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P.P. Zelena[1], Yu.M. Yumyna[1], I.V. Dombrovska[1], I.M. Malynovska[2], V.V. Shepelevych[1], L.M. Grytsenko[1], N.V. Yavorska[1], V.K. Pozur[1], L.M. Skivka[1]

[1]Educational and Scientific Centre "Institute of Biology" of Taras Shevchenko National University of Kyiv, 64/13, Volodymyrska Str., Kyiv, Ukraine, 01601,
Phone +380679988064 ; Fax +380445253841; e-mail: realmed@i.com.ua
[2]National research centre "Institute of agriculture of the National Academy of Agrarian Sciences of Ukraine, 2-b, Mashynobudivnykiv str., Chabany, Kyevo-Svyatoshunskyi district, Kyiv region, Ukraine, 08162

EFFECT OF ESSENTIAL OILS OF *ANIBA ROSAEODORA*, *LAURUS NOBILIS*, *SYZYGIUM AROMATICUM*, *MENTHA PIPERITA* AND *LAVANDULA ANGUSTIFOLIA* ON GROWTH OF MICROMYCETES

Aim: *to investigate the effect of essential oils (EOs) of rosewood Aniba rosaeodora Ducke, laurel Laurus nobilis L, clove Syzygium aromaticum (L.) Merr. &L.M. Perry, mint Mentha piperita L. and lavender Lavandula angustifolia Mill. on the mycelial growth and spore germination of Alternaria alternata (Fr.) Kiessl, Aspergillus ochraceus G. Wilh. and Penicillium chrysogenum Thom was performed.* ***Methods:*** *tested fungi were isolated from contaminated carrot seeds, stored tomatos and spikelets of wheat. Agar diffusion method and modified agar diffusion and vapor assay were used.* ***Results:*** *EOs of rosewood, clove and laurel exerted a potent dose dependent inhibitory effect on mycelial growth of tested fungi. Mycelium of micromycetes was more sensitive to the fungistatic action of EOs than their conidia. This EO at the concentrations of 1.5µl/ml caused prolonged total inhibition of mycelial growth and spore germination of all tested fungi.* ***Conclusion:*** *these findings support the potential use of EO of Aniba rosaeodora as well as EOs of Laurus nobilis and Syzygium aromaticum for natural food protection against mold infestation.*

K e y w o r d s : essential oil, antimicrobial activity, micromycetes.

In spite of the introduction of new antifungal drugs, they are limited in number. The increase of fungal resistance to classical drugs justify the search for new strategies. Essential oils are one of the most promising groups of natural compounds from which a new prototype of antifungal agents may be developed. The antifungal activity of plant essential oils (EOs) are commonly used in medicine [9]. Relatively new fields of application for EO antifungal effect are stored product protection against mold infestation and post harvest preservation of fruits and vegetables [5, 8, 10]. The possibility of application of fungicidal and fungistatic potentials of EOs in agriculture and food industry is being extensively investigated [1, 2]. Particularly, EOs of Mentha piperita and Lavandula angustifolia showed strong antifungal activity

against post-harvest phytopathogenic fungi [3]. Syzygium aromaticum EO exibited potential antifungal capacity against fungi causing spoilage of bakery products [4]. Antifungal activity of many EOs with broad spectrum of biological effects such as EOs from the plants belonging to Lauraceae family, is vitrually not studied [11, 13]. The application of EOs as medicinal agents or food preservatives requires detailed knowledge about their properties, i.e., the minimum inhibitory concentration (MIC), the range of target organisms etc.

The purpose of this paper was to investigate the effect of EOs of *Aniba rosaeodora* Ducke*, Laurus nobilis* L*, Syzygium aromaticum* (L.) Merr. &L.M. Perry*, Mentha piperita* L. *and Lavandula angustifolia* Mill. on mycelial growth and spore germination of *Alt. alternata* (Fr.) Kiessl*, A. ochraceus* G. Wilh. and *P.chrysogenum* Thom.

Materials and Methods

Alternaria alternata 37/8, *Aspergillus ochraceus* 5/7, *Penicillium chrysogenum* 10/3 were stock cultures of the Department of Microbiology and Genegal Immunology of the ESC "Institute of Biology" of Taras Shevchenko National University of Kyiv. Tested fungi were isolated from contaminated carrot seeds, stored tomatos and spikelets of wheat.

We have used EO of *Aniba rosaeodora* Ducke obtained from "Aromatika", (Ether-oil seed plant, Alushta, Ukraine) and EOs of *Laurus nobilis* L., *Syzygium aromaticum* (L.) Merr. &L.M. Perry, *Mentha piperita* L., *Lavandula angustifolia* Mill. obtained from PE firm "Nikitskiy sad" (Yalta, Ukraine) in our experiments.

