Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Sporicidal properties of a composite nanomaterial based on silver and copper nanoparticles and chlorhexidine against the anthrax pathogen

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Article info
Received 07.03.2026
Received in revised form 09.04.2026
Accepted 02.05.2026

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Derevianko, S. V., Bolotin, V. I., Ukhovskyi, V. V., Arefiev, V. L., Demchenko, N. R., Romanko, M. Y., Kaplunenko, V. H., & Demchenko, A. M. (2026). Sporicidal properties of a composite nanomaterial based on silver and copper nanoparticles and chlorhexidine against the anthrax pathogen. Regulatory Mechanisms in Biosystems, 17(3), e26063. doi:10.15421/0226063

The search for new substances with bactericidal and sporicidal properties and the development of modern antimicrobial preparations and disinfectants is one of the most important tasks in microbiology. In this work the sporicidal properties of nanoparticles (NPs) and composite nanomaterials (NMs) against the *Bacillus anthracis* strain "SB" were studied. The sporicidal properties of chelated Zn NPs, Se/I NPs, NMs based on Ag/Cu NPs, CHH, and NMs based on Ag/Cu NPs combined with CHH were studied at concentrations ranging from 0.0125 to 250 mg/cm³ and exposure times of 2, 6, 12, and 24 hours using spores of *B. anthracis* strain "SB". Viability of spores was assessed on Petri dishes with tryptic soy agar. Colony counting was performed after 24 hours of incubation at 37 ± 0.5 °C. It was established that upon contact of *B. anthracis* "SB" strain spores with CHH at a concentration of 380 μg/cm³ for 2 hours, high statistically significant sporicidal activity (100 %) was observed. At a concentration of 38 μg/cm³, CHH caused a significant reduction in spore viability by 89.9%. High sporicidal activity was exhibited by Ag/Cu NPs at concentrations of 250 and 25 μg/cm³ of each element, causing statistically significant spore inactivation of 100% and 78.9%, respectively. At a concentration of 2.5 μg/cm³, a significant reduction in spore viability by 19.7% was observed. NMs based on Ag/Cu NPs and CHH at concentrations of 125, 125, and 190 μg/cm³ of each composite respectively, caused 100% loss of spore viability of the *B. anthracis* "SB" strain. With a combination of particles at a concentration of 1.25 μg/cm³ (Ag), 1.25 μg/cm³ (Cu), 1.90 μg/cm³ (CHH), the spore titer was significantly reduced by 56.4%. On tryptic soy medium, the *B. anthracis* "SB" strain forms opaque white colonies with irregular edges, 2–3 mm in size. No changes in colony morphology were observed under the action of CHH. Upon contact with NMs based on Ag/Cu NPs and NMs based on Ag/Cu NPs combined with CHH, a 2–4-fold reduction in colony size compared to the control was observed. Thus, all tested substances demonstrated high sporicidal activity. The highest sporicidal activity was shown by NMs based on Ag/Cu NPs combined with CHH. The influence of the above-mentioned NMs on colony morphology was noted. These investigated NMs can be used for the development of methods for soil sanitation and decontamination of environmental objects, as well as for the development of antimicrobial agents against the anthrax pathogen.

Keywords: nanoparticles; chlorhexidine hydrochloride; composite nanomaterial; sporicidal properties; *Bacillus anthracis.*

Introduction

Anthrax remains one of the most dangerous zoonotic diseases in the world. It is recorded in animals and humans in numerous countries across all continents and represents not only a veterinary but also a significant medical, economic, political, and military concern (Goel, 2015; Finke et al., 2020). The causative agent is the gram-positive, facultatively anaerobic, rod-shaped bacteria *Bacillus anthracis*, which forms highly stable, heat-resistant spores remaining viable for decades (Baweja et al., 2008). The bacteria are non-hemolytic, non-motile, and capsulated (Apriliana et al., 2025).

Vegetative forms are generally susceptible to penicillin, tetracycline, ciprofloxacin, doxycycline, pefloxacin, levofloxacin, teicoplanin, vancomycin, clindamycin, imipenem, and rifampicin (Apriliana et al., 2025). However, antibiotic resistance has been described. Among 96 *B. anthracis* isolates collected in France (1994–2000), 11.5% were resistant to penicillin G and amoxicillin, and all were resistant to co-trimoxazole (Cavallo et al., 2002). Susceptibility testing of three Ukrainian isolates (BA-C-12-Sm, BA-C-10-Chern, BA-D-12-Mel) from the National Center for Microorganism Strains against 43 antibiotics showed high sensitivity to norfloxacin, ampicillin/sulbactam, and tetracycline, whereas metronidazole and nystatin exhibited no inhibitory effect.

