by steam distillation method.

Juniper essential oils is made from pre-mash or dried cone-berries 2 years (blue color) common juniper – *Juniperus communis* L. in Latin (family – *Cupressaceae*) by steam distillation method [6].

Eucalyptus essential oil is made from eucalyptus ball – *Eucalyptus globulus* L. in Latine (family – *Myrtaceae*) by steam distillation method. Essential oil from various types of eucalyptus is usually subjected to additional distillation or rectification, obtaining various grades of oil with a lower (70-75%) and higher (80-85%) cineol content [6,7].

The estimation of plant essential oil concentration was calculated in accordance with manufacturer recommendations ("Aromatika" firm). The volume of lecture-hall investigated is 120 m³, and its space is 30 m². Inasmuch as 1 drop of essential oil is equal 0.06 ml = 60 µl, therefore for

treatment the room with the space 30 m² it is needed 600 µl. According to manufacturer recommendations, we added 10 ml of drops or 600 µl (0.6 ml) of essential oil to the aroma-lamp.

B. Test organisms

For this investigation, we took standard stains commonly found in the air microflora. The selected test organisms used to evaluate the antimicrobial activity of the essential oils were as follows: Gram positive (*Staphylococcus aureus* UCM B-904), and Gram negative (*Pseudomonas aeruginosa* UCM B-907, *Escherichia coli* UCM B-906), all purchased from the collection of Ukrainian Institute of Microbiology and Virology named by D.K. Zabolotnoho NAS of Ukraine.

During the experiment, we incubated culture of investigated strains in the incubator at 30 °C for 24 hours, afterwards suspended test-culture in sterile saline (NaCl 0,85%), making to a concentration of 0,5 CFU/ml. Characteristics of cultural properties of chosen microorganisms present in the table 1 [9].

D. Methods

The first part of investigation was carried out at the ecobiosafety laboratory of Scientific-Research Institute of National Aviation University. The assessment of air microflora contamination by microorganisms in the lecture-halls conducted by

determining the total number of bacteria in 1 m³ (CFU). Determination of the total number of microorganisms occurred in lecture-hall of university after classes in triple repetition for systematic reliability of the results.

The first part of investigation includes:

1. Sedimentation method (Koch's Pour Plate method) which involve settling of air microflora on the surface of the culture medium [10].

2. The method of direct counting colonies by V. Omelianskii rule, according to which the number of microorganisms, there are in 10 liters, sediments on the area of 100 cm² for 5 minutes.

Statistical processing of data made on the Microsoft Office Exel (with adequacy 95%). Indicators of standard deviations were calculated by conventional procedures. The morphology of microorganisms was studied on microscope "Mikmed 2" (with zoom × 1500).

The second part of investigation was carried out at the Institute of Microbiology and Virology D.K. Zabolotnoho NAS of Ukraine where we got microorganisms strains for our research.

3. Disc-diffusion method of investigating air microflora, which is widely used for epidemiological control resistance. The sensitivity of microorganisms to different substances identify only in pure culture.

Table 1

Characteristics of cultural properties of test microorganisms

Test microorganisms	For growing in dense culture medium		For growing in liquid culture medium		For microscoping study of smear agar culture, painted by Gram method
	conditions of cultivation	description of culture	conditions of cultivation	description of culture	
<i>Staphylococcus aureus</i>	Medium № 1, T° (36 ± 1) °C, 18-20 hours, then at room temperature 24 hours	Smooth colonies with smooth edges, with a uniform golden pigmentation	Nutrient borth, pH 7,2- 7,4, 18-20 hours	Uniform turbidity of broth without pellicle and mucous sediment at the bottom	Uniform in size and location in the form of cocci bunch with well-expressed gram positive color
<i>Pseudomonas aeruginosa</i>	Nutrient agar, pH 7,2-7,4, T° (36 ± 1) °C, 18-20 hours	The broad, vague, transparent colonies with ragged edge	Nutrient borth, pH 7,2-7,4 18-20 hours	A noticeable turbidity, thick pellicle, medium can take the yellow-green color	Single sticks or short chains with well-expressed gram negative color
<i>Escherichia coli</i>	Medium №1, T°(36 ± 1) °C, 18-20 hours	Small gray-blue colonies which are easily merge together	Nutrient borth, pH 7,2-7,4, 18-20 hours	A significant turbidity of the medium, a small sediment, pellicle on the surface of the borth is usually absent	Single flat red colonies medium-sized, dark metallic luster and with well-expressed gram negative color

5. Results and discussion

Investigation the effect of plant essential oils on the total number of microorganisms in the air of the interior premises and study their antimicrobial properties were carried out at the ecobiosafety laboratory of Scientific-Research Institute of National Aviation University and the Institute of Microbiology and Virology D.K Zabolotnoho NAS of Ukraine where we got microorganisms strains for our research. All investigations were repeated three times and were conducted statistical analysis of taken results by conventional methods.

With the help of sedimentation method in the air educational multimedia audience of university after classes, we determined the total number of microorganisms in colony forming units (CFU) and the impact on this indicator of plant essential oils. According to experimental research were identified that investigated essential oils exhibit fungicidal properties.

