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Effect of constant electric field interaction on antiseptic properties of eucalyptus oil used against *Escherichia coli*

Abstract *An issue that continues to be the focus of attention of medical and veterinary practitioners, biotechnologists and animal and human nutrition scientists are the ways and mechanisms of transferring antibiotic resistance in bacteria and limiting the use of antibiotics. The mechanism of antimicrobial action of antibiotics includes inhibition of: DNA synthesis, RNA synthesis or protein synthesis [1]. Products of natural origin are widely used in the cosmetic, perfume or pharmaceutical industries. A characteristic feature of essential oils is their intense fragrance and diverse composition. These oils contain up to several hundred components in their composition [5]. The purpose of this study was to determine the effect of exposure to a constant electric field of varying voltage and exposure time on a biological substance on the aseptic properties of selected eucalyptus oil. The scope of the work included subjecting eucalyptus oil to constant electric field interaction For each interaction combination, the degree of antiseptic properties against Escherichia coli ATCC 25912 strain was determined. Constant electric field interaction affects the antiseptic properties of eucalyptus oil used against Escherichia coli ATCC 25922. An inversely proportional relationship between inhibition zone values and optical density values was noted.*

Streszczenie. *Zagadnieniem nadal pozostającym w centrum uwagi lekarzy medycyny i weterynarii, biotechnologów oraz naukowców zajmujących się żywieniem zwierząt i człowieka są sposoby oraz mechanizmy transferu antybiotykooporności bakterii i ograniczenie stosowania antybiotyków. Mechanizm działania przeciwdrobnoustrojowego antybiotyków polega między innymi na hamowaniu: syntezy DNA, syntezy RNA czy syntezy białek [1]. Produkty pochodzenia naturalnego mają powszechne zastosowanie w przemyśle kosmetycznym, perfumeryjnym czy farmaceutycznym. Cechą charakterystyczną olejków eterycznych jest intensywny zapach i zróżnicowany skład. Olejki te zawierają w swym składzie nawet do kilkuset składników [5]. Celem badań było określenie wpływu oddziaływania stałego pola elektrycznego o zróżnicowanym napięciu oraz czasie oddziaływania na substancję biologiczną na właściwości aseptyczne wybranych olejku eukaliptusowego. Zakres pracy obejmował poddanie olejku eukaliptusowego oddziaływaniu stałego pola elektrycznego Dla każdej kombinacji oddziaływania został określony stopień właściwości antyseptycznych w stosunku do szczepu Escherichia coli ATCC 25912. Oddziaływanie stałego pola elektrycznego wpływa na właściwości antyseptyczne olejku eukaliptusowego wykorzystanego przeciwko Escherichii coli ATCC 25922. Odnotowano odwrotnie proporcjonalną zależność między wartościami strefy zahamowania wzrostu a wartościami gęstości optycznej. (**Wpływ oddziaływania stałego pola elektrycznego na właściwości antyseptyczne olejku eukaliptusowego wykorzystanego przeciwko Escherichia coli**)*

Keywords: electric field, antiseptic properties, essential oils, Escherichia coli
Słowa kluczowe: pole elektryczne, właściwości antyseptyczne, olejki eteryczne, Escherichia coli

Introduction

An issue that continues to be a focus of attention for medical and veterinary practitioners, biotechnologists and animal and human nutrition scientists are the ways and mechanisms of transferring antibiotic resistance in bacteria and reducing the use of antibiotics. Substances with antimicrobial activity, primarily involving bacteria, are naturally occurring compounds, mainly produced by microorganisms. Although antibiotics have found, starting with the discovery of penicillin and its implementation in the treatment of bacterial diseases of humans and animals, increasing use. The mechanism of antimicrobial action of antibiotics includes inhibition of: DNA synthesis, RNA synthesis, protein synthesis, murine synthesis and cytoplasmic membrane function. Unlike antibiotics, which have, as shown above, well-defined targets of action within the bacterial cell and specific ways of specific actions, disinfectants, or biocidal products, do not have specific targets of negative effects on the functions of the bacterial cell. Instead, they have broader areas of bactericidal [1]. They act as electrophilic or membrane-active agents, depending on the concentration used [2]. They react by inactivating enzymes critical to bacterial metabolism and thus inhibit growth and reproduction, up to cell lysis, occurring after several hours of exposure. In addition to bacteria with innate antibiotic resistance, there are bacterial strains that are susceptible, with the ability to acquire antibiotic resistance as a result of previously occurring genomic variation with the effect of changes in cellular metabolism, expressed by the emergence of the ability to produce broad-spectrum enzymes, including those that inactivate antibiotics. The second important factor in the mechanism of antibiotic resistance is that bacteria possess eflux pumps. Located in the cytoplasmic membrane, the pumps are proteins that displace or transport toxic substances outside the bacterial cell. They are an important tool for the occurrence of antibiotic resistance, including the onset of multi-antibiotic resistance. Resistance of a bacterial cell to different antibiotics simultaneously refers to the presence of different gene determinants of resistance on a common genetic element. In this case, one antibiotic can select bacterial resistance to other antibiotics as well [3-5].

