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COMPARATIVE STUDY OF THE FUNGICIDAL ACTIVITY OF Ce, La, Ag CATIONS AND CONTAINING THEM NANOCOMPOSITES

In this study, a comparative analysis of the antifungal activity of cations of cerium, lanthanum and silver, as well as nanosized oxides and silver-containing composites based on rare earth elements (REE), was performed. The oxides were synthesized by chemical precipitation followed by thermal treatment at 400-600 °C and characterized using X-ray diffraction, scanning electron microscopy, energy-dispersive spectroscopy and electrokinetic methods. Cerium dioxide formed a fluorite-type crystal structure, while lanthanum oxide crystallized in a hexagonal system. Silver formed metallic clusters on oxide surfaces due to Ce^{+}/Ce^{4+} oxidation and Ag^{+}/Ag^{0} reduction processes. Antifungal activity was evaluated against Aspergillus niger, Candida albicans, Cyberlindnera jadinii, Mrakia aquatica, and Penicillium polonicum using the agar diffusion method. Silver nitrate solutions showed the strongest inhibitory effect against all tested fungi. Unmodified oxides demonstrated limited activity, inhibiting only P. polonicum. In contrast, silver-modified oxides exhibited enhanced antifungal activity against most tested strains. Overall, silver cations and containing them nanocomposites showed larger inhibition zones compared to the antibiotic control, indicating their high potential as antifungal agents.

Key words: *cerium dioxide, lanthanum oxide, silver, nanocomposites, rare earth elements, micromycetes, fungicidal activity*

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Statement of the problem

Today, the usage of nanobiomaterials represents a promising strategy for the development of modern biomedical products [1, 2]. Among nanoscale structures successfully used in the creation of biosensors, drug delivery systems, and contrast agents, rare earth (REE) metal oxides are becoming widespread [3]. It should be noted that among the wide variety of rare earth oxides, cerium dioxide [4] has acquired the greatest practical use in technology and biomedicine, which is characterized by the fact that cerium cations on the surface of CeO_2 nanoparticles are in the oxidation states Ce^{3+}/Ce^{4+}, which gives the structure redox properties; In oxygen-deficient environments, cerium oxide acts as a source of oxygen. After the oxide surface has released oxygen, Ce^{4+} is reduced to Ce^{3+} using a residual electron, which, in turn, leads to the formation of oxygen vacancies [5]. In addition, the redox properties of the particles provide for the reduction of noble metal clusters and even promote the formation of core-shell structures [6]. The inclusion of noble metals, in particular silver, into the structure of cerium dioxide not only gives the particles photocatalytic activity [7], but also enhances the bactericidal activity of the CeO_2&Ag nanocomposite [8]. A less common rare earth oxide used in biomedical research is lanthanum oxide, which can crystallize in several crystallographic forms, which affects not only the structure of the particles, but also their morphology, size, and physicochemical properties. The La_2O_3 structure has a strong ability to absorb hydroxyl radicals [9]. In [10], it was shown that lanthanum-doped CeO_2 exhibited significant in vitro bactericidal activity against Escherichia coli at lanthanum concentrations of 4–6 wt %, and it was demonstrated that the La-CeO_2 composite acts as an inhibitor of E. coli β-lactamase and E. Additionally, the quantum dots demonstrated significant photocatalytic degradation of methylene blue in an alkaline medium. Modifying lanthanum oxide particles with silver broadens the range of functional applications for composites, imparting antioxidant and significant antibacterial effects in vitro [11]. In recent years, more complex composite systems have become increasingly popular; their multicomponent nature further expands the spectrum of so-called useful properties of materials. For example, a ternary system based on cerium dioxide, lanthanum oxide, and silver has shown high photocatalytic activity in the decomposition of the organic dye Malachite Green under visible light conditions [12].

Thus, the creation of nanoscale structures of rare earth oxides and composites based on them, which include cations of REE and noble metals, and the study of their biological activity, in particular, the effect on the growth processes of fungal mycelium, is an urgent task for the creation of new antifungal drugs.