There wasn't determined any fungi growth in any Petri glasses were treated by essential oil vapors. It

was determined that all essential oils are inhibit the microorganisms growth at use concentrations of 5; 10; 12,5 and 15 mcl per 1 m³ of air. The most effective was lavender and eucalyptus essential oils, the least effective was juniper (Table 2, Figure 1). Changes of antimicrobial effect with the increasing of lavender essential oil concentration in the air environment of the investigated audience are represented. The total number of microorganisms in comparison with control was decreased in 4 times per 1 m³ of air at a minimum concentration of 5 mcl/m³ (control - 191 000 CFU/m³, taken results - 51 000 CFU/m³) after treatment the audience by lavender essential oil evaporation.

The number of microorganisms after eucalyptus essential oil treatment was decreased in 3 times. The least effective was juniper (166 000 CFU/m³). The average number of colony forming units in the result of processing at concentrations of 5 and 10 mcl/m³ according to calculation was 115 000, which near 1.7 times less than in the control.

Table 2

Numerical indicators of microorganisms after air treating by plant essential oil vapor

Concentration of essential oil	The number of microorganisms, CFU/m ³ (% from control)				
	Control	Juniper	Composition	Eucalyptus	Lavender
5 mcl/m ³	3275	2855 (13%)	1976 (40%)	1098 (66%)	878 (73%)
10 mcl/m ³	3270	2800 (14%)	1900 (42%)	1000 (67%)	850 (74%)
12,5 mcl/m ³	3108	2650 (15%)	1800 (42%)	987 (68%)	800 (74%)
15 mcl/m ³	3300	2500 (24%)	1756 (47%)	950 (71%)	730 (78%)

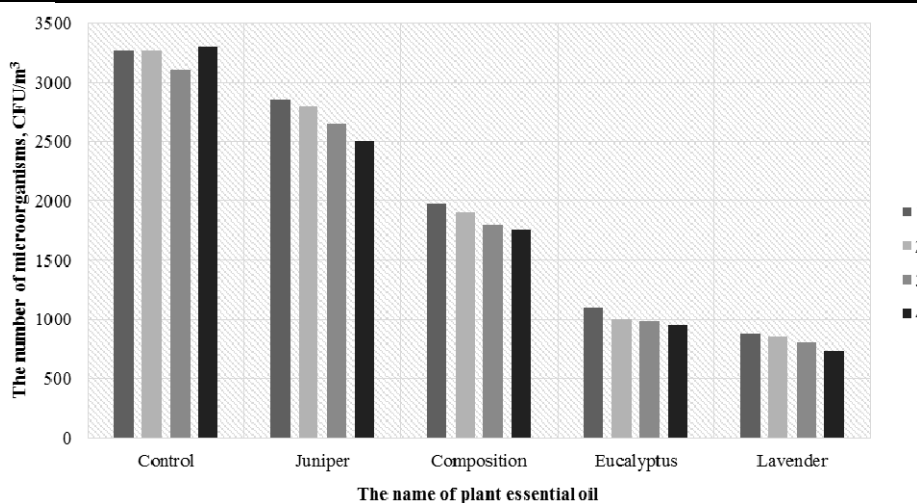


Fig. 1. Antimicrobial properties of investigated essential oils: 1–5 mcl/m³ concentration of essential oil; 2–10 mcl/m³ concentration of essential oil; 3–12.5 mcl/m³ concentration of essential oil; 4–15 mcl/m³ concentration of essential oil

This may indicate antagonism, which means that the impact of some components of essential oils could lead to a weakening of the antimicrobial properties of others.

Since the composition of three essential oils not proved sufficiently effective in the second stage of the experiment, in determining the antimicrobial activity of essential oils it was decided not to use. According to cultural features, all selected colonies of microorganisms were round, white or yellow with a matte or glossy and straight edge. By the morphological features, microorganisms were gram-negative bacilli or gram-positive cocci. The result of investigation of antibacterial properties is shown in the table 3.

Table 3

Antimicrobial activity of plant essential oils, determined by disc-diffusion method

Test microorganisms	Diameter of inhibition zone (mean value), mm		
	Lavender	Eucalyptus	Juniper
<i>S. aureus</i>	20	10	0
<i>E. coli</i>	15	11	0
<i>P. aeruginosa</i>	4	5	1

According to these results, we may conclude that the essential oils of lavender and eucalyptus have antimicrobial activity against to all the strains investigated. The highest antimicrobial activity was observed in relation to *Staphylococcus aureus*, and less to gram-negative bacteria.

6. Conclusions

It has been investigated that plant essential oils affect on the total number of microorganisms in the air microflora of an lecture-hall and have abilities to antimicrobial activity for separate species of microorganisms.

It has been determined that investigated essential oils (lavender, eucalyptus, and juniper) inhibit the microorganisms to increase. The most efficient essential oils were lavender and eucalyptus, the least effective was juniper.

It was established that all studied essential oils show fungicidal properties.