Products of natural origin are widely used in the cosmetic, perfume, pharmaceutical and, more recently, agricultural industries. These include essential oils, which are volatile mixtures of organic substances, secreted from plants or their parts [5]. The hallmarks of essential oils are their intense fragrance and varied composition. These oils contain in their composition up to several hundred components-chemical compounds especially from the group of terpenoids. The action of oils is multifaceted and determined, to a fairly large extent, by the properties of the dominant component. Each type of oil shows a slightly different composition, this is due to changes in such factors as temperature, insolation, humidity, etc. This variability is also a consequence of metabolic processes in plants, which are dynamic, because they adapt the plant to specific environmental factors [6]. Studies testify to the antimicrobial properties of eucalyptus essential oils against a wide range of microorganisms. This is mainly true for several species of eucalyptus , especially on E. citriodora oil, which has been shown to have a broad spectrum of antifungal activity. Other studies have also focused mainly on the antifungal properties of eucalyptus essential oils while only a few studies have examined their effects against pathogenic and food spoilage microorganisms [6, 8-14].

The purpose of this study was to determine the effect of exposure to a constant electric field of varying voltage and

exposure time on a biological substance on the aseptic properties of selected eucalyptus oil.

Material and methods

The scope of the work included subjecting eucalyptus oil to a constant electric field interaction For each interaction combination, the degree of antiseptic properties against *Escherichia coli* ATCC 25912 strain was determined.The study included the determination of growth inhibition zones and optical density of *Escherichia coli* ATCC 25912.

Cultures were carried out on Trypticasein Soy LAB - AGAR solid medium (TSA) for in Petri dishes to restore vital functions. All steps were carried out under sterile conditions in a laminar flow chamber to prevent unwanted contamination. The plates were incubated for 24 hours at 37°C. Bacterial suspensions 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 Brain Heart Infusion Broth (BHI), the collected microbial colonies were introduced using a sterile ezy. The suspension was stirred using a vortex type centrifuge. Optical density was measured using a DEN- 1B densitometer.

Fig 1. Densitomete DEN - 1B

Three essential oils were used in the study: eucalyptus, fir and peppermint. Four samples each were prepared from the essential oils in ten replicates: a control sample and three samples exposed to a constant electric field. The samples were then exposed to an electric field (Figure 2) at three variants of voltage densities of 2.2 kV/cm, 4.2 kV/cm and 8.6 kV/cm, and at 3 variants of exposure, i.e. stimulation times of 1, 2 and 3 hours