A.alternata, A.ochraceus and *P. chrysogenum* have been precultured on PDA slant at 28 °C for 14 days.

The assessment of mycelia growth was performed as it was described by Marinelli et al., 2012 [7]. The EOs were dissolved in Tween 80 and then incorporated in the PDA at 0.5; 1.0; 1.5; 3.0; 5.0; 10.0 µl/ml (the concentration of Tween 80 did not exceed 0.1%), were vigorously agitated and poured sterilized Petri dishes. Growth medium for control probes contained 0.1% of Tween 80. PDA with EO as well as control PDA were inoculated with 6 mm plugs from fungi cultures (7 days old). The Petri dishes were subsequently sealed with the use of parafilm and incubated at 28 °C. The fungi growth was recorded after 6, 15 and 21 days. Radial growth of tested fungi was evaluated by the easurement of colony diameter. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control probes in which colonia diameter was 90 mm using the Abbott's formula: $T= (D_k – D_e)/ D_k \times 100$, where D_k – an average diameter of fungal colony from control samples, D_e – an average diameter of fungal colony from treatment samples [14].

The effect of EO on spore germination was tested in PDA with the use of agar diffusion and vapor assay as it was described earlier with some modifications [6]. Conidia were taken from the slants with the use of sterile saline contained of 0.05% Tween 80. Mycelia were removed by filtration through sterile gauze. One ml of adjusted to $1x10^8$ conidia/ml conidial suspension was added to 100 ml of agar medium containing 1% peptone, 1% glucose, 1% agarose at 50 °C. EOs at the volume of 10, 20, 30, 60, 100 and 200 µl were aseptically pipetted onto sterile 6-mm paper discs

(Becton Dickinson). The EO impregnated paper discs were then aseptically placed in the center of the Petri dish with PDA (20 ml). Agar media containing conidia (10 ml) was then overlaid on the surface of PDA. The Petri dishes were subsequently sealed with the use of parafilm and incubated at 28°. The diameter of inhibition zone was recorded after 6, 14 and 21 days of culturing as well as after 6 month.

All the experiments were performed in quadruplicate. Each experiment was repeated four times. The data are presented as M± SD.

Results and discussion

EOs exerted dose dependent inhibitory effect on mycelial growth of Alt. alternata, *A.ochraceus* and *P. chrysogenum* as it was determined by the agar dilution method (Table 1). EOs of mint and lavender demonstrated weak fungistatic activity towards Alt. alternata and *P. chrysogenum* only at the concentrations of ≥10 μl/ml. EOs of laurel and cloves exerted inhibitory effect for 17 days when they were applied at the concentrations of ≥ 1.5 μl/ml. The most potent fungistatic activity was registered for rosewod EO.

Micromycetes had distinct sensitivity to EOs. Mycelial growth of Alt. alternata and *A. ochraceus* was more sensitive to inhibitory effect of rosewood oil than that of *P. chrysogenum*. *A. ochraceus* was sensitive only to the impact of rosewood and clove EOs. Sessou P. et al. have also demonstrated high fungistatic activity of clove EO against fungal isolates from foodstuff and they regard this EO as the most promising agent to be used as additive in substitution of synthetic chemicals ones to extend shelf life time of cheese [12].

The inhibitory effect of EOs on mycelial growth of tested fungi was weakening with the course of time. Fungistatic effect of EOs of clove, mint and lavender after 15 days of cultivation was 2–2.5 times lower than that after 6 days of cultivation. The inhibitory effect of EOs of rosewood and laurel was more stable over time. Inhibitory effect of rosewood EO was the most prolonged (up to 24 days). However, the effect of this EO used at the concentrations of 0,5 μl/ml and 1,0 μl/ml after 21 days of cultivation was at the average 1.8 times lower than that after 15-day treatment. The mycelial growth of all fungi was inhibited 100% when treated with rosewood EO at the concentration of 1.5 μl/ml independently of the duration of cultivation.

The numerous literature data indicate that EOs are highly effective in vapor phase against micromycetes [4, 6, 15]. Modified agar diffusion and vapor method was developed to minimize the evaporation of volatile fraction of EOs, which was pipetted onto a paper disc. Being applied to the surface of a paper disc EOs of rosewood, laurel and clove inhibited spore germination of all tested fungi (Table 2). The most expressed fungistatic activity was registered for *Aniba rosaeodora* EO. The inhibitory effect of this EO on spore germination detected by modified agar diffusion and vapor method was comparable to those on mycelial growth of tested fungi. Rosewood oil (total volume 30 μl) hampered spore germination of *P. chrysogenum* up to the fourteenth day of cultivation, *A. ochraceus* – up to the 21-th day of cultivation. We did not observe spore germination of Alt. alternata treated with roseewood EO (total volume 30 μl) throughout 6 months.

