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Evaluation of fungistatic properties of selected essential oils obtained from the *Lamiaceae* family plants

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Abstract: Evaluation of fungistatic properties of selected essential oils obtained from plants from the Lamiaceae spp. The paper presents the results of research on fungistatic properties of selected essential oils from plants from the Lamiaceae spp. against the fungus Coniophora puteana. The influence of solutions of essential oils has been analyzed: mint, rosemary and thyme on the growth was analyzed. It has been shown that essential oils have fungistatic properties.

Keywords: essential oil, natural wood preservatives, *Coniophora puteana*

INTRODUCTION

The legal provisions introduced by the European Union regarding restrictions on the use of chemical active substances in wood preservatives have led to research into alternative methods of wood protection, based on natural products with low or zero environmental toxicity. New generation specimens are to be favorable to people and their surroundings. Currently, there are, among others research on the effectiveness of substances of natural origin acting on wood degrading agents. The list of measures examined includes vegetable oils, essential oils or natural resins.

One of the alternative sources of biocides of natural origin may be essential oils. These are substances obtained from plants, currently used mainly in the cosmetics industry and aromatherapy. A number of studies on essential oils have shown that some of them show fungistatic properties.

The aim of the work was to examine the fungistatic properties of selected essential oils obtained from plants belonging to the *Lamiaceae* family. to the fungus *Coniophora puteana* (Schumach.) P. Karst.

CHARACTERISTICS OF ESSENTIAL OILS

Essential oils are mainly liquid and fragrant mixtures of chemical compounds. They are obtained from the entire plant or part of plants usually through a distillation process (Klimek 1957). Each essential oil consists of a few to a dozen components in the form of chemical compounds, usually belonging to the terpenes group (Lamer-Zarewska et al. 2007). The most characteristic components of essential oils are: esters, aliphatic or terpene alcohols, aldehydes, ketones, oxides and lactose (Klimek 1957). Most essential oils do not dissolve in water, however, they dissolve quite easily in alcohols, ethers, chloroform or in liquid fats. They are characterized by high boiling points, from 100ºC to even around 300ºC. A very characteristic feature of essential oils is their strong aroma (Drygas and Śniegocki 1971).

Essential oils have a wide range of applications, from the pharmaceutical, food and cosmetic and perfumery industries (Rumińska et al. 1990).

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS

Numerous studies show that essential oils have antimicrobial properties that inhibit the growth of bacteria and fungi (Hołderna-Kędzia 2010). They are natural, multi-component substances produced by plants, inter alia, to protect against microbes. Attempts to use essential oils as microbial corrosion inhibitors are effective. The biocidal ability of oils can be

used to inhibit the growth of microorganisms that inhabit the surface of a variety of materials (Kunicka-Styczyńska and Maroszyńska 2012).

Research on antimicrobial activity of essential oils concerned pathogenic microorganisms that threaten people, plants and animals. Experiments were carried out on *Aspergillus niger* mold fungus and *Candida albicans*, considered to be human pathogens. The results of the experiments show that for some oils the fungi are very sensitive (Abbasoglu and Kusemenoglu 1994, Garg and Jain 1992, Hili et al. 1997, Dwiwedi et al. 1996, cited in Kalemba 2000), and more resistant to others (Langezaal et al. 1992, Stassi et al. 1996, Kalodera et al. 1997, quoted in Kalemba 2000). All of the proven essential oils showed biocidal properties against at least one microorganism. The higher concentration of the oil increased the resistance of samples against microorganisms (Kalemba 2000).

Antimicrobial activity of essential oils is associated with their chemical composition. The composition of the oil depends on: the plant species, the part of the plant from which it is obtained, the stage of plant development and habitat conditions. Each of these factors affects the quality of the essential oil and its properties. The chemical composition of the oil also plays a very important role in its antimicrobial activity. The lipid nature of the hydrocarbon backbone and the hydrophilic nature of functional groups have an impact on the intensity of the protective properties of the individual components of the essential oils. Studies have shown that essential oils of which the main components are phenols most strongly affecting microorganisms. They are referred to as antiseptics (Kohlmünzer 2007). These are oils: thyme, carbohydrate, oregano, clove and cinnamon. The main components of sage oils (thujas, camphor) and mint oils (menton, carvone) are ketones, which give them high efficiency in protection against degrading agents. The oils in which the dominant substance is alcohol are less active against microorganisms. This group includes geranium oils (geraniol and citronello) (Kędzia 2009), mint (menton) and lavender oil (linalool and its acetate) (Kalemba 2000).