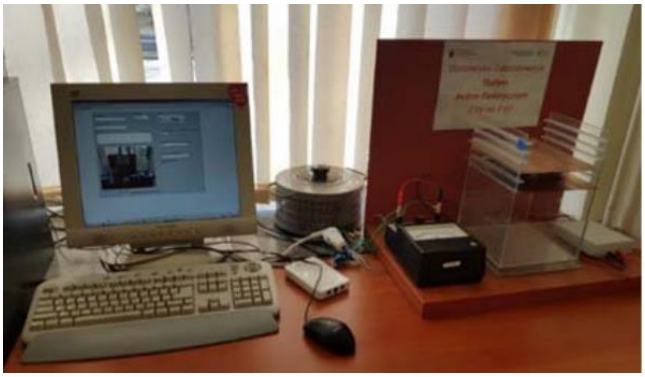

Fig 2. Constant electric field exposure site

In order to determine the response of the tested microbial strains - stimulation or inhibition of growth, 1 ml of previously prepared suspensions were introduced into 2 ml test tubes with an optical density of 0.5 McF, 1 ml each of the tested essential oils exposed to a constant electric field were introduced. Control tests were also performed. The susceptibility of microorganisms to the selected essential oils was tested according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines. The diffusion-well method was chosen for the test. The compound diffuses radially, its highest concentration is at the edge of the well and decreases with increasing distance from the well. A measure of the killing activity of the test substance is the size of the zone of inhibition of microbial growth, measured in millimeters. The larger the diameter of the zone of inhibition of microbial growth, the greater the biocidal activity of the test substance. Petri dishes with Mueller Hinton 2 LAB -AGAR medium were inoculated with a suspension of Escherichia coli ATCC 25912 (with an optical density of 0.5 McF), and then wells were cut with scales and agar fragments were removed. 0.3 ml each of test oil solutions were introduced into the wells. Petri dishes along with test tubes were incubated for 24 hours at 37°C. After the incubation period, the inhibition zones of the test microorganisms were measured with a ruler (Figure 3) and the optical density with a densitometer.

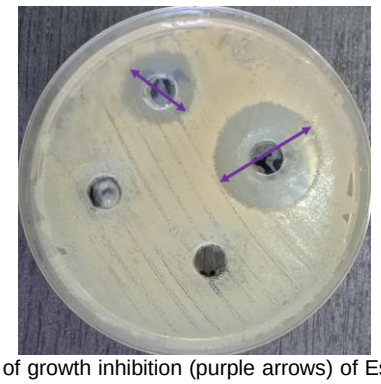

Fig 3. Zones of growth inhibition (purple arrows) of Escherichia coli ATCC 25912 obtained after exposure to a constant electric field

Results

It was observed that with increasing exposure time to an electric field of 2.2 kV/cm, the diameter of growth inhibition of *Escherichia coli* ATCC 25922 decreased. The highest diameter of growth inhibition was obtained for the control sample, which was 31.8 mm, while the lowest at three-hour exposure was 21.2 mm. The largest decrease in the size of the diameter of growth inhibition occurred between the control sample and the three-hour exposure sample, which was 10.6 mm. The figure 4 shows the diameters of the zones of growth inhibition of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 2.2 kV/cm.

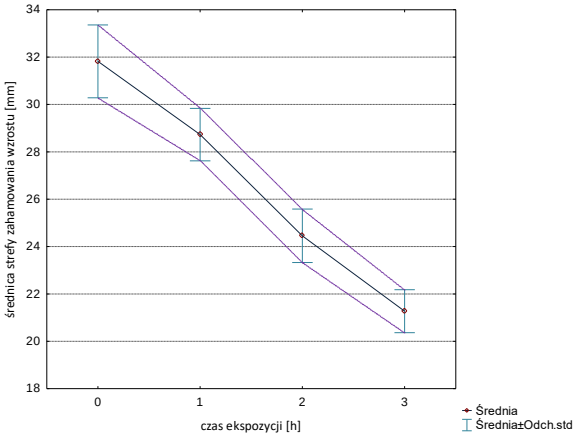

Fig 4. The diameter of the zone of growth inhibition of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 2.2 kV/cm.

It was observed that the diameter of growth inhibition of *Escherichia coli* ATCC 25922 decreased with increasing exposure time to an electric field of 4.2 kV/cm (Figure 5). The highest diameter of growth inhibition was obtained for the control sample, which was 31.8 mm, while the lowest at three-hour exposure was 12.3 mm. The largest decrease in the size of the diameter of growth inhibition occurred between the control sample and the three-hour exposure sample, which was 19.5 mm.