Table 1

The effect of essential oils of *Aniba rosaeodora*, *Laurus nobilis, Syzygium aromaticum, Mentha piperita, Lavandula angustifolia* on mycelial growth of *Alt. alternata, A. ochraceus* and *P. chrysogenum* determined by an agar dilution assay

Microorganism	Mycelial growth inhibition (%)														
	**Aniba rosaeodora* essential oil (µl/ml)			***Laurus nobilis* essential oil (µl/ml)			***Syzygium aromaticum* essential oil (µl/ml)			****Mentha piperita* essential oil (µl/ml)			****Lavandula angustifolia* essential oil (µl/ml)		
	0.5	1.0	1.5	1.5	3.0	5.0	1.5	3.0	5.0	3	5	10.0	3	5	10.0
	After 6 days of cultivation														
Alt. alternata	100	100	100	0	25.4±2.5	79.5±3.5	0	0	21.3±1.9	0	0	40.5±4.3	0	0	0
A. ochraceus	100	100	100	0	0	30.2±1.6	30.8±2.4	67.8±3.6	80.4±3.7	0	0	0	0	0	0
P. chrysogenum	34.6±1.1	100	100	0	44.7±3.6	81.6±4.5	21.3±1.5	54.9±2.3	72.1±2.2	0	0	36.9±2.8	0	0	56.4±3.6
	After 15 days of cultivation														
Alt. alternata	69.7±4.1	81.3±2.6	100	0	14.6±1.8	52.9±2.8	0	0	0	0	0	18.2±2.0	0	0	0
A. ochraceus	70.0±4.6	90.1±3.3	100	0	0	26.2±1.1	16.4±0.9	20.6±2.4	32.2±2.4	0	0	0	0	0	0
P. chrysogenum	25.6±1.5	83.3±4.1	100	0	0	77.3±3.2	16.5±1.8	18.3±2.9	35.4±2.8	0	0	18.4±1.8	0	0	31.2±3.2

Notes: tested micromycetes were cultured on PDA at 28 °C.

*- fungistatic effect of rosewood EO was registered up to 23 days of cultivation.

** – fungistatic effect of these EOs used at the concentrations < 1.5 µl/ml was absent. The inhibitory action leasted for 17 days and has ceased then.

*** – fungistatic effect of these EOs used at the concentrations < 3.0 µl/ml was absent. The inhibitory action leasted for 17 days and has ceased then.

Table 2

The effect of essential oils of *Aniba rosaeodora*, *Laurus nobilis*, *Syzygium aromaticum* on spore germination of *Alt. alternata, A. ochraceus* and *P. chrysogenum* determined by modified agar diffusion and vapor assay

Microorganism	Inhibition zone (mm)								
	Aniba rosaeodora essential oil (µl)			*Laurus nobilis*** essential oil (µl)			*Syzygium aromaticum*** essential oil (µl)		
	10	20	30	20	60	100	20	60	100
Alt. alternata	55.0±1.5	TI*	TI	0	20.0±1.0	47.6±0.5	0	0	15.1±0.6
A. ochraceus	TI	TI	TI	0	0	20.3±0.5	18.2±0.4	45.0±0.7	50.3±0.5
P. chrysogenum	27.0±2.0	55.0±1.0	TI	0	34.5±0.8	40.0±0.6	19.0±2.0	33.4±0.4	49.5±0.5

Notes: confluent growth was registered on the control untreated Petri dishes (diameter 90 mm).
* – TI – total inhibition
** – inhibitory effect of these EOs applied to the surface of paper disc at the volume of < 20 µl was absent.

Conclusion

Thus, EOs of rosewood, laurel and clove inhibited mycelial growth of Alt. alternata, *A. ochraceus* and *P. chrysogenum* that were isolated from contaminated carrot seeds, stored tomatos and spikelets of wheat. Significant inhibition of spore germination of tested fungi was observed in the probes treated with rosewood EO. Moderate inhibitory effect on spore germination was also registered for EOs of clove and laurel. Conidia of Alt. alternata and *A. ochraceus* were less sensitive to EOs than vegetative tissues of these fungi. The most potent fungistatic effect was registered for *Aniba rosaeodora* EO. Our findings support the potential use of essential oils of *Aniba rosaeodora*, *Laurus nobilis* and *Syzygium aromaticum* for natural food protection against mold infestation.

