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Effect of electromagnetic stimulation of the active substance of selected plants on their antiseptic potential

***Abstract**. Currently, there is a significant increase in antibiotic resistance and associated multiresistance among microorganisms. An alternative may be the use of essential oils, which possess bactericidal properties. This paper discusses the effect of electromagnetic stimulation of a plant biologically active substance on the degree of their aseptic efficiency with respect to selected strains of Candida krusei ATCC 14243, Enterococcus faecalis ATCC 29212, Paseudomonas aeruginosa ATCC 278596 and Lactobacillus spp. It was found that the application of electromagnetic stimulation of essential oils affects their characteristics of antiseptic effect. Depending on the applied combination of 40 mT and 80 mT magnetic flux density and stimulation time, a progressive or regressive effect of the electromagnetic field was noted. In the case of 40 mT electromagnetic field irrespective of the stimulation time, a significant decrease in the antimicrobial properties of tea tree oil was noted.*

***Streszczenie.** Obecnie odnotowywany jest znaczący wzrost antybiotykoodporności oraz związanej z nią wieloporności wśród drobnoustrojów. Alternatywą może być wykorzystanie olejków eterycznych, posiadających właściwości bakteriobójcze. W artykule omówiono wpływ stymulacji elektromagnetycznej substancji biologicznie czynnej roślin na stopień ich efektywności aseptycznej w odniesieniu do wybranych szczepów Candida krusei ATCC 14243, Enterococcus faecalis ATCC 29212, Paseudomonas aeruginosa ATCC 278596 i Lactobacillus spp.* Stwierdzono, że *zastosowanie stymulacji elektromagnetycznej olejków eterycznych wpływa na ich charakterystykę oddziaływania antyseptycznego. W zależności od zastosowanej kombinacji indukcji pola magnetycznego 40 mT oraz 80 mT i czasu stymulacji odnotowywano progresywne lub regresywne oddziaływanie pola elektromagnetycznego, a w przypadku oddziaływania pola elektromagnetycznego o indukcji 40 mT bez względu na czas stymulacji odnotowano znaczne zmniejszenie właściwości przeciwdrobnoustrojowych olejku z drzewa herbacianego (**Wpływ stymulacji elektromagnetycznej substancji czynnej wybranych roślin na ich możliwości antyseptyczne**)*

Keywords: electromagnetic field, essential oils, biological substance, antiseptic properties
Słowa kluczowe: pole elektromagnetyczne, olejki eteryczne, substancja biologiczna, właściwości antyseptyczne

Introduction

The use of plant essential oils and extracts as an additive to foodstuffs exerts antioxidant or antimicrobial activity. It should be noted that among compounds of natural origin, essential oils from aromatic and medicinal plants exhibit biological activity. Bacteria such as: *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella spp, Shigella, Enterococcus sp*. and *Escherichia coli* exhibit multidrug resistance which justifies the use of natural antiseptics. Plant extracts and essential oils have antifungal, antibacterial and antiviral properties and research is being conducted on their potential use as a source of new antimicrobial compounds, food preservation promoting agents and alternatives in the treatment of infectious diseases [1]. Today, about 3000 different essential oils are known, of which about 300 are of commercial importance, used mainly in the aroma and flavor market [2]. In nature, essential oils play an important role in protecting plants from pests, pathogenic microorganisms and also in repelling and reducing the appetite of certain herbivores [3]. The chemical composition of the essential oils of plants varies from species to species and is dependent on geographic location, environment, stage of maturity of the plant and the method of extraction of the oil. The difference in chemical composition of oils is directly correlated with differences in their biological activity [4, 5].

The use of essential oils against bacteria, leads to an increase in the permeability of their cell membrane [6,7]. This phenomenon is associated with loss of ions and an associated decrease in membrane potential, as well as changes in proton pump function and depletion of the ATP pool [8, 9]. In addition, essential oils penetrating the cell wall and membrane of microorganisms cause changes in the structural arrangement of the lipid-protein layer [10, 11]. Changes in plasmalemma continuity affect the functioning of basic metabolic processes such as synthesis of structural macromolecules, nutrient utilization and energy metabolism [7]. The same is true for the action of essential oils against fungi. They are capable of destroying the cell wall and also damaging mitochondrial membranes. Changes in the electrical potential of mitochondria lead to damage of lipids and proteins and to a decrease in the amount of nucleic acids [12].