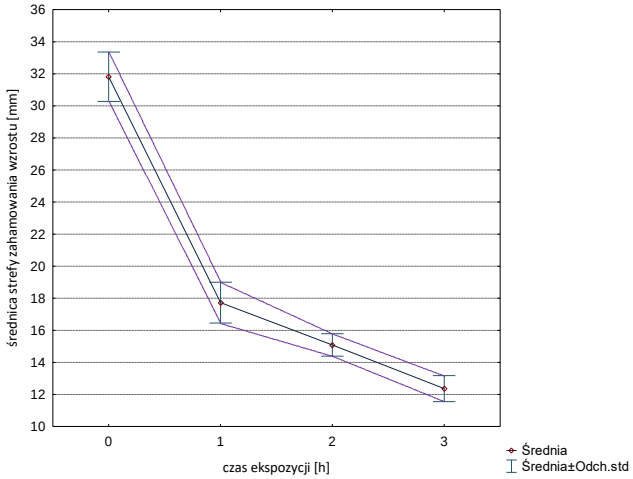

Fig 5. The diameter of the zone of growth inhibition of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 4.2 kV/cm.

It was observed that the diameter of growth inhibition of *Escherichia coli* ATCC 25922 decreased with increasing exposure time to an electric field of 8.6 kV/cm (Figure 6). The highest diamaeter of growth inhibition was obtained for the control sample, which was 31.8 mm, while the lowest at three-hour exposure was 9 mm. The largest decrease in the size of the growth inhibition diameter occurred between the control sample and the three-hour exposure sample, which was 22.8 mm.

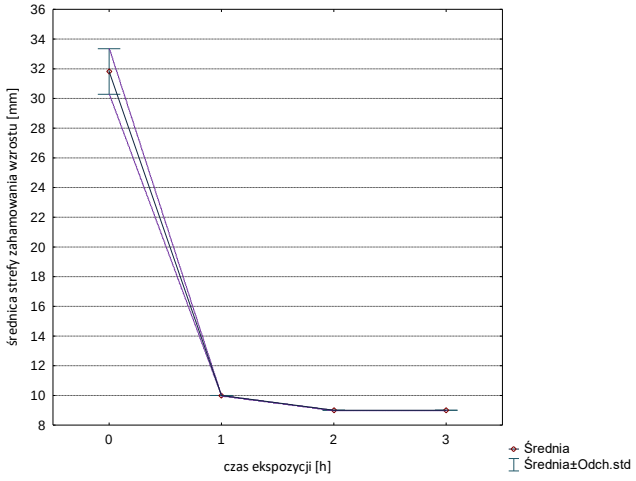

Fig 6. The diameter of the zone of growth inhibition of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 8.6 kV/cm.

It was observed that the optical density of *Escherichia coli* ATCC 25922 increased with increasing exposure time to an electric field of 2.2 kV/cm (Figure 7). The highest optical density was obtained for the sample exposed for three hours, which was 1.9 McF, while the lowest for the control sample was 0.94 McF. The largest increase in optical density values occurred between the control sample and the sample subjected to three-hour exposure and amounted to 0.94 McF.

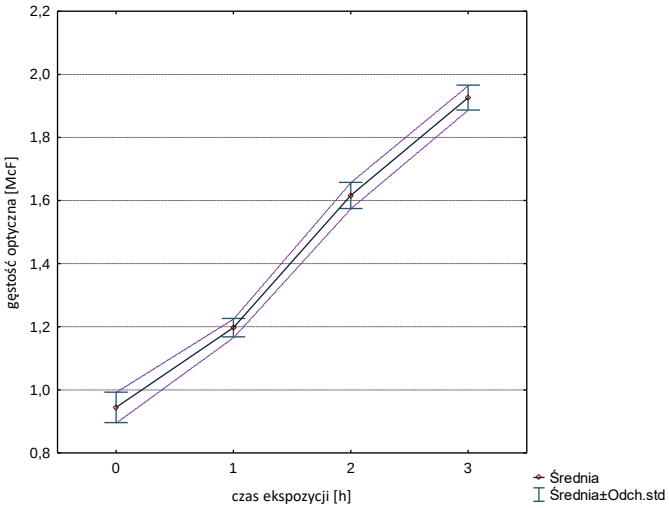

Fig 7: Optical density of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 2.2 kV/cm.

It was observed that the optical density of *Escherichia coli* ATCC 25922 increased with increasing exposure time to an electric field of 4.2 kV/cm (Figure 8). The highest optical density was obtained for the sample exposed for three hours, which was 5.4 McF, while the lowest for the control sample was 0.94 McF. The largest increase in optical density values occurred between the control sample and the sample subjected to three-hour exposure and amounted to 4.46 McF.