For surface decontamination of anthrax-contaminated objects, the following disinfectants are officially recommended: 10% hot sodium hydroxide; 4% formaldehyde; chlorine-based preparations containing ≥5% active chlorine; 10% dichloroisocyanuric acid sodium salt (10% active chlorine); 10% iodine monochloride (wooden surfaces only); 7% hydrogen peroxide + 0.2% OP-10; 2% glutaraldehyde. With the exception of iodine monochloride, hydrogen peroxide, and glutaraldehyde, disinfection is performed three times at 1-hour intervals (1–2 L/m² depending on facility type) (Whitney et al., 2003).

A novel integrated soil decontamination method against *B. anthracis* spores has been developed at the Institute of Veterinary Medicine of the NAAS of Ukraine. The protocol combines a germinant mixture (0.05% each of inosine, L-histidine, L-valine, L-serine) with a quaternary ammonium compound-based disinfectant (1:1 mixture of 0.5% benzalkonium chloride and 0.5% didecyldimethylammonium chloride), achieving 99.9% spore inactivation (Yim et al., 2021; Bezymennyi & Tarasov, 2023; Nuradji & Adji, 2023).

Among emerging approaches, the use of nanoparticles (NPs) and composite nanomaterials (NMs) is particularly promising. Antimicrobial properties of Ag, Au, and Cu NPs are best characterized (Naika, 2015; Holubnycha et al., 2024), followed by ZnO, TiO, MgO, I, S, Se NPs (Demchenko et al., 2020; Dop et al., 2023; Feghi et al., 2023; You et al., 2023; Mondal et al., 2024). Recent advances in nanotechnology open broad prospects for the production and use of composite nanomaterials (NMs). This direction is highly promising. It has been established that composite NMs consisting of NPs of several chemical elements exhibit a synergistic antibacterial effect (Vidic et al., 2013).

A hypothesis was proposed about possible synergy between silver and copper nanoparticles. The antibacterial effect of mixtures of CuO nanoparticles (CuO, $CuO–NH_2$, CuO–COOH) with silver nanoparticles (uncoated and coated) was studied. Combinations of CuO and Ag NPs were more effective than Cu or Ag NPs alone against a wide range of bacteria, including antibiotic-resistant strains such as gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, and gram-positive *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus dysgalactiae* (Vasiliev et al., 2023).

Several commercial and experimental disinfectants based on Ag/Cu NPs have been developed (e.g., "Shumerian Silver") with proven activity against *E. coli*, *S. aureus*, and surface biofilms. Other high-performance composite NMs include FeO@SiO/Schiff-base/Cu(II), ZnO-CuO/chitosan, and Se/CuO systems (Jose et al., 2022; Abou Baker & Abbas 2023; Abbasi et al., 2024; Azadi et al., 2024), showing high antibacterial activity against a wide range of bacteria (*S. aureus*, *E. faecalis*, *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*), including antibiotic-resistant strains (Asaad et al., 2025). Chlorhexidine-containing calcium phosphate/fluoride nanocomposites effectively inhibit *Streptococcus mutans* biofilm formation and metabolism (Cheng et al., 2012).

Despite extensive data on antibacterial NPs, information on their sporicidal activity against *B. anthracis* remains limited (Demchenko et al., 2024; Tsakiridou et al., 2024). Carbon-doped TiO NPs [TiO(C)] have shown visible-light-activated sporicidal efficacy against *B. anthracis* (Sun et al., 2016). Given the above, the aim of the present study was to evaluate the sporicidal properties of nanoparticles and composite nanomaterials against *B. anthracis* strain "SB".

Materials and methods

The study utilized composite nanomaterials (NMs) based on citric acid-chelated Zn nanoparticles (initial concentration 4 mg/cm^3), Se/I nanoparticles (concentrations 42 μg/cm^3 and 112 μg/cm^3 for Se and I, respectively), and Ag/Cu nanoparticles (500 μg/cm^3 of each element). All nanoparticles were produced by pulsed laser ablation of high-purity metal and non-metal granules in deionized water. Chlorhexidine hydrochloride (CHH) at a concentration of 760 μg/cm^3 was used as a pharmaceutical-grade analytical standard substance manufactured by GlaxoSmithKline Pharmaceuticals S.A. (Fig. 1).