Analysis of previous research

Although silver is widely used in the development of materials for biomedical applications, the effectiveness of cationic forms of silver, metallic silver particles and composites modified with silver on biological systems remains a matter of debate. Silver nanoparticles are among the most extensively studied categories of nanomaterials, which are successfully used to create new and improved biomaterials and biotechnologies, with applications in the pharmaceutical and cosmetics industries, anti-infective therapy and wound care, and the food and textile industries [13]. Silver is used in sensor and diagnostic platforms, restorative and regenerative biomaterials, and so on. Silver nanoparticles are used as therapeutic agents in modern and alternative cancer treatment strategies, as antimicrobial agents, coatings for biomedical devices, drug delivery carriers, imaging probes, and in diagnostic and optoelectronic platforms, as they possess unique physical and optical properties and biochemical functionality that depend on the particles' size [14].

At the same time, ionic forms of silver have not been left out of researchers' attention [15]. Advances in coordination chemistry open up significant opportunities for the development of new compounds that may include silver ions. In particular, such compounds exhibit increased efficiency and an expanded spectrum of antimicrobial action.

Silver nitrate, silver sulfadiazine, and silver sulfathiazole are most often used as drugs. In-depth studies of their physicochemical, microbiological, cytotoxic, and genotoxic properties are ongoing and are associated with certain difficulties. Systemic use of drugs containing silver in the form of salts or coordination compounds can cause generalized or local argyria. However, this does not exclude local use, i.e., introduction into body cavities. They promote wound healing, eliminate skin lesions, or have a beneficial effect on the eye, etc. However, recent publications have discussed the results of studies indicating the potential environmental, health and biosafety hazards of silver nanoparticles [16]. According to in vitro cell culture studies, silver nanoparticles have been reported to be toxic to several human cell lines. Silver particles (10 nm) are cytotoxic and can cross the blood-brain barrier in mice via the circulatory system. They tend to accumulate in the internal organs of animals. Thus, the effects of nanosized silver are ambiguous; they may kill microorganisms but cause cytotoxicity in mammalian cells.

In addition, the use of rare-earth elements in the form of particles and aquahydroxoforms are becoming increasingly widespread in the development of materials for biomedical applications. Rare-earth elements (REEs) have recently attracted considerable attention in biomedicine, agriculture and animal husbandry.In particular, a review article [17] examined the interaction between biological molecules and REEs, which may provide valuable insights into their biological effects. It also examined pharmaceutical complexes of REEs and attempted to establish a link between the fundamental chemistry of REEs and their biological effects, which will significantly advance the field of lanthanide chemical biology. Based on the results of recent advances, it is expected that new initiatives in the near future will lead to a marked improvement in our understanding of the role of REEs in living organisms, as well as to the expanded use of the unique properties of REEs for the development of new applications in diagnostic procedures and the creation of powerful medical devices [18].

In our previous studies, cerium and lanthanum oxide particles and silver-modified composites based on them were synthesized [19]. The resulting structures were investigated for bactericidal activity [20] and showed that CeO_2, TiO_2 and La_2O_3 with an Ag concentration of 4 wt.% inhibited the growth of prokaryotic cells E. coli, Bacillus sp. and S. aureus compared to pure oxides. Replication of influenza A and herpes viruses was completely inhibited by the CeO_2–Ag (2.5 wt.%) and La_2O_3–Ag (2.5 wt.%) nanocomposites. At the same time, silver-modified nanosized cerium and lanthanum oxides, as well as a ternary composite based on them, demonstrated virucidal activity. When irradiated with UV light for 30 minutes, they effectively inhibited influenza A virus, but did not change their antiviral properties against HAdV2 and HSV-1/US. CeO_2-Ag particles ($pH_{pzc} > 5$) were excellent photosensitizers, leading to the inactivation of the viral RNA genome [21].

The aim of the study is to compare the effect of nanoscale particles of cerium and lanthanum oxides, their silver-modified composites, and solutions of inorganic salts of the corresponding metals on the inhibition of fungal growth.