Technological advances in recent decades have resulted in the creation of artificial electromagnetic fields [13]. This has allowed research on the biological effects of electromagnetic fields in living organisms [14-20]. The mechanisms of electromagnetic field interactions at the cellular level are not sufficiently understood, determining the effects of interactions using simple organisms such as bacteria and yeast may provide many new characteristics and previously unknown relationships [21-23]. The magnitude and direction of these changes occurring under the influence of an electromagnetic field depend on the energy and spatial distribution of the field. The application of appropriately selected values of field parameters, stimulates or inhibits the activity of microorganisms [24, 25].

The aim of the study was to determine the effect of electromagnetic field on the characteristics of antiseptic properties of biologically active substance which was subjected to such stimulation. Two levels of the magnetic flux density were used in the study viz: 40 mT and 80 mT and three time intervals of exposure of the biological substance to the electromagnetic field i.e.: one, two and three hours.

Material and methodls

Candida krusei ATCC 14243, *Enterococcus faecalis* ATCC 29212, *Paseudomonas aeruginosa* ATCC 278596 and *Lactobacillus spp*. strains from the strain collection of the Laboratory of Experimental Research Techniques of Raw and Biological Products. Reduction cultures were performed on TSA solid medium for bacteria and Sabouraud medium for yeast in Petri dishes for restoration of vital functions. All steps were performed under sterile conditions in a laminar flow cabinet to prevent unwanted contamination. The plates were incubated for 24 hours (bacteria) and 48 hours (yeast) at 37 °C. Suspensions of microorganisms with an optical density of 0.5 on the McFarland scale were prepared. Into round-bottomed sterile tubes, filled with 10 ml of sterile liquid BHI medium, the collected microbial colonies were introduced using a sterile ezzle. The mixture was mixed using a vortex type centrifuge. The optical density was measured using a DEN-18 densitometer.

Two essential oils were used in this study: tea tree and cedarwood. From the essential oils, 4 samples each were prepared in ten repetitions: a control sample and three stimulated samples of 1h, 2h and 3h. Then, the samples were subjected to electromagnetic field (Figure 1) with a frequency of 50 Hz in two variants of magnetic flux density of 40 mT and 80 mT and in 3 variants of interaction, i.e. stimulation time of 1, 2 and 3 hours.

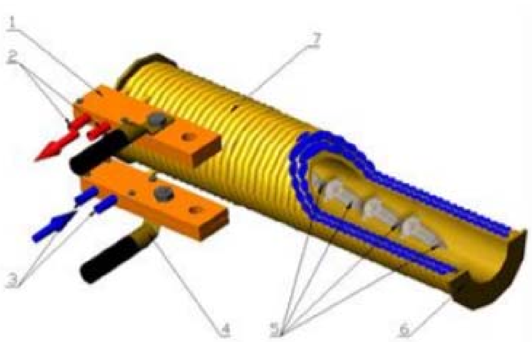

Figure 1: Schematic of solenoid for electromagnetic stimulation: 1 - supply terminals; 2 - cooling water outlet; 3 - cooling water inlet; 4 - supply cables; 5 - samples; 6 - carcass; 7 - turns [26]

Figures 2a and 2b show the distribution of the electromagnetic field around the solenoid used in this study, which was made of 108 turns. The turns were made of copper tube with a diameter of 8mm and a wall thickness of 1mm. The calculations assumed: current 310A, frequency 50Hz.