The substance was initially dissolved in a minimal volume of dimethyl sulfoxide (DMSO). Composite NMs based on Ag/Cu NPs combined with CHH were prepared by mixing the components in a 1:1 volume ratio. A commercially available disinfectant containing 50 g of potassium peroxymonosulfate per 100 g of product served as a positive control. Excipients: sodium chloride, sulfamic acid, malic acid, polyphosphate, sodium dodecylbenzenesulfonate, dye, lemon fragrance. Working solution: 40 g dissolved in 10 L of water. Control series included equivalent concentrations of DMSO and citric acid as present in the tested colloidal nanoparticle solutions, as well as sterile distilled water.

Sporicidal activity was evaluated using spores of the vaccine strain *B. anthracis* "SB" (deposited and maintained in the National Collection of Microorganism Strains at the State Scientific-Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine). Experiments were performed with a spore suspension of *B. anthracis* "SB" at a concentration of 1.5–3.0 × 106 CFU/cm^3. Test substances were added to the spore suspension in a 1:1 (v/v) ratio at various final concentrations.

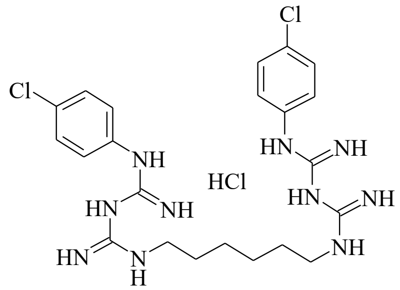

Fig. 1. Structural formula of chlorhexidine hydrochloride (CHH)

Controls consisted of sterile distilled water, DMSO solution, citric acid solution, and potassium peroxymonosulfate solution, all mixed 1:1 with the spore suspension. Exposure time was 2 hours at 22 ± 0.5 °C; selected experiments were also conducted at 6, 12, and 24 hours. Each experiment was performed in quadruplicate. Tryptic soy agar was used as the growth medium. Results were recorded after 24 hours of incubation at 37 ± 0.5 °C. Colony-forming units (CFU) were counted visually, and the number of viable spores per cm^3 was calculated using the formula:

$$A = \frac{X}{0{,}1} \times 10^n$$

where A – number of CFU per 1 cm^3 of the tested culture; X – number of colonies formed on solid nutrient medium after inoculation; n – dilution factor.

Atomic charges and molecular orbital energies were calculated using Chem3D software (ChemOffice package, PerkinElmer Informatics Inc.). Prior to quantum-chemical calculations, molecular geometry was optimized by the MM2 method (minimum RMS gradient = 0.010; step interval = 2; frame interval = 10; target temperature = 300 K; all parameters finalized; job type: minimize energy to a minimum RMS gradient of 0.010, display every iteration). Molecular orbital energies were computed using the Hückel method. Significance of differences was determined using Student's t-test ($P < 0.05$).

Results

Sporicidal activity of composite nanomaterials (NMs) based on citric acid-chelated Zn NPs was investigated at concentrations ranging from 2 to 200 μg/cm^3, Se/I NPs at concentrations from 2.1/5.6 to 0.021/0.056 μg/cm^3 (Se/I, respectively), and Ag/Cu NPs at concentrations from 250 to 0.25 μg/cm^3 of each element. Exposure time was 2 hours at 22 ± 1 °C against spores of *B. anthracis* "SB" (Table 1).

Citric acid-chelated Zn NPs at 200 and 20 μg/cm^3 significantly reduced viable spore titer by 23.6% and 28.7%, respectively. At 2.0 μg/cm^3, no significant effect was observed. Se/I NPs at 2.1/5.6 and 0.21/0.56 μg/cm^3 caused a significant 28.3% reduction in viable spore titer after 2 h exposure. At the lowest concentration (0.021/0.056 μg/cm^3), a non-significant reduction of 21.9% was recorded. The highest sporicidal activity was exhibited by citric acid-chelated Ag/Cu NPs. At 250 μg/cm^3 of each element, complete (100%) spore inactivation was achieved. At 25/25 and 2.5/2.5 μg/cm^3, viable spore titer was significantly reduced by 77.2% and 11.0%, respectively. At 0.25/0.25 μg/cm^3, no significant effect on spore viability was observed.