Methodology

Synthesis and characterization of nanoparticles

The particles were synthesised by the chemical precipitation method using solutions of $Ce(NO_3)_3$, $CeCl_3$, $La(NO_3)_3$, and $AgNO_3$ in an alkaline medium, with controlled addition of auxiliary substances (urea and potassium hydroxide) to the suspensioni. Cerium and lanthanum hydroxides and their mixture were precipitated separately, and a solution of silver nitrate in a mass ratio of 2 mass.% was introduced into the suspension, along with the reducing agent hydroxylamine. The hydroxide precipitates were dried at 110 °C and then ерун цуку calcined at T = 400 °C for cerium hydroxide and at 600 °C for lanthanum oxide for 2 hours., solutions of inorganic cerium and lanthanum salts were prepared with a concentration of 1 g/dm³ at a pH of 6.5–7 to compare the effectiveness of the biological action on the fungal growth.

Characterization of particles was carried out by X-ray phase analysis, scanning electron microscopy and energy dispersive spectroscopy. Electron micrographs of samples were recorded on a MIRA3 TESCAN scanning electron microscope. Determination of the phase composition of the obtained samples was carried out by powder X-ray diffraction analysis on a DRON-3 device with copper anode radiation. To determine the phase composition, the database JSPDS International Center for Diffraction Data 1999 was used. The zero charge point was measured according to the standard titration method,