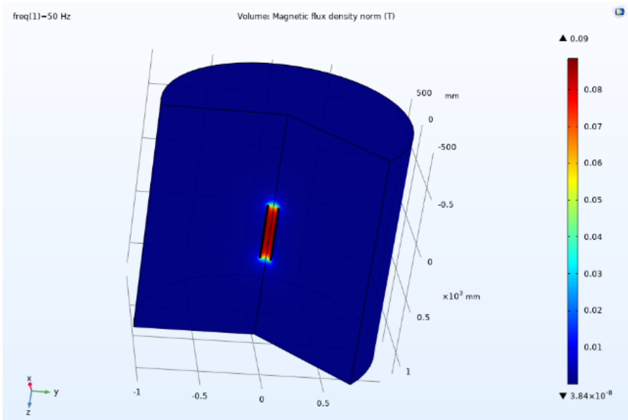

Figure 2a. Distribution of the magnetic flux density around the solenoid

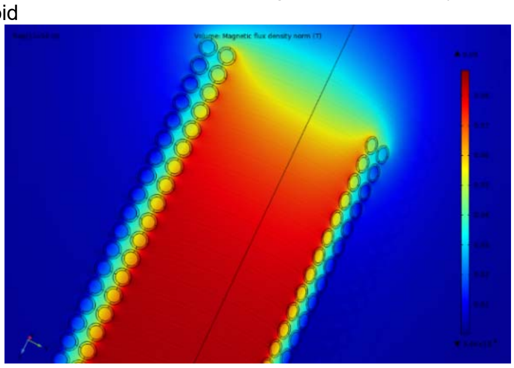

Figure 2b. Magnetic flux density distribution inside the solenoid

In order to determine the response of the tested microbial strains - stimulation or inhibition of growth, suspensions with an optical density of 0.5 McF of each microorganism were prepared and introduced 2ml each into test tubes, from which 1 ml of the tested infusions exposed to the electromagnetic field were then introduced. The same procedure was followed for essential oils. The cultures were incubated for 24 hours at 37°C. After the incubation period, the optical density was read using a densitometer. The sensitivity of microorganisms to selected essential oils was tested according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines. The diffusion-well method was chosen for the study. The compound diffuses radially, its highest concentration is at the edge of the well and decreases with increasing distance from the well. The measure of the killing activity of the test substance is the size of the growth inhibition zone of microorganisms measured in millimeters. The larger the diameter of the microbial growth inhibition zone, the greater the biocidal activity of the test substance. Petri dishes with Mueller Hinton 2 LAB -AGAR medium were inoculated with suspensions of the tested microorganisms (with an optical density of 0.5 McF), then the wells were cut with the help of scales (Figure 3) and the agar fragments were removed. 0.3 ml each of the solutions of the test oils were introduced into the wells. The plates were incubated for 24 hours at 37 °C. After the incubation period, the growth inhibition zones of the tested microorganisms were measured using a ruler.

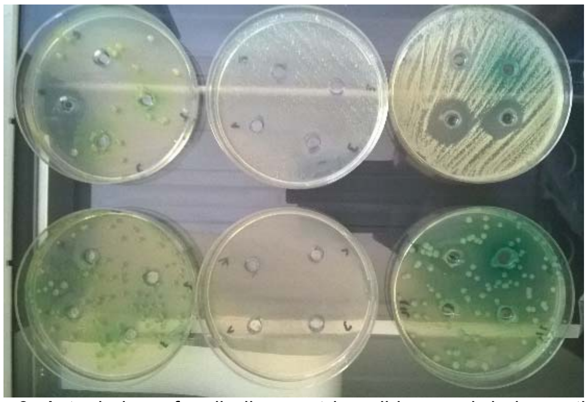

Fig. 3. Actual view of well alignment in solid ground during optical identification of the interaction Surface

Results

The study analyzed the antiseptic properties of two essential oils: tea tree and cedarwood. The level of antiseptic properties was identified by the value of optical density and the surface area constituting the zone of inhibition of microbial growth, which constituted the experimental material.