Thus, composite NMs based on citric acid-chelated Ag/Cu NPs demonstrated the highest sporicidal activity and were selected for further investigation. In the subsequent experiment, Ag/Cu NPs were tested at concentrations from 250 to 0.25 μg/cm^3 with exposure times of 2, 6, 12, and 24 hours at 22 ± 1 °C (Tables 2 and 3).

The viable spore titer in the control decreased naturally over the experiment duration, reaching 66.0% lower at 24 h compared to the initial value (Table 2). Ag/Cu NPs at 250 μg/cm^3 of each element achieved 100% spore inactivation already after 2 h, confirming the previous experiment. At 25 μg/cm^3 of each element, a statistically significant reduction in viable spore titer was observed at all exposure times (2, 6, 12, and 24 h). At 2.5 µg/cm³, significant reduction occurred only at 2 and 6 h. At the lowest concentration (0.25 µg/cm³), no change was seen at 2 h, a 30.5% reduction at 6 h, and paradoxically an increase in viable titer by 45.0% and 53.4% at 12 and 24 h, respectively (Table 3). However, both the increase and decrease at this concentration were not statistically significant in most cases.

At the next stage, the sporicidal properties of a heterocyclic compound (CHH) and a composite nanomaterial based on Ag/Cu NPs and CHH were studied. Given the energy of the lowest unoccupied molecular orbital (E_LUMO = +0.366), the studied compound exhibits nucleophilic properties and can form bonds with positively charged particles. The nucleophilic centers are nitrogen atoms bearing negative charges: N2 (−0.71146), N4 (−0.85463), N7 (−0.88918), N9 (−0.82385). The formation of such a complex increases the activity of its components.

Potassium peroxymonosulfate, DMSO solution, and citric acid were used as controls. It was found that CHH at 380 µg/cm³ possesses sporicidal activity, resulting in 100% reduction of viable *B. anthracis* "SB" spores compared to control already after 2 hours at room temperature (Table 4).

The same high sporicidal activity was shown by Ag/Cu NPs at 250 µg/cm³ (100% loss of spore viability) and at 25 µg/cm³ (75%). Composite NMs based on Ag/Cu NPs and CHH at concentrations of 125/125/190 and 12.5/12.5/19 µg/cm³ caused 100% loss of viability of *B. anthracis* "SB" spores. The disinfectant based on potassium peroxymonosulfate (positive control) at 1000 µg/cm³ reduced spore viability by 100%. Citric acid and DMSO at the used concentrations had no significant effect on *B. anthracis* "SB" spore activity.

Table 1
Sporicidal activity of Zn NPs, Se/I NPs, and Ag/Cu NPs against *B. anthracis* "SB" spores at different concentrations (exposure 2 h, temperature 22 ± 1 °C, n = 4)

Tested substance	NPs	NPs concentration, µg/cm³	Colony-forming units, CFU/cm³	Difference vs control, %
Control		0	59.25 ± 3.04	0
Zn		200.0	45.25 ± 3.94*	–23.6
		20.0	42.25 ± 1.49*	–28.7
		2.0	61.75 ± 2.78*	3.0
Se/I	Se	2.1	42.5 ± 1.32*	–28.3
	I	5.6		
	Se	0.21	42.5 ± 2.06*	–28.3
	I	0.56		
	Se	0.021	46.25 ± 1.84	–22.0
	I	0.056		
Ag/Cu	Ag	250.0	0	100
	Cu	250.0		
	Ag	25.0	13.5 ± 1.76**	–77.2
	Cu	25.0		
	Ag	2.5	52.75 ± 1.38*	–11.0
	Cu	2.5		
	Ag	0.25	60.25 ± 1.93	1.7
	Cu	0.25		

Note: * – the difference between the experiment and the control is statistically significant at P < 0.05, ** – at P < 0.001.

Table 2
Effect of Ag/Cu NPs on viability of *B. anthracis* "SB" spores at different concentrations and exposure times (temperature 22 ± 1 °C, n = 4)

NPs	NPs concentration, µg/cm³	Viable spores at different exposure, CFU/cm³			
		2 h	6 h	12 h	24 h
Control		75.75 ± 4.85	53.25 ± 3.97	32.75 ± 2.25	25.75 ± 4.92
Ag	250	0	0	0	0
Cu	250				
Ag	25	13.50 ± 1.55*	12.75 ± 1.49*	18.0 ± 3.63*	10.0 ± 1.22*
Cu	25				
Ag	2.5	49.0 ± 3.39*	32.50 ± 0.64*	47.25 ± 4.77	18.75 ± 2.95
Cu	2.5				
Ag	0.25	71.75 ± 3.70	37 ± 2.12	47.5 ± 2.99	39.5 ± 3.12
Cu	0.25				

Note: * – the difference between the experiment and the control is statistically significant at P < 0.05.