Research on the relationship between the composition of essential oils and their antimicrobial activity did not yield a clear result (Lis-Balchin 1996, Patkar et al. 1993, quoted in Kalemba 2000). Probably not only the chemical composition, but also proportions, synergistic and antagonistic effects affect the activity of essential oils (Kalemba 2000). The antimicrobial activity of linalool essential oils has also been proven. The influence of thyme, basil and marjoram oil on molds *A. niger* and P. epansum was investigated. The fungistatic and fungicidal activity of thyme and marjoram oil has been confirmed, thanks to which indicated oils can be used to limit the development of filamentous fungi. However, this is not the effect of lilanol as the main antimicrobial agent (Kunicka-Styczyńska and Maroszyńska 2012).

MATERIALS

Peppermint oil

Mentha arvensis mint oil - Japanese mint oil [PN-ISO 4720: 1999]. The content of the substance in the composition of the oil specified by the manufacturer: Limonene and Linalool. Peppermint oil has a wide range of applications. It is used in the cosmetics industry for the production of toothpaste, confectionery for making sweets and medicine as disinfectant.

Rosemary oil

Rosemary oil *Rosmarinus offiinalis* [PN- ISO 4720: 1999]. Composition provided by the manufacturer: Limonene and Linalool. Essentially, rosemary oil is used in the cosmetics industry for scenting soaps, in the food industry as an aroma and in pharmacy for the production of rosemary ointment. It has a strengthening, diastolic and bactericidal effect (especially on streptococcus and staphylococci). It is also used to repel insects.

Thyme oil

Thyme oil, *Thymus vulgaris* [PN-ISO 4720: 1999]. According to the manufacturer, the oil includes: D-Limonene and Linalool. Thyme oil is used as a strong disinfectant and also as an ingredient in the treatment of respiratory diseases and oral care preparations. It has antispasmodic and anti-inflammatory properties, has a disinfecting effect on the skin, destroys both bacteria, yeasts and parasitic fungi.

Fungus

Coniophora puteana (Schumach.) P. Karst. came from the collection of pure cultures of the SGGW Department of Wood Protection. The C. puteana fungus is the main test species used to determine the effectiveness of wood protection against basidiomycetes (PN EN 113: 2000).

METHODS

Eleven solutions of ethanol oil with concentrations: 0.1; 0.5; 1; 5; 10; 15; 20; 25; thirty; 40; 50 µl / l for each oil (mint, rosemary and thyme). Using automatic pipettes, 1 milliliter of solution was prepared for each concentration.

The tests were carried out in Petri dishes with a diameter of 90 mm. Each of the solutions of the oils was introduced into sterile agar-maltose medium contained in the plates in the amount of 10 ml of medium and 50 µl ethanolic solution of the essential oil. There were two samples for each concentration of the oil solution. There were two control samples marked with Ke letters for each oil. Control samples contained 10 ml of medium and 50 µl of ethanol. In addition, two control samples were prepared without the addition of ethanol. They were marked with the letters K.

The dosing of individual components was carried out in a laminar chamber under sterile conditions. A 3 mm diameter inoculum was centrally inoculated onto the medium with the oil solution and stored in an incubator at the temperature of 23 ± 2 ° C and 65 ± 5% relative humidity.

Two horizontal (L1) and vertical (L2) measuring lines were drawn on Petri dishes. Each sample was marked with the symbols M- peppermint oil, R- rosemary oil, T- thyme oil, K- control sample with medium and Ke - control sample with medium and ethanol. Measurements were taken every 48 hours. The measurement consisted of reading the dimensions of the diameter of the fungus on the measuring lines with a measuring cup with an accuracy of 1 mm. Measurements were taken every 48 hours. After inoculation, until the surface of the mycelium medium is overgrown with mycelium on Ke samples.