In the research of antimicrobial activity of essential oils with the help of disco-diffusion method, it has been determined that the essential oils of lavender and eucalyptus have antimicrobial activity against to all the strains investigated: *Staphylococcus aureus* UCM B-904, *Pseudomonas*

aeruginosa UCM B-907, *Escherichia coli* UCM B-906, representatives of the most common cause of inflammatory diseases.

The highest antimicrobial activity was observed in relation to *Staphylococcus aureus*, and less to gram-negative bacteria.

Based on the research, recommendations on practical application of taking results for treatment and recovery of air at lecture-hall with the implementation of plant essential oils, as effective prevention of seasonal diseases were developed.

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Застосування рослинних ефірних олій для покращення якості повітря аудиторій університету

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Мета: Аналіз забруднення повітря мікроорганізмами в навчальних та дослідження антимікробної активності рослинних ефірних олій для покращення екологічного стану аудиторій. **Методи дослідження:** Для встановлення загальної кількості мікроорганізмів та оцінки забруднення повітря було використано Седиментаційний метод (чашковий метод Коха). Колонії мікроорганізмів підраховувалися за правилом В.Л. Омелянського. Статистичну обробку даних проводили за допомогою програмних пакетів Microsoft Office Excel з достовірністю 95%. Показники стандартних відхилень обчислювали за загальноприйнятими формулами. Морфологію клітин вивчали на мікроскопі «Мікмед-2» (збільшення $\times 1500$). Вплив рослинних ефірних олій на обрані штами мікроорганізмів (*Staphylococcus aureus* УКМ Б-904, *Pseudomonas aeruginosa* УКМ Б-907, *Escherichia coli* УКМ Б-906) оцінювали за допомогою диско-дифузійного методу. **Результати:** Під час досліду були виділені круглі, білі, жовті з матовою або блискучою поверхнею та рівним краєм колонії мікроорганізмів. За морфологічними ознаками мікроорганізми виявилися грамнегативними паличками або грампозитивними коками. Було встановлено, що ефективність рослинних ефірних олій проявляється у малих концентраціях і змінюється не суттєво при її збільшенні. При вивченні антимікробної активності ефірних олій диско-дифузійним методом визначено, що ефірні олії лаванди та евкаліпту мали антибактеріальну активність по відношенні до всіх досліджуваних штамів. **Висновки:** Досліджено, що рослинні ефірні олії (ялівцева, лавандова, евкаекаліп та їх композиція) зменшує кількість мікроорганізмів в повітрі навчальних аудиторій в концентраціях 5-15 мкм/м³ і можуть бути ефективним засобом оздоровлення мікрофлори повітря приміщень. За результатами досліджень можна рекомендувати використовувати рослинні ефірні олії лаванди та евалкапту в концентраціях 5 мкл/м³ повітря після занять.

Ключові слова: Антимікробні властивості; ароматерапія; біологічна активність; ефірні олії; мікрофлора повітря; фітонциди рослин

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Применение растительных эфирных масел для улучшения качества воздуха аудиторий университета

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Цель: Анализ загрязнения воздуха микроорганизмами в учебных заведениях и исследование антимикробной активности растительных эфирных масел для улучшения экологического состояния аудиторий. **Методы исследования:** Для установления общего количества микроорганизмов и оценки

загрязнения воздуха было использовано Седиментационный метод (чашечный метод Коха). Колонии микроорганизмов подсчитывали по правилу В.Л. Омелянского. Статистическую обработку данных проводили с помощью программных пакетов Microsoft Office Excel с достоверностью 95%. Показатели стандартных отклонений рассчитывали по общепринятым формулам. Морфологию клеток изучали на микроскопе «Микмед-2» (увеличение $\times 1500$). Влияние растительных эфирных масел на выбранные штампы микроорганизмов (*Staphylococcus aureus* УКМ Б-904, *Pseudomonas aeruginosa* УКМ Б-907, *Escherichia coli* УКМ Б-906) оценивали с помощью диско-диффузного метода.

Результаты: Во время исследования были выделены круглые, белые, желтые с матовой или блестящей поверхностью и ровным краем колонии микроорганизмов. За морфологическими признаками микроорганизмы оказались грамнегативными палочками и грампозитивными коками. Было установлено, что эффективность растительных эфирных масел проявляется в малых концентрациях и меняется не существенно при её изменении. При изучении антимикробной активности эфирных масел диско-диффузным методом определено, что эфирные масла лаванды и эвкалипта имели антибактериальную активность по отношению ко всем исследуемым штаммам.

Выводы: Исследовано, что растительные эфирные масла (можжевельника, лаванды, эвкалипта и их композиции) уменьшает количество микроорганизмов в воздухе учебных аудиторий в концентрациях 5-15 мкм/м³ и могут быть эффективным средством оздоровления микрофлоры воздуха помещений. За результатами исследований можно рекомендовать использовать растительные эфирные масла лаванды и эвкалипта в концентрациях 5 мкл/м³ воздуха после занятий.

Ключевые слова: антимикробные свойства; ароматерапия; биологическая активность; эфирные масла; микрофлора воздуха; фитонциды растений

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