Table 3
Percentage difference in viable spore count of *B. anthracis* "SB" relative to control at different Ag/Cu NP concentrations and exposure times (temperature 22 ± 1 °C, n = 4)

NPs	NPs concentration, µg/cm³	Difference vs control at different exposure, %			
		2 h	6 h	12 h	24 h
Ag	250	–100	–100	–100	–100
Cu	250				
Ag	25	–82.2*	–76.1*	–45.0*	–61.2*
Cu	25				
Ag	2.5	–64.7*	–39.0*	44.3	–27.2
Cu	2.5				
Ag	0.25	–0.1	–30.5	45.0	53.4
Cu	0.25				

Note: see Table 2.

At the next stage, minimal active concentrations of CHH and composite NMs based on Ag/Cu NPs and CHH against *B. anthracis* "SB" spores were determined using ten-fold serial dilutions. Potassium peroxymonosulfate-based disinfectant in ten-fold dilutions was used as a control. Exposure was 2 hours at 22 ± 1 °C (Table 5).

It was found that CHH at 380 µg/cm³ completely (100%) inactivated *B. anthracis* "SB" spores. At 38 µg/cm³, CHH caused a significant reduction in spore viability by 89.9%. High sporicidal activity was shown by Ag/Cu NPs at 250 and 25 µg/cm³ of each element (100% and 78.9% inactivation, respectively). At 2.5 µg/cm³, a significant reduction of 19.7% was recorded. At higher dilutions (0.25 and 0.025 µg/cm³), a non-significant increase in spore titer by 22.3% and 28.4% was observed. Composite NMs based on Ag/Cu NPs and CHH at 125/125/190 and 12.5/12.5/19 µg/cm³ caused 100% loss of spore viability. At 1.25/1.25/1.90 µg/cm³, the spore titer was significantly reduced by 56.4%.

Table 4
Determination of sporicidal properties of CHH and composite NMs based on Ag/Cu NPs and CHH against *B. anthracis* "SB" spores (exposure 2 h, temperature 22 ± 1 °C, n = 4)

Tested substance	Substance or NPs	Concentration, µg/cm³	CFU/cm³	Difference vs control, %
Control		0	73.25 ± 1.38	-
CHH		380.0	0	–100
Ag/Cu	Ag	250.0	0	–100
	Cu	250.0		
	Ag	25.0	18.25 ± 1.03**	–75.1
	Cu	25.0		
Ag/Cu/CHH	Ag	125.0	0	–100
	Cu	125.0		
	CHH	190.0		
	Ag	12.5	0	–100
	Cu	12.5		
	CHH	19.0		
Potassium peroxymonosulfate		1000.0	0	–100
DMSO		2750.0	69.75 ± 4.39	–4.8
Citric acid		25000.0	70.5 ± 1.71	–3.7

Note: see Table 1.

The disinfectant based on potassium peroxymonosulfate (positive control) at concentrations from 10 to 1000 µg/cm³ reduced spore viability by 100%. At 0.01 to 1 µg/cm³, it significantly reduced viability by 66.1%, 34.0%, and 13.8%, respectively. On solid medium, *B. anthracis* "SB" forms opaque white colonies with irregular edges, 2–3 mm in size (Fig. 2).

Under the influence of heterocyclic CHH, a decrease in the number of colonies is observed without changes in their morphology. When Ag/Cu NP-based NMs and Ag/Cu NP + CHH composite NMs are applied, a decrease in spore viability is also observed. Colony sizes vary from 0.5 to 2.0 mm, which is significantly smaller than in the control.