RESULTS

Control samples

Table 1 presents a list of dimensions of the mycelium diameter of the *C. puteana* fungus for control samples. Plates with medium were marked with K. However, samples with medium and ethanol were marked with the Ke symbol. The control plates were included in the studies as a reference point for the remaining preparations.

Table 1. The average value of linear measurements of *C. puteana* mycelium on control samples.

Symbol of samples	Day							
	2	4	6	8	10	12	14	16
	[mm]							
K	12	23	33	45	61	74	81	90
Ke	10	23	33	43	56	70	81	90

Mycelium growth on the medium with the addition of a mint oil solution

Considering the average results of measurements of *C. puteana* mycelium diameter (Table 2) the highest increase was recorded for samples M1, M2, M2 and M10. These plates contained solutions of peppermint oil in ethanol at a concentration of 0.1; 0.5; 1 and 40 µl/l. At the 16th day, the cellar fungus grew over the entire surface of the tiles, and the mycelium reached average dimensions of 90 mm. The smallest increase was recorded with M9 samples, i.e. a solution concentration of 30 µl/l. The mycelium has reached an average dimension of 87 mm.

Analyzing the results of measurements of mycelial growth on test and control samples, it can be concluded that the concentration of peppermint oil with ethanol affects the development of the fungus. The results indicate that the higher the concentration of the oil in the solution, the mycelial growth decreases. In the case of the concentration of the mint oil with a value of 0.1 µl/l the fungus develops similarly to the control sample, whereas in the plates where the concentration is 40 or 50 µl/l, a significant decrease in the development of the mycelium is visible.

Table 2. The average value of linear measurements of mycelial diameter of the basement fungus with the applied solution of mint oil in ethanol.

Symbol of samples	Concentration of the solution	Day							
		2	4	6	8	10	12	14	16
	[µl/l]	[mm]							
M1	0,1	8	19	31	42	56	70	84	90
M2	0,5	10	24	39	51	66	79	89	90
M3	1	7	18	30	43	61	73	83	90
M4	5	9	18	29	43	57	70	83	89
M5	10	7	18	27	39	53	69	81	88
M6	15	10	21	37	50	64	76	85	89
M7	20	9	19	30	42	56	69	84	89
M8	25	10	22	32	44	59	74	82	89
M9	30	9	17	25	37	50	67	78	87
M10	40	11	24	37	52	68	82	90	90
M11	50	8	15	24	34	49	64	80	88

Mycelium growth on the medium with the addition of rosemary oil

Analyzing the average results of measurements of linear diameter of the fungus (Table 3), the highest growth value at day 16 was obtained by sample R1, with a concentration of 0.1 µl/l. The value of the mycelium diameter was 90 mm. Equally high increase was recorded for samples with the symbols R8 (concentration 25 µl/l) and R9 (concentration 30 µl/l). The value of the mycelium diameter was 89 mm. The smallest increase was read from a 0.5 µl/l sample with the R2 symbol.

The results obtained with samples of rosemary solution with ethanol give different values. On the basis of the obtained data, it is not possible to conclusively state the relationship between the specific concentration of the rosemary solution and the impact on the development of *C. puteana*. The measurements show that most of the concentrations slow down the development of the mycelium, and each of the samples gives a similar value. The average difference between day 2 and day 16 is 78 mm.

Mycelium growth on the medium with the addition of a thyme oil solution

Referring to the averaged results of measurements of the linear diameter of the fungus (Table 4), the highest growth value at day 16 was obtained by the T2 sample, with a concentration of 0.5. The value of mycelium diameter was 89 mm. An equally high increase was observed in the sample with the T1 symbols, the solution concentration was 0.1 µl/l, the average linear dimension of the mycelium diameter was 86 mm. The smallest increase was recorded for the sample at 50 with the symbol T11.

Table.3. The average value of linear measurements of the mycelial diameter of the basement fungus with the applied solution of rosemary oil in ethanol.