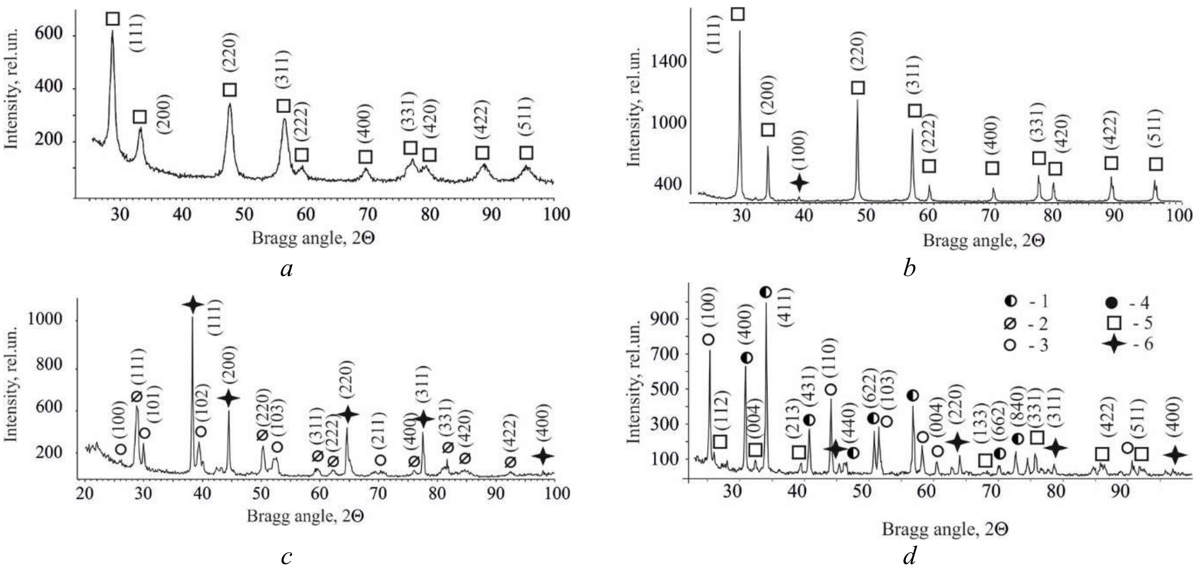
Antifungal activity

The antifungal activity was evaluated using agar well diffusion against *Aspergillus niger* Tiegh. VURV-F 822, *Candida albicans* (C.P. Robin) Berkhout N-023, *Cyberlindnera jadinii* (Sartory, R. Sartory, Jos. Weill & J. Mey.) Minter N-022, *Mrakia aquatica* (E.B.G. Jones & Slooff) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout N-021, *Penicillium polonicum* K.W. Zaleski VURV-F 823. All tested fungal strains were grown on plates with malt extract agar medium (Scharlau Chemie S.A., Spain). *Aspergillus niger* and *P. polonicum* were incubated at 26±1°C for 3-5 days until sporulation, then the spore suspension in concentration of 1×10^6 spores/mL was prepared with sterile distilled water. *Candida albicans, C. jadinii* and *M. aquatica* were incubated at 37±1°C for 24 h, then the standard 0.5 McFarland fungal suspensions were prepared. One milliliter of each fungal suspension was uniformly distributed on agar MEA plates. The wells were cut with a sterile cork-borer; 50 µL of the sample suspensions at 10 mg/ml concentrations in 10% DMSO were transferred to each well and the plates were incubated at the indicated temperatures for 24-72 hours. Discs containing amphotericin B (10µg) were used as positive controls and 10% DMSO was used as a negative control. All tests were performed in triplicate and the antifungal activity was expressed as the mean of inhibition diameters (mm).

Results and discussion

1. Characterisation of cerium and lanthanum oxide particles and composites based on them

The study of the structure of nanomaterials, conducted by the method of X-ray phase analysis, indicates that heat treatment of cerium hydroxide precipitate at a temperature of 400 °C leads to the formation of cerium dioxide nanopowder (JSPDS No. 34-0394), Fig. 1a. The crystal lattice parameter of cerium dioxide is a = 0.5397 nm, the size of primary particles or the coherent scattering region (CSR) is 7.0 nm. According to energy dispersive spectroscopy, the cerium content is 68.7 wt.%, oxygen – 18.2 wt.%; the content of auxiliary substances – (carbon and potassium) – 132.1 wt.%. When introducing agrantum nitrate into a suspension of cerium hydroxide with subsequent heat treatment of the precipitate leads to the formation of metallic silver clusters on the surface of cerium dioxide particles (JSPDS No. 4-0783). (Fig. 1b). This is accompanied by a decrease in the crystal lattice parameters a = 0.5393 nm and the CSR – 6.5 nm. According to the EDS data, the mass content of cerium is 77.1%, oxygen – 17.5, and argentum – 1.9%. For particles of lanthanum dioxide (JSPDS No. 05-0602), modified with silver, the crystal lattice parameters are a = 0.3937 nm and c = 0.6129 nm, and the CSR of the parameter is 12.5 nm. (Fig. 1c). The mass content of elements according to the UDS is: lanthanum – 72.0 wt.%, oxygen – 23.3 wt. %, argentum – 2.1 wt.%, sulfur – 2.6 wt.%. The cerium dioxide sample supplemented with lanthanum and silver consists of phases of two lanthanum oxides belonging to the cubic system, space group la3 and trigonal system with space group P3m1 (JCPDS file No. 02-0688), cerium dioxide with space group Fm3m (JCPDS file No. 34-0394) and reduced silver (JCPDS file No. 04-0783) (Fig. 1e). According to the EDS data, the cerium content is 78.5 wt.%, oxygen – 16.8 wt.%, lanthanum – 1.67 wt.%, argentum – 2.1 wt.%, sodium – 0.93 wt.%.



a – CeO_2, 400 °C; *b* – CeO_2&Ag, 400 °C; *c* – La_2O_3&A, 600 °C; *d* – CeO_2&La_2O_3&Ag , 600 °C

Fig. 1 – X-ray diffraction patterns of rare-earth oxide powders and silver-modified composites based on them. Numbers correspond to phases: *1* – La_2O_3 (P3m1); *2* – LaO (F); *3* – La_2O_3 (la3); *4* – $La(OH)_3$ (P63/m); *5* – CeO_2 (Fm3m); *6* – Ag^0