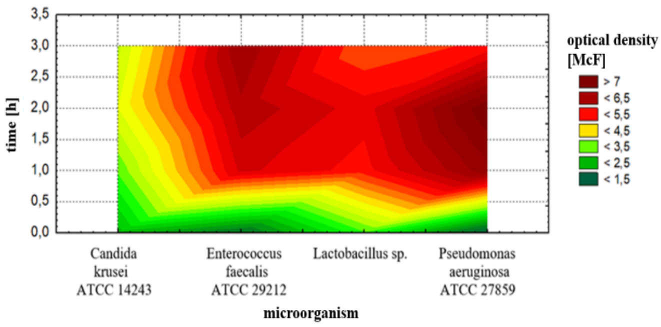

Fig. 4. Sensitivity of the tested microorganisms to tea tree oil tea tree oil subjected to the action of electromagnetic field with the flux density of 40 mT

In the case of electromagnetic field stimulation of tea oil with a flux density of 40 mT, it was noted that there was no statistically significant effect of this stimulation on the antiseptic properties of the biologically active substance at each time interval of exposure of this substance to the electromagnetic field. The exception in this case is *Candida krusei* strain ATCC 14243, where the growth characteristics of microorganisms were different (Figure 4). The highest values of optical density for strains were recorded after two hours of stimulation and they were for *Pseudomonas aeruginosa* ATCC 27859 - 7.2 McF, for *Candida krusei* ATCC 14243 - 4 McF and for *Lactobacillus sp*. - 5.62 McF. In case of *Enterococcus faecalis* ATCC 29212 the highest value of optical density was obtained after three hours of stimulation - 6.5 McF.

After the application of cedar oil subjected to the influence of an electromagnetic field with a flux density of 40 mT, it was found that regardless of the time of stimulation of the active substance as well as the strains of microorganisms against which the electromagnetically stimulated cedar oil was applied, an increase in the optical density of the examined microorganisms was recorded. The highest values of optical density for all tested microorganisms were recorded after three-hour stimulation with electromagnetic field (Fig. 5). The optical density values were respectively for *Pseudomonas aeruginosa* ATCC 27859 - 6.74 McF, for *Enterococcus faecalis* ATCC 29212 - 6.99 McF, for *Candida krusei* ATCC 14243 - 4.97 McF and for *Lactobacillus sp*. - 5.97 McF.

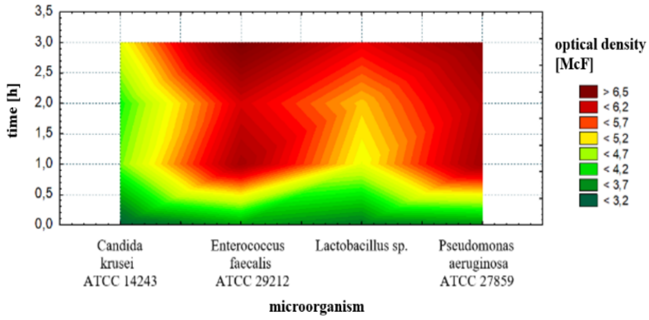

Fig. 5. Sensitivity of the tested microorganisms to the oil of cedar wood subjected to the action of electromagnetic field with the flux density of 40 mT

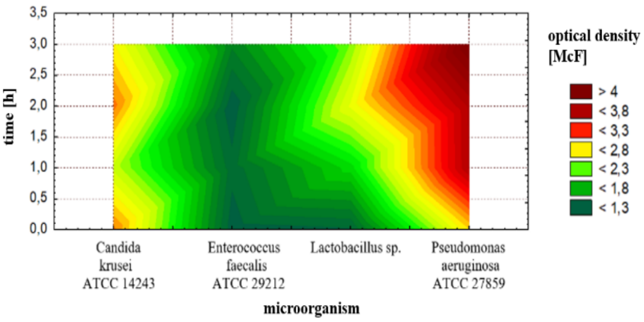

Fig. 6. Sensitivity of the tested microorganisms to tea tree oil tea tree oil subjected to 80 mT magnetic flux density

After the application of tea tree oil subjected to 80 mT electromagnetic field against microorganism strains, differences in its effect were observed (Fig.6). An increase in optical density relative to control samples was noted for *Pseudomonas aeruginosa* ATCC 27859 and *Lactobacillus sp*. strains regardless of the stimulation time used. In case of *Enterococcus faecalis* ATCC 29212 the increase of optical density was noted after one and three hours of stimulation. For *Candida krusei* ATCC 14243 the increase of optical density was observed only after two hours stimulation. In the other cases the optical density values were lower than the control samples. The highest values of optical density were registered at three hours stimulation for *Pseudomonas aeruginosa* ATCC 27859 strain - 4.22 McF. On the other hand, the lowest value of optical density was recorded for *Enterococcus faecalis* ATCC 29212 strain at two hours stimulation - 1.22 McF.