Discussion

During the study, the sporicidal properties of nanomaterials (NMs) based on Zn NPs, Se/I NPs, and Ag/Cu NPs were investigated against the *B. anthracis* strain "SB". It was established that Zn NP-based NMs exhibited low sporicidal activity at concentrations of 200 and 20 µg/cm³, significantly reducing the titer of viable spores by 23.63% and 28.69% CFU/cm³, respectively. In contrast to their high bactericidal effect (8 log reduction) previously observed against another *Bacillus* species, namely *B. subtilis* IMV B-7023 at 100 µg/mL, these nanoparticles were less active against *B. anthracis* spores. Additionally, Zn NPs showed high activity against soil anaerobic sulfate-reducing bacteria of the genus *Desulfovibrio* at 300 µg/mL, reducing viability by five orders of magnitude (Demchenko et al., 2020). At a Zn NP concentration of 2.0 µg/cm³, a slight non-significant increase in viable spore titer was observed. Thus, unlike previous studies, Zn NPs were less active against anthrax spores and were not used in further experiments.

It is known that Se/I NPs can exhibit both growth-stimulating and antimicrobial activity. This nanocomposite at concentrations of 2–400 µg/mL has been reported to completely inactivate methicillin-resistant *Staphylococcus aureus*. Literature data also indicate that Se/I NPs at 100 µg/mL significantly increase the viable cell titer of *B. subtilis* IMV B-7023 by up to 20%. In our study, Se/I NPs at concentrations of 2.1/5.6 and 0.21/0.56 µg/cm³ caused a significant 28.3% reduction in anthrax spore titer compared to control, which was insufficient for further investigation.

The most promising proved to be Ag/Cu NPs. At 250 µg/cm³ of each element, Ag/Cu NPs achieved 100% inactivation of *B. anthracis* "SB" spores. At concentrations of 25/25 and 2.5/2.5 µg/cm³, a significant reduction in spore viability of 77.2% and 11.0%, respectively, was recorded, consistent with other studies. For example, Ag NPs at 10–195 µg/mL showed bactericidal activity against *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Holubnycha et al., 2024). CuO NPs exhibit strong antibacterial activity against *Klebsiella aerogenes*, *Pseudomonas desmolyticum*, *Escherichia coli*, and *Staphylococcus aureus* at 5–1000 µg/mL (Naika et al., 2015). Ag/Cu NPs at 25–2800 µg/mL inactivated *Escherichia coli* and *Staphylococcus aureus*. Thus, the silver-copper composite was the most promising and was selected for further research.

Table 5

Determination of sporicidal properties of CHH and composite NMs based on Ag/Cu NPs and CHH against *B. anthracis* "SB" spores at different concentrations (exposure 2 h, temperature 22 ± 1 °C, n = 4)

Tested substance	Substance or NPs	Concentration, µg/cm³	CFU/cm³	Difference vs control, %
Control		0	54.5 ± 3.66	0
CHH	CHH	380	0	–100
		38	5.5 ± 0.29***	–89.9
		3.8	50.25 ± 3.07	–7.8
		0.38	54.5 ± 5.06	0
		0.038	62.0 ± 4.42	13.8
Ag/Cu	Ag	250	0	–100
	Cu	250		
	Ag	25	11.5 ± 2.4**	–78.9
	Cu	25		
	Ag	2.5	43.75 ± 1.03*	–19.7
	Cu	2.5		
	Ag	0.25	67 ± 1.41	22.3
	Cu	0.25		
	Ag	0.025	70.0 ± 2.92	28.4
	Cu	0.025		
Ag/Cu/CHH	Ag	125	0	–100
	Cu	125		
	CHH	190		
	Ag	12.5	0	–100
	Cu	12.5		
	CHH	19		
	Ag	1.25	23.75±0.48**	–56.4
	Cu	1.25		
	CHH	1.90		
	Ag	0.125	62.25±4.07	14.2
	Cu	0.125		
	CHH	0.190		
	Ag	0.0125	68.5±5.98	25.7
	Cu	0.0125		
	CHH	0.0190		
Potassium peroxymo-nosulfate	Potassium peroxymono sulfate	1000	0	–100
		100	0	–100
		10	0	–100
		1	18.5±2.75***	–66.1
		0.1	35.75±2.95**	–34.0
		0.01	47.0±1.29*	–13.8

Note: * – the difference between the experiment and the control is statistically significant at $P < 0.05$, ** – at $P < 0.001$; *** – at $P < 0.0001$.