П.П. Зелена[1], Ю.М. Юмина[1], І.В. Домбровська[1], І.М. Малиновська[2], В.В. Шепелевич[1], Л.М. Гриценко[1], Н.В. Яворська[1], В.К. Позур[1], Л.М. Сківка[1]

[1] ННЦ «Інститут біології» Київського національного університету імені Тараса Шевченка, вул. Володимирська, 64/13, Київ, Україна, 01601
[2] Національний науковий центр «Інститут землеробства НААН України», вул. Машинобудівників, 2-б, смт Чабани, Києво-Святошинський район, Київська обл., 08162, Україна

ВПЛИВ ЕФІРНИХ ОЛІЙ *ANIBA ROSAEODORA, LAURUS NOBILIS, SYZYGIUM AROMATICUM, MENTHA PIPERITA* ТА *LAVANDULA ANGUSTIFOLIA* НА РІСТ МІКРОМІЦЕТІВ

Реферат

Мета: *дослідити вплив ефірних олій (ЕО) трояндового дерева Aniba rosaeodora Ducke, лавра Laurus nobilis L, гвоздики Syzygium aromaticum (L.) Merr. &L.M. Perry,*

м'яти Mentha piperita L. та лаванди Lavandula angustifolia Mill. на ріст міцелію та проростання спор Alternaria alternata (Fr.) Kiessl, Aspergillus ochraceus G. Wilh. and Penicillium chrysogenum Thom. **Методи:** *гриби виділені з інфікованого насіння моркви, зіпсованої плодоовочевої продукції (томати), закладеної на зберігання, та колосся пшениці. У дослідженнях застосовували метод серійних розведень у щільному поживному середовищі та модифікований метод паперових дисків. Результати: ЕО трояндового дерева, лавра та гвоздики справляли сильний дозозалежний гальмівний вплив на ріст міцелію тестованих грибів. Міцелій грибів був більш чутливим до фунгістатичної дії ЕО, ніж їх спори. ЕО трояндового дерева у концентрації 1,5 мкг/мл спричиняла довготривале повне інгібування росту міцелію та проростання спор усіх тестованих грибів.* **Висновок:** *отримані результати свідчать на користь можливості застосування ЕО Aniba rosaeodora, а також ЕО Laurus nobilis та Syzygium aromaticum як природного засобу захисту продуктів харчування від зараження цвільовими грибами.*

К л ю ч о в і с л о в а : ефірні олії, антимікробна активність, мікроміцети.

П.П. Зеленая[1], Ю.М. Юмина[1], И.В. Домбровская[1], И.М. Малиновская[1], В.В. Шепелевич[1], Л.М. Гриценко[1], Н.В. Яворская[1], В.К. Позур[1], Л.М. Скивка[1]

[1]УНЦ "Институт биологии" Киевского национального университета имени Тараса Шевченко ул. Владимирская, 64/13, Киев, Украина, 01601

[2] Национальный научный центр «Институт земледелия НААН Украины», ул. Машиностроителей, 2-б, пгт Чабаны, Киево-Святошинский район, Киевская обл., 08162, Украина

ВЛИЯНИЕ ЭФИРНЫХ МАСЕЛ *ANIBA ROSAEODORA, LAURUS NOBILIS*, *SYZYGIUM AROMATICUM*, *MENTHA PIPERITA* И *LAVANDULA ANGUSTIFOLIA* НА РОСТ МИКРОМИЦЕТОВ

Реферат

Цель: *исследовать влияние эфирных масел (ЭМ) розового дерева Aniba rosaeodora Ducke, лавра Laurus nobilis L, гвоздики Syzygium aromaticum (L.) Merr. &L.M. Perry, мяты Mentha piperita L. и лаванды Lavandula angustifolia Mill. на рост мицелия и прорастание спор грибов Alternaria alternata (Fr.) Kiessl, Aspergillus ochraceus G. Wilh. и Penicillium chrysogenum Thom. Методы: грибы выделены из инфицированных семян моркови, испорченной плодоовощной продукции (томаты), заложенной на хранение, и колосьев пшеницы. В исследованиях применяли метод серийных разведений в плотной питательной среде и модифицированный метод бумажных дисков. Результаты: ЭМ розового дерева, лавра и гвоздики оказывали сильное ингибиторное действие на рост мицелия тестируемых грибов. Мицелий грибов был более чувствителен к фунгистатическому действию ЭМ, чем их споры. ЭМ розового дерева в концентрации 1,5 мкг/мл вызывало продолжительное тотальное угнетение роста мицелия и прорастания спор всех тестированных грибов.* **Вывод:** *полученные результаты свидетельствуют в пользу возможности применения ЭМ Aniba rosaeodora, а также ЭМ Laurus nobilis и Syzygium aromaticum в качестве природного средства защиты продуктов питания от заражения плесневыми грибами.*

К л ю ч е в ы е с л о в а **:** *эфирные масла, антимикробная активность, микромицеты.*

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