The morphology of the particles is presented in SEM images (Fig. 2). Cerium dioxide particles formed by the precipitation of nitrate solutions (Fig. 2a) and cerium chloride (Fig. 2b) are characterized by lower particle crystallinity and spherical shape of aggregates, while the development of faces is observed for particles obtained in a cerium nitrate solution. Fig. 2c shows aggregates of cerium dioxide with reduced silver clusters. The size of the aggregates varies from 200 nm to 1 μm. Lanthanum oxide is a weakly crystallized plate (Fig. 2d). When silver is introduced into the system, the degree of crystallinity increases and polyphase nature of oxidized forms of lanthanum is manifested, which is associated with the low temperature of heat treatment of the powder (Fig. 2e). Silver is reduced on the surface of lanthanum oxide in the form of clusters. The ternary system is characterized by heterogeneity and crystallization of all components of the composite with a decrease in the size of the components (Fig. 2f).

2. Antifungal well-diffusion assay

The results of the evaluation of antifungal activity are provided in the table 1. Overall, the highest activity with the largest inhibition zones against all tested fungi was exhibited by $AgNO_3$. In contrast, cerium and lanthanum salts demonstrated moderate and selective activity. $Ce(NO_3)_3$ and $LaCl_3$ showed similar inhibition patterns, being active against *C. jadinii, M. aquatica* and *P. polonicum*. $CeCl_3$ displayed slightly higher activity, including a moderate effect against *C. albicans.*

CeO_2 exhibited limited antifungal activity, inhibiting only *P. polonicum* (17 mm). The observed targeting can be attributed to species-specific structural and physiological differences, for instance, variations in cell wall structure and antioxidant defense mechanisms [22, 23]. At the same time, none of the tested fungi were sensitive to CeO_2&La_2O_3 and La_2O_3, indicating that the combination of oxides alone did not enhance antifungal properties.

Among cerium and lanthanum oxides, their silver-modified composites demonstrated significantly improved activity. The CeO_2&Ag composite showed strong inhibition of all tested fungi, particularly against *C. jadinii* (29 mm) and *M. aquatica* (27 mm), although its activity against *A. niger* (19 mm) remained lower than that of $AgNO_3$. The CeO_2&La_2O_3&Ag composite exhibited relatively moderate activity, which could be presumably related to changes in silver availability or surface interactions.

Notably, all silver-containing systems outperformed the reference antifungal agent Amphotericin B, which showed relatively low inhibition zones (Table 1). Overall, the results indicate that silver plays a dominant role in antifungal activity, while rare earth oxides contribute mainly through synergistic effects.