Also, after the application of cedar oil subjected to an electromagnetic field of 80 mT against the strains of microorganisms, differences were found in its effect in comparison with the oil where such an interaction was not present. An increase in optical density in comparison with control samples, i.e. without stimulation, was noted for *Enterococcus faecalis* ATCC 29212 strain regardless of the stimulation time applied. In case of *Pseudomonas aeruginosa* ATCC 27859 and *Lactobacillus sp*. the increase of optical density was noted after two hours and three hours of stimulation. However, for *Candida krusei* ATCC 14243 strain, the increase in optical density was observed only after one-hour and three-hour stimulation (Figure 7). In other cases, optical density values lower than control samples were obtained. The highest values of optical density were registered at three-hour stimulation for *Pseudomonas aeruginosa* strain ATCC 27859 - 6.75 McF. The lowest value of optical density was recorded for Lactobacillus sp. strain at two hours stimulation - 0.66 McF.

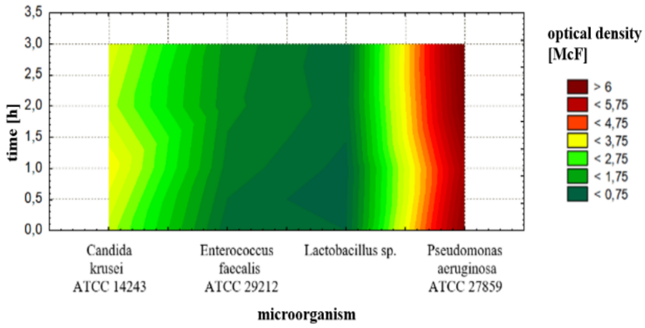

Fig. 7. Sensitivity of the tested microorganisms to the oil of cedar tree subjected to an electromagnetic field of 80 mT

After application of tea tree oil subjected to magnetic flux density of 40 and 80 mT against microbial strains, significant differences were found in the diameter of the growth inhibition zones recorded (Fig. 8, 9).

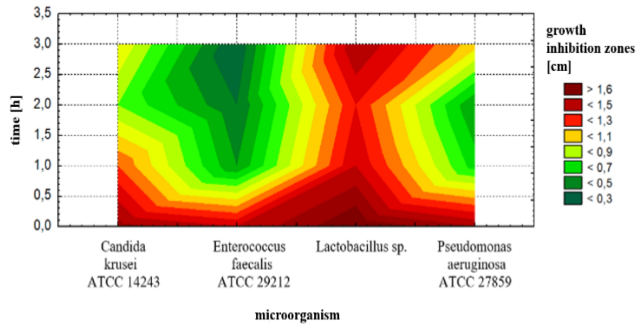

Fig. 8. Diameters of growth inhibition zones of the tested microorganisms induced by tea tree oil subjected to 40 mT magnetic flux density

In the case of tea oil subjected to 40 mT stimulation, a decrease in the diameter of growth inhibition zones was observed for all microorganisms tested (Fig.8).
The smallest zones of growth inhibition were observed *Pseudomonas aeruginosa* ATCC 27859, *Candida krusei* ATCC 14243 and *Lactobacillus sp*. after two hours of

stimulation. They were 0.5; 0.8 and 1.3 cm, respectively. For *Enterococcus faecalis* strain ATCC 29212, the lowest diameter was 0.3 cm after three hours of stimulation. For tea oil subjected to 80 mT stimulation, a decrease in the diameter of the zones of growth inhibition was also observed for all microorganisms tested (Figure 9).