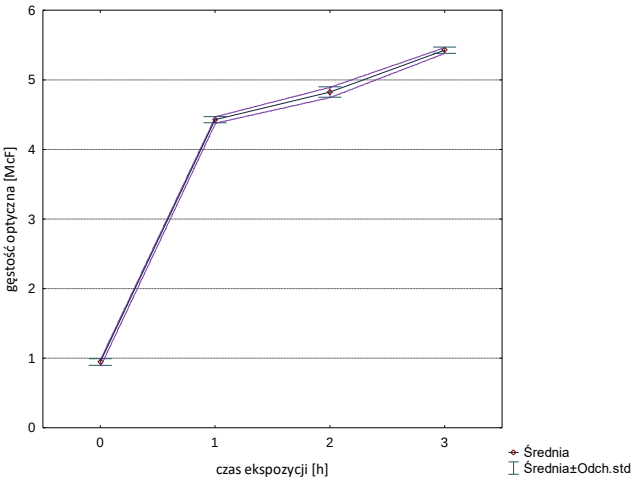

Fig 8: Optical density of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 4.2 kV/cm.

It was observed that the optical density of *Escherichia coli* ATCC 25922 increased with increasing exposure time to an electric field of 8.6 kV/cm (Figure 9). The highest optical density was obtained for the sample exposed for three hours, which was 7.8 McF, while the lowest for the control sample was 0.94 McF. The highest increase in optical density values occurred between the control sample and the sample subjected to three-hour exposure, which was 6.86 McF.

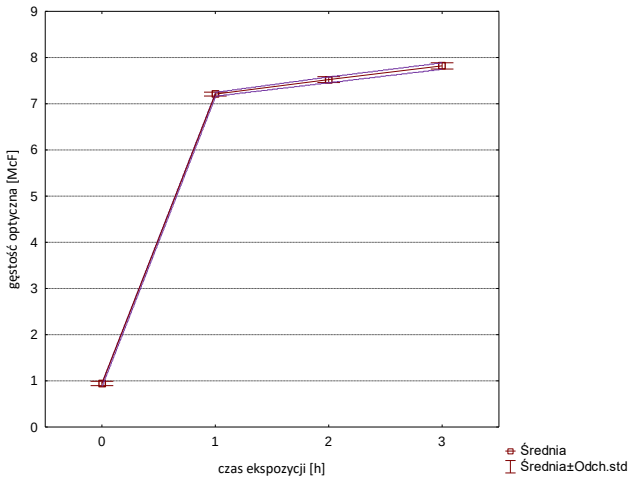

Fig 9: Optical density of *Escherichia coli* ATCC 25922 obtained for eucalyptus oil exposed to a constant electric field of 8.6 kV/cm.

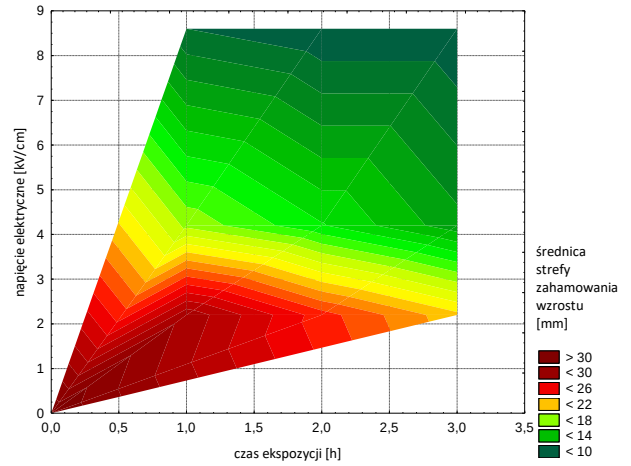

Fig 10: Relationship between electric voltage and exposure time of a constant electric field and the diameter of the zone of inhibition of *Escherichia coli* ATCC 25922