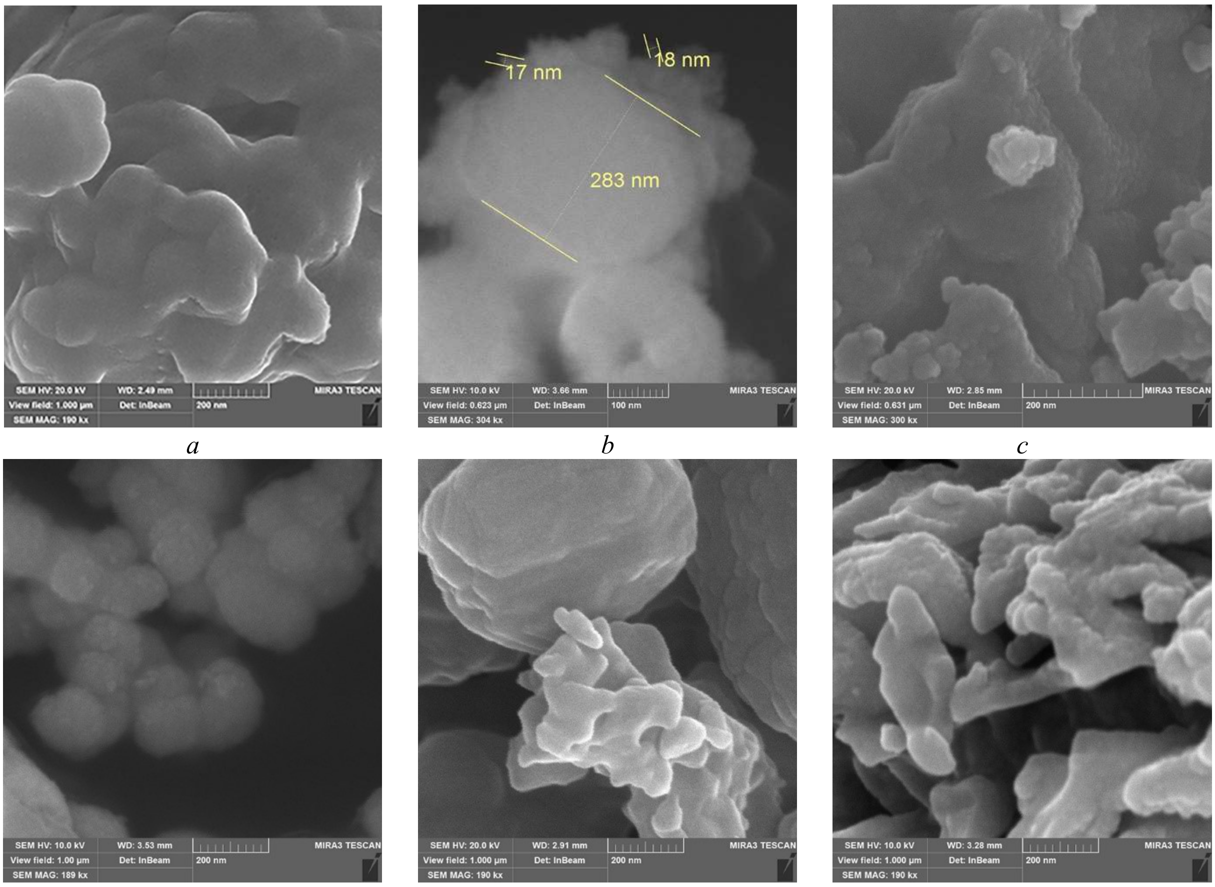


Fig. 2 – SEM images of the nanoparticles used for the study of fungal growth inhibition: *a* – CeO_2 formed in $Ce(NO_3)_3$ solution; *b* – CeO_2 formed in $CeCl_3$ solution; *c* – CeO_2&Ag; *d* – La_2O_3; *e* – La_2O_3&Ag; *f* – CeO_2&La_2O_3&Ag

Table 1 – Antifungal activity of the samples by well diffusion method

The reactive component	Growth inhibition zone diameter (mm)				
	Candida albicans	*Cyberlindnera jadinii*	*Mrakia aquatica*	*Penicillium polonicum*	*Aspergillus niger*
$AgNO_{3(aq)}$*	–	20	17	14	–
$Ce(NO_3)_{3(aq)}$*	–	19	17	14	–
$LaCl_{3\,(aq)}$*	13	20	18	14	–
$CeCl_{3\,(aq)}$*	17	29	27	23	19
CeO_2&$Ag_{(s)}$**	–	–	–	–	–
CeO_2&$La_2O_{3(s)}$**	–	–	–	17	–
$CeO_{2(s)}$**	–	–	–	–	–
$La_2O_{3(s)}$**	–	17	–	15	14
CeO_2&'La_2O_3&$Ag_{(s)}$**	12	9	13	10	12
Amphotericin B	–	20	17	14	–

* aq – salt solution
** s – solid phase, nanoparticles

Fig. 3 – Antifungal activity by the agar well diffusion method showing inhibition of *Penicillium polonicum* (*A*), *Mrakia aquatica* (*B*), *Cyberlindnera jadinii* (*C*)

Prospects for further research. Further research will focus on determining the contribution of the anionic components of solutions, in particular nitrates and chlorides, to the inhibition of fungal growth.