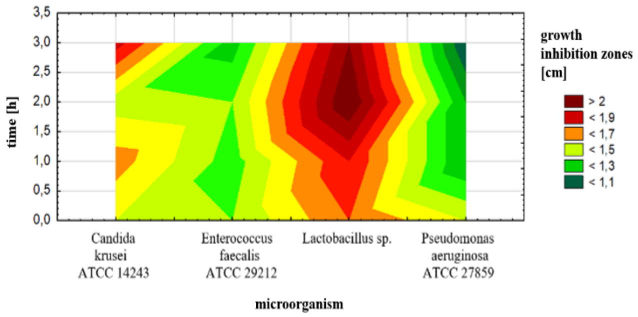

Fig. 9. Diameters of growth inhibition zones of the tested microorganisms induced by tea tree oil subjected to 80 mT magnetic flux density

The smallest zones of growth inhibition after one hour of oil stimulation were shown for the strain *Lactobacillus sp.* - 1.8 cm. After a two-hour stimulation, the lowest diameter of the zone of inhibition was obtained for *Candida krusei* ATCC 14243 strain - 1.45 cm. In contrast, three-hour oil stimulation resulted in the lowest inhibition diameters in *Pseudomonas aeruginosa* ATCC 27859 (1 cm) and *Enterococcus faecalis* ATCC 29212 (1.25 cm) strains.

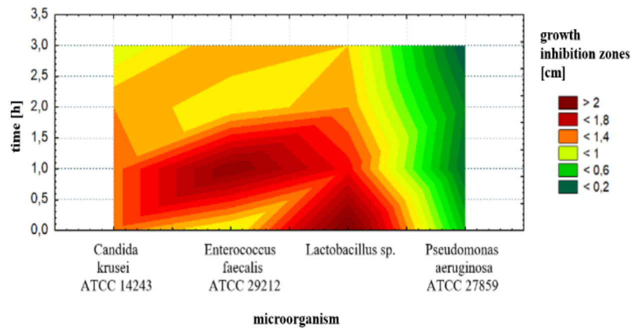

Fig. 10. Diameters of growth inhibition zones of the tested microorganisms induced by cedar oil subjected to an electromagnetic field with a flux density of 40 mT

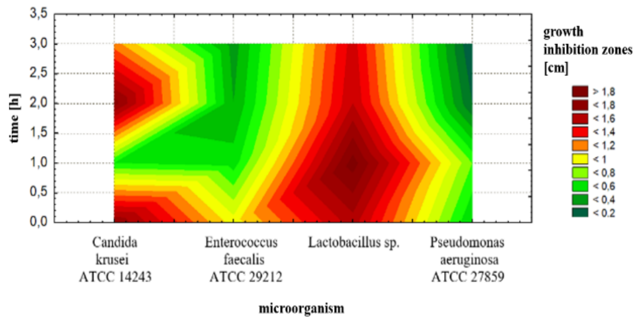

Fig. 11. Diameters of growth inhibition zones of tested microorganisms induced by cedar oil subjected to electromagnetic field with a flux density of 80 mT

After application of cedar oil subjected to a magnetic flux density of 40 and 80 mT, the diameter of the growth inhibition zones in both cases decreased with the length of stimulation time. The lowest diameters of growth inhibition zones when the oil was stimulated with a 40 mT magnetic field were obtained after three hours of stimulation in all strains tested (Figure 10). However, when the oil was stimulated with 80 mT field, the lowest diameters were recorded after three hours in *Pseudomonas aeruginosa* ATCC 27859, *Enterococcus faecalis* ATCC 29212 and *Lactobacillus sp.* (Fig.11). They were 0.1; 0.35 and 1.45 cm, respectively. For *Candida krusei* ATCC 14243 strain, the lowest diameter was obtained at one hour stimulation - 0.6 cm.

Conclusion

The use of electromagnetic stimulation of essential oils affects their antiseptic properties. Depending on the tested strain of microorganisms, the properties of electromagnetically stimulated oils change. The parameters of the applied electromagnetic stimulation are also important. In the case of electromagnetic field stimulation at a flux density of 80 mT, an increase in the antiseptic properties of both tested oils was observed, manifested by a decrease in the value of optical density of the tested microbial suspensions. However, the application of electromagnetic field stimulation at a magnetic flux density of 40 mT completely deprived the tested essential oils of antimicrobial properties.

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