Symbol of samples	Concentration of the solution	Day							
		2	4	6	8	10	12	14	16
	[µl/l]	[mm]							
R1	0,1	10	17	31	45	60	74	84	90
R2	0,5	4	7	14	23	34	49	61	71
R3	1	9	21	35	47	61	78	84	88
R4	5	8	15	23	33	47	60	73	83
R5	10	10	20	32	46	60	73	84	88
R6	15	9	18	31	41	59	74	82	87
R7	20	8	16	26	42	61	73	86	88
R8	25	9	18	33	47	63	78	87	89
R9	30	8	16	27	41	58	73	84	89
R10	40	10	19	32	44	59	72	83	88
R11	50	8	14	27	41	58	73	83	88

Table 4. The average value of linear measurements of the mycelium diameter of the fungus growing on the nutrient medium with the addition of thyme oil in ethanol.

Symbol of samples	Concentration of the solution	Day							
		2	4	6	8	10	12	14	16
	[µl/l]	[mm]							
T1	0,1	8	17	31	42	57	71	81	86
T2	0,5	10	22	35	47	62	75	84	89
T3	1	9	19	24	43	56	68	78	88
T4	5	6	13	19	26	32	36	42	49
T5	10	5	10	18	29	40	53	68	78
T6	15	4	4	4	4	4	4	4	10
T7	20	4	4	4	8	14	21	27	31
T8	25	5	10	15	17	20	24	25	26
T9	30	4	4	7	13	21	32	43	51
T10	40	4	4	5	5	5	5	9	13
T11	50	4	4	4	4	4	4	4	4

Considering the effect of a solution of thyme oil with ethanol on the growth of *C. puteana* mycelium diameter, a certain relationship is evident. Namely, the higher the concentration of thyme oil solution, the lower the mycelium growth or its lack. Mycelium

growth gradually stops with increasing concentration until the mycelium growth stops. The measurements show that a concentration of 50 µl/l inhibits fungal growth.

SUMMARY

Summing up the analysis of the results, it should be noted that each of the tested solutions of oils differently affects the growth of mycelium diameter of the *C. puteana* fungus. In the case of peppermint oil, it was noted that the concentration of the solution has an effect on reducing the growth of mycelium, but is not as strong as with thyme oil. In one of the samples (T11), the mycelium developed completely. Comparing these two tiles with each other, thyme oil has a stronger fungistatic effect. It is more effective and at lower concentrations than peppermint oil inhibits fungal growth. In the case of peppermint oil, mycelium measurements show a different relationship. The results show that the oil solution affects the slowdown in the development of mycelium, and the growth of mycelium is not as dynamic as in the case of the other two samples. No relation was noticed more clearly between the concentration of the essential oil solution and inhibition of growth of the fungus. Of the three considered solutions of oils, thyme oil obtained the best results in terms of inhibiting mycelial growth.

CONCLUSIONS

1. The addition of a solution of peppermint, rosemary and thyme essential oils to the agar-maltose medium causes a slowdown in the development of *C. puteana* mycelium.
2. Peppermint oil has low fungistatic properties towards *C. puteana*.

REFERENCES

1. ABBASOGLU U., KUSEMENOGLU S., 1994: Antibacterial and antifungal studies on *Achillea* L. species. J. Fac. Pharm. Gazi. 11:177-181, Cyt. za: Kalemba D. 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
2. AVATO P., VITALI C., MONGONELLI P., TAVA A., 1997: Antimicrobial activity of polyacetylenes from Bells perennis and their synthetic dericatives. Planta Med. 63:503-507,. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
3. DRYGAS A., ŚNIEGOCKI L., 1971: Towaroznawstwo zielarskie i środków leczniczych. PWSZ, Warszawa.
4. DWIWEDI S.K., PANDEY V.N., 1996: Effect of essential oils of some higher plants on *Aspergillus flavus* Link. infesting stored seeds of guar (*Cyamopsistetragonoloba L. (Taub.).* Flavour Fragr. J., 6: 295-297. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
5. GARG S.C., JAIN R. 1992: Biological activity of the essential oil of *Piper betle* L. J. Essent. Oil Res. 4: 601-606. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
6. HILI P., EVANS C.S., VENESS R.G., 1997: Antimicrobal action of essential oils: the effect of dimethylsulfoxidole on the activity of cinnamon oil. Lett. Appl. Microbiol. 24: 269-275. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
7. HOŁDERNA-KĘDZIA E., 2010: Działanie substancji olejkowych na bakterie i grzyby. Postępy Fitoterapii 1/2010: 3-8.
8. KALEMBA D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.