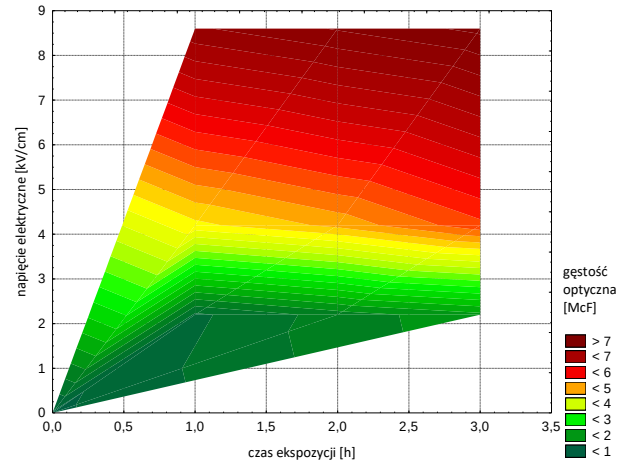

Fig 11. Relationship between electric voltage and time of exposure of a constant electric field and optical density of *Escherichia coli* ATCC 25922

The red area of the graph (Figure 10) represents the size of the diameter of the zone of inhibition of *Escherichia coli* ATCC 25922 above 22 mm. High values of the diameter of the growth inhibition zone were recorded when an electric voltage of up to 3.5 kV/cm and an exposure time of up to 2.5 hours were applied. A gradual decrease in the size of the diameter of the zone of growth inhibition of *Escherichia coli* (yellow turning to green) was observed when the electric field was exposed at voltages above 3.5 kV/cm regardless of the exposure time.

The red area of the graph (Figure 11) represents the magnitude of the optical density of *Escherichia coli* ATCC 25922 above 6 McF. High values of optical density were recorded when an electric voltage between 0.7 and 3 kV/cm and an exposure time of more than four hours were applied. Low optical density values of *Escherichia coli* (green) up to 3 McF were observed at electric field exposures of up to three hours regardless of electric field voltage.

A one-way analysis of variance (ANOVA) was performed, for which Statistica 13 (StatSoft, Inc., Tulsa, OK, USA) was used. The significance of differences between the averages was verified using Scheffe's test (α=0.05). The results are shown in Table 1. Four homogeneous groups were identified for both the size of the zone of inhibition and the optical density of the bacterial suspension. The first homogeneous group included the size of the growth inhibition zone and optical density obtained for the control sample. The second included values obtained using an electric voltage of 2.2 kV/cm, while the third group included values obtained using an electric voltage of 4.2 kV/cm. Meanwhile, the fourth group included values recorded using an electric voltage of 8.6 kV/cm. This shows that the size of the zone of inhibition as well as the optical density of the bacterial suspension varied between homogeneous groups.

Table 1. The size of the zone of growth inhibition and the optical density of *Escherichia coli* ATCC 25922 depending on the applied electric voltage.

Electric voltage [kV/cm]	Size of stunted zone [mm]	Optical density [McF]
0	31,8[a]	0,94[a]
2,2	24,8[b]	1,58[b]
4,2	14,97[c]	4,9[c]
8,6	9,3[d]	7,5[d]

- calculations made at the significance level of α=0.05
a - first homogeneous group, b - second homogeneous group, c - third homogeneous group, d - fourth homogeneous group

Three homogeneous groups were distinguished for the size of the inhibition zone The first homogeneous group included the size of the inhibition zone obtained for the control sample. The second included the values obtained with one hour of electric field exposure, while the third group included the values obtained with two and three hours of exposure. This shows that the size of the zone of growth inhibition varied between homogeneous groups. As for the optical density of the bacterial suspension, two homogeneous groups were distinguished. The first group included the optical density value obtained for the control sample, while the other values were included in the second homogeneous group. This shows the variation of optical density values between homogeneous groups.

Table 2. The size of the zone of growth inhibition and optical density of *Escherichia coli* ATCC 25922 depending on the applied electric field exposure time.

Exposure time [h]	Size of stunted zone [mm]	Optical density [McF]
0	31,8[a]	0,94[a]
1	18,8[b]	4,3[b]
2	16,2b[c]	4,7[b]
3	14,2[c]	5,1[b]

- calculations made at the significance level of α=0.05
a - first homogeneous group, b - second homogeneous group, c - third homogeneous group

Conclusion

The effect of a constant electric field affects the antiseptic properties of eucalyptus oil used against Escherichia coli ATCC 25922. A loss of antiseptic properties of eucalyptus oil was observed after stimulation with a constant electric field, with the strongest effect recorded when an electric voltage of 8.6 kV/cm was applied regardless of the stimulation time of the material. An inversely proportional relationship was also recorded between the inhibition zone values and the optical density values.

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