Conclusion. To conduct a comparative analysis of the effect of cerium, lanthanum and silver on the inhibition of fungal growth, solutions of inorganic salts of the corresponding metals and nanosized particles of cerium and lanthanum oxides and their composites modified with silver were selected. The synthesis of nanoparticles was carried out by chemical precipitation of inorganic salts in the presence of auxiliary substances. The phase composition of the particles after heat treatment of the solutions corresponds to cerium and lanthanum oxides, silver forms metal clusters on the surface of the particles. The particle size varies within 6.5-12.5 nm. The degree of crystallinity of the composites increases in the presence of silver. According to the EDS data, in addition to the main elements of : Ce, La, O, and Ag, the particles contain insignificant amounts of auxiliary elements, in particular, C, S, N, and K.
Overall, silver-containing composites exhibited the highest antifungal activity, with $AgNO_3$ showing the strongest and broad-spectrum effect, while cerium and lanthanum composites demonstrated moderate and species-specific activity. The incorporation of silver significantly enhanced the antifungal properties.

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ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ФУНГІЦИДНОЇ АКТИВНОСТІ КАТІОНІВ Ce, La, Ag ТА НАНОКОМПОЗИТІВ НА ЇХ ОСНОВІ

На сьогоднішній день актуальною задачею наукової спільноти є розробка новітніх матеріалів, які характеризуються високою активністю при знешкодженні патогенних організмів: бактерій, грибів, вірусів тощо. В той час як бактерицидна, фунгіцидна і віруліцидна активність срібла відома вже впродовж сторіччя, біологічна активність наноматеріалів досліджується відносно нещодавно. В нашому дослідженні було проведено порівняльний аналіз активності катіонів церію, лантану та срібла, а також нанорозмірних оксидів та срібловмісних композитів на основі рідкісноземельних елементів (РЗЕ). Синтез оксидів проводили хімічним методом осадження в слабколужному середовищі з додаванням допоміжних речовин, що забезпечують регулювання процесом зародкоутворення та росту частинок. Отримані осади підлягали термообробці за температур 400-600 °С. Фізико-хімічну характеристику частинок проводили методами рентгенофазового аналізу (РФА), сканувальної електронної мікроскопії, енергодисперсійної спектроскопії, електрокінетичних досліджень. За даними РФА структури на основі діоксиду церію утворюють кристалічну решітку типу флюориту, а оксид лантану кристалізується в гексагональній сингонії. Срібло утворює металічні кластери на поверхні частинок діоксиду церію і нанокомпозиту на основі оксидів лантану і церію, що обумовлене окисно-відновною реакцією за рахунок окиснення церію (Ce^{3+}/Ce^{4+}) і відновлення срібла (Ag^{+}/Ag^{0}). Для отримання катіонів церію, лантану і аргентуму використовували неорганічні солі (нітрати і хлориди) з регулюванням значення pH середовища. Для проведення біологічних досліджень були обрані гриби Aspergillus niger, Candida albicans, Cyberlindnera jadinii, Mrakia aquaticata Penicillium polonicит. Антифунгальну активність визначали загальноприйнятим методом дифузії в агар з використанням лунок. Результати досліджень враховували за розміром зон затримки росту колоній грибів. Отримані дані свідчать про те, що найбільше ріст усіх обраних для експерименту видів грибів пригнічують розчини нітрату арґентуму, в той час як нітрат і хлорид арґентуму проявляють активність проти C. jadinii, M. aquatica та P. polonicит. Немодифіковані сріблом частинки оксидів церію і лантану, а також їх подвійні композити демонструють затримку росту лише P. polonicит. Водночас, формування кластерів срібла на поверхні перелічених оксидів сприяє до прояву антифунгальної активності для всіх протестованих штамів грибів. Виключенням є потрійна система CeO_2-La_2O_3-Ag, яка призводить до затримки росту C. jadinii, P. polonicит, A. niger. Порівняння активності катіонів і оксидів РЗЕ модифікованих сріблом вказує на збільшення зон пригнічення росту колоній грибів у порівнянні з антибіотичним засобом, використаним в якості контролю.

Таким чином, використання модифікованих сріблом оксидів РЗЕ, зокрема, церію і лантану, а також розчинів, які містять РЗЕ і арґентум може бути доцільним для створення засобів, які характеризуються фунгіцидною активністю.

***Ключові слова**: діоксид церію, оксид лантану, срібло, нанокомпозити, рідкісноземельні елементи, мікроміцети, фунгіцидна активність*

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