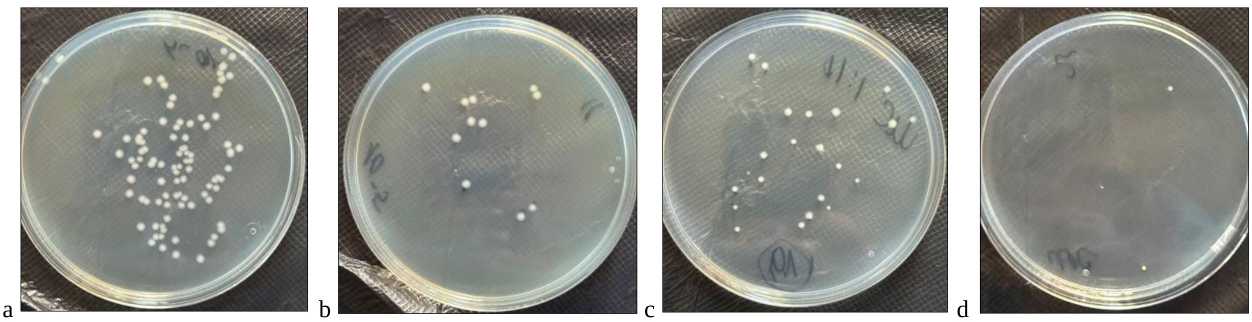

Fig. 2. Morphology of *B. anthracis* "SB" colonies: *a* – without test substances; *b* – under the action of CHH; *c* – under the action of NMs based on Ag/Cu NPs; *d* – under the action of NMs based on Ag/Cu NPs and CHH

When Ag/Cu NPs were applied at concentrations of 25 to 250 µg/cm³ with exposure times of 2, 6, 12, and 24 hours, the titer of viable anthrax spores decreased from 45% to 100%. At lower concentrations, both statistically significant reductions and non-significant increases (44.3–53.4%) were observed. This increase can be explained by the strain's characteristics: during storage at 22 ± 1 °C for 12–24 hours, the control lost 66% of its activity. It can be assumed that Ag/Cu NPs at 0.25–2.5 µg/cm³ stabilize anthrax spores, demonstrating a clear dose–effect relationship.

A novel composite nanomaterial based on Ag/Cu NPs and a heterocyclic compound (CHH) was developed. Its sporicidal activity, as well as that of its individual components, was determined and compared with a commercial disinfectant based on potassium peroxymonosulfate. Both the newly developed composite nanomaterial and CHH demonstrated high sporicidal activity. Auxiliary components (citric acid and DMSO) showed no sporicidal effect against *B. anthracis* "SB".

It was proven that CHH at 380 µg/cm³ inactivated 100% of spores, and at 38 µg/cm³ reduced viability by 89.9%. Ag/Cu NPs at 250 and 25 µg/cm³ of each element caused 100% and 78.9% spore inactivation, respectively. The composite NMs based on Ag/Cu NPs and CHH at concentrations of 125/125/190 and 12.5/12.5/19 µg/cm³ resulted in 100% loss of spore viability. At 1.25/1.25/1.90 µg/cm³, the composite significantly reduced spore titer by 56.4%. Thus, this nanocomposite exhibited higher sporicidal activity even at lower concentrations compared to CHH or Ag/Cu NPs alone.

Recently, intensive development of nanocomposites combining nanoparticles and chemically synthesized compounds has been ongoing. A similar compound based on ZnO NPs and a cationic heterocyclic triazoloazepinium derivative was previously created and tested against *Desulfovibrio* spp., reducing bacterial load by 72.4% (Demchenko et al., 2024).

Thus, the development of NMs based on nanoparticles and heterocyclic chemical compounds with bactericidal and sporicidal properties is a promising research direction, particularly for soil and environmental object decontamination contaminated with anthrax spores. One key advantage of such materials is the absence of bacterial resistance development due to their unique physicochemical properties and multiple mechanisms of action, making them a viable alternative to traditional antimicrobial agents (AlQurashi et al., 2025).

Conclusions

All tested substances showed high sporicidal activity. The highest sporicidal activity was demonstrated by the composite nanomaterial based on Ag/Cu NPs and the heterocyclic compound CHH. A new nanomaterial combining Ag/Cu NPs and CHH was developed, showing superior sporicidal activity. The influence of these NMs on colony morphology was also noted. The investigated nanomaterials can be used for developing methods of soil and environmental object decontamination, as well as for creating antimicrobial agents against the anthrax pathogen.

The authors extend their sincere appreciation to the National Research Foundation of Ukraine for providing funds to carry out the research work.

The work was carried out within the framework of the project under registration number 2023.04/0141 "Development of a monitoring system, molecular genetic control of *B. anthracis* and new approaches to soil and environmental remediation" under the National Research Foundation of Ukraine funding.

The authors declare that they have no conflict of interest.

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