9. KALEMBA D., 2009: Przeciwbakteryjne i przeciwgrzybiczne właściwości olejków eterycznych. Postępy Mikrobiologii 38: 185-203.
10. KALODERA Z., PEPELJNAK S., BLAZEVIC N., PETRAK T. 1997: Chemical composition and antimicrobial activity of Tanacetumparthenium essential oil. Pharmazie, 52: 885-886. Cyt. za: Kalemba D. 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
11. KĘDZIA E., 2009: Przeciwgrzybicze działanie olejku gerianiowego (Oleum gerianii). Postępy Fitoterapii 4/2009: 217-221.
12. KLIMEK R., 1957: Olejki eteryczne. Wydawnictwo Przemysłu lekkiego i spożywczego. Warszawa.
13. KOHLMÜNZER S. 2007: Farmakognozja. Wydawnictwo Lekarskie PZWL, Warszawa.
14. KUNICKA-STYCZYŃSKA A., MAROSZYŃSKA M., 2012a: Aktywność antydrobnoustrojowa olejków eterycznych zawierających linanol. Ochrona przed korozją 9s/A, Sigma-Not, Warszawa.
15. KUNICKA-STYCZYŃSKA A., MAROSZYŃSKA M., 2012b: Aktywność olejków eterycznych wobec pleśni *Aspergillus Niger.* Ochrona przed korozją 9s/A, Sigma-Not, Warszawa.
16. LAMER-ZAREWSKA E., KOWAL-GERCZAK B., NIEDWOROK J. (red.) 2007: Fitoterapia i leki roślinne. PZWL, Warszawa.
17. LANGEZAAL C.R., CHANDRA A., SCHEFFER J.J.C., 1992: Antimicrobial screening of essential oils of some HumulusLupulus L. cultivars. Pharm. Weekbl. Sci. Ed. 14: 353-356, 1992. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
18. LIS-BALCHIN M., DEANS S.G., HART S., 1996: Bioactivity of generanium oils from different commercial sources. J. Essent. Oil Res. 8: 281-290. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
19. PATKAR K.L., USHA C.M., SHETTY H.S., PASTER N., LACEY J.: Effect of spice essential oils on growth and aflatoxion B1 production by *Aspergillus flavus*. Lett. Appl. Microbiol. 17: 49-51, 1993. Cyt. za: Kalemba D.: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. Politechnika Łódzka , 2000
20. RUMIŃSKA A., SUCHORSKA K., WĘGLARZ Z., 1990 : Rośliny lecznicze i specjalne.
SGGW_AR, Warszawa.
21. SEN S., TASCIOGLU C., TIRAK K., 2009: Fixation, leachibiliy and decay resistance of wood treated with some commercial extracts and wood preservative salts. IntBiodeteriorBiodegrad 63:135–141. Cyt. za Singh A.P, Singh T., 2012: A review on natural products as wood protectant. Wood Sci Technol 46, 851-870, Springer Verlag.
22. SINGH A.P, SINGH T., 2012: A review on natural products as wood protectant. Wood SciTechnol 46: 851-870, Springer Verlag.
23. STASSI V., VERYKOKIDOU E., LOUKIS A., HARVALA C., PHILIANOS S., 1996: The antbacterial activity of the essential oils of four Juniperus species growing wild in Greece. Flavour Fragr. J. 11: 71-74. Cyt. za: Kalemba D., 2000: Składniki i właściwości biologiczne olejków eterycznych z roślin z rodzaju Solidago i Artemisia. PŁ, Łódź.
24. PN- ISO 4720: 1999. Olejki eteryczne. Nazewnictwo. PKN, Warszawa.

Streszczenie: *Ocena właściwości fungistatycznych wybranych olejków eterycznych pozyskanych z roślin z rodziny Lamiaceae.* W artykule przedstawiono wyniki badań właściwości fungistatycznych wybranych olejków eterycznych roślin z rodziny Lamiaceae wobec grzyba *Coniophora puteana*. Przeanalizowano wpływ roztworów olejków eterycznych: miętowego, rozmarynowego oraz tymiankowego na wzrost grzybni. Wykazano, że olejki eteryczne posiadają właściwości fungistatyczne.

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