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RESEARCH ARTICLE

Antibacterial Effect of Different Concentrations of Silver Nanoparticles

A Ebru Borum[1*] and Ertan Güneş[2]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey
[2]Vocational School of Technical Sciences, Uludag University, Bursa, Turkey
*Corresponding author: ebruborum@balikesir.edu.tr

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A B S T R A C T

Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. Silver has long been known to show a strong antimicrobial effect to microorganisms. The antimicrobial effect of 30 and 100 ppm silver nanoparticles were investigated against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Paenibacillus larvae*, *Candida albicans* and *Aspergillus niger*. The microorganisms were diluted with sterile distilled water and prepared dilutions of 10^6 of test microorganisms. Dilutions of microorganisms cultured to blood agar base and incubated at 37°C for 24 hours. One mL dilution of 10^6 of all of microorganisms was centrifuged at 3500 rpm for 20 minutes then 30 and 100 ppm of silver nanoparticle solutions were added. Samples were inoculated in blood agar for different time intervals *i.e.*, 0, 2, 5, 10, 30, 60 minutes and 24 hour. Antibacterial activity of silver nanoparticles against various microorganisms was detected at 0, 2, 5, 10, 30, 60 minutes and 24 hours. As results, yeast, fungi and bacteria were inhibited at 30 and 100 ppm. But, *P. larvae* were not inhibited, while *B. subtilis* also could not be inhibited at 30 ppm. The antibacterial activity of 100 ppm was stronger than the antibacterial activity of 30 ppm of nanoparticles. Nanosilver is very effective to important pathogens.

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INTRODUCTION

Silver is used in medical and surgical equipment such as endotracheal tubes, surgical meshes, catheters, dental filling materials, bandages, medical dressings and a topical cream to prevent burn-associated infections (Silver, 2003). Silver has long been known to show a strong antimicrobial effect to microorganisms (Liau *et al*., 1997). The nanosilver is effective against bacteria resistant to antibiotics, fungi and virus (Feng *et al*., 2000; Radzig *et al*., 2013). Silver nanoparticles are reported to be effective against fungi and bacteria as well as multidrug-resistant bacteria *i.e.*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (S. *aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus vulgaris* (*P. vulgaris*), *Bacillus subtilis* (*B. subtilis*), *Aspergillus niger* (*A. niger*), *Candida albicans* (*C. albicans*), *Penicillium citrinum* (*Pen. citrinum*) (Kim *et al*., 2007; Lara *et al*., 2010; Marambio-Jones and Hoek 2010; Lalueza *et al*., 2011).

Silver nanoparticles show great antibacterial effectiveness on important foodborne pathogens (include: *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Vibrio parahaemolyticus*) (Zhang *et al*., 2016). Also, nanosilver has antifungal effect on *Candida albicans*, *Candida glabrata*, *Candida crusei*, *Candida parapsilosis* and *Trichophyton mentagrophytes* (Kim *et al*., 2008). Silver nanoparticles have antiviral effect on human immunodeficiency virus-I (Lara *et al*., 2010) and herpes simplex (Barm-pinto *et al*., 2009).

Silver has been used as in creams, wound dressing, different medical devices, food containers, and water disinfection for antimicrobial agent. The new strains of bacteria were resistant to antibiotics. Therefore, new bactericides were development. The nanosilver is very effective for multidrug-resistant bacteria (Morones *et al*., 2005).

Silver in ionized form or in nanoparticles have got excellent antimicrobial, antifungal activities and was used for coating medical devices for preventing biofilm

formation by pathogenic bacteria, water purification, (Bandyopadhyay *et al*., 2008; Chang *et al*., 2008; El-Naggar *et al*., 2016) and wound dressing for the promotion of healing (Feng *et al*., 2000; Abboud *et al*., 2014).

In this study, antimicrobial effect of 30 and 100 ppm silver nanoparticles were determined against *E. coli, S. aureus, S. typhimurium, Ent. faecalis, B. cereus, B. subtilis, P. larvae, C. albicans, A. niger*. We detected antibacterial effect of nanosilver on *P. Larvae*, which causes American foulbrood for honeybees the first time. We compared the efficacy of 30 and 100 ppm concentrations of silver.

MATERIALS AND METHODS

*Escherichia coli (*ATCC 25922), *Staphylococcus aureus (*ATCC 25923), *Salmonella typhimurium* (CCM 5445), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 6633), *Bacillus subtilis* (ATCC 6051), *Paenibacillus larvae* (ATCC 25747), *Candida albicans* (ATCC 90028), *Aspergillus niger* (Clinical isolate, Uludag University, Medicine Faculty) were used for of antibacterial and antifungal activity of nanosilver. Bacteria and *C. albicans* were purchased from American Type Culture Collection (ATCC). The activity of nanosilver on *P. larvae* was determined for the first time in this study. *E. coli, S. aureus, S. typhimurium, Ent. faecalis, B. cereus, B. subtilis, P. larva*e and *C. albicans* were incubated in Fluid Thioglycollate Medium at 37°C for 24 hours and *A. niger* at 37°C for 7 days. The cells were washed twice and centrifuged at 3500 rpm for 20 minutes then suspended in distilled water, obtaining a final concentration of 10^6 cells/100 ml (Sondi and Salopek-Sondi, 2004). Each culture was divided into two replicates. The cultures were then supplemented with 100 µl of 30 ppm and 100 ppm nanosilver solutions, respectively. Samples with different concentration of nanosilver were inoculated on blood agar at the exposure time of 0, 2, 5, 10, 30, 60 minutes and 24 hours. The cultures were incubated at 37°C for 24 hours. The number of bacteria was determined by counting the colonies.

Antifungal effect of nanosilver for *A. niger* was determined according to The National Committee for Clinical Laboratory Standards (NCCLS) M29 method. *A. niger* was incubated Potato dextose agar at 37°C for 7 days. After, *A. niger* was inoculated to 1 ml of 0.85% sterile saline and, added 0.01 ml of Tween 20. Suspension of *A. niger* was homogenized with vortex for 15 seconds Spacciapoli *et al*. (2001). 1 ml dilution of 10^6 of *A. niger* was centrifuged at 3500 rpm for 20 minutes. A 100 µl of 30 and 100 ppm silver solutions were added to two different cultures. Samples with nanosilver were inoculated on Potato Dextrose Agar (Bragg and Rannie, 1974) at 2, 5, 10, 30, and 60 minutes. The cultures were incubated at 37°C for 7 days. Plates without silver nanoparticles were used as negative control. We counted microorganisms growing on plates as colonies following Ki-Young *et al*. (2007) and Samarajeewa *et al*. (2017).

RESULTS AND DISCUSSION

Thirty ppm of nanosilver solution inhibited the growth of *S. typhimurium* in 2 minutes. *E. coli, S. aureus, A. niger, C. albicans, Ent. faecalis* and *B. cereus* were inhibited on different time intervals of the exposure. But *S. aureus* was not inhibited in 24 hours time interval of exposure. *P. larvae* were not inhibited even in 60 min and 24 hours exposure. *Bacillus subtilis* was not inhibited by 30 ppm of nanosilver solution. Micro-organisms and inhibition periods are shown in Table 1.

Ent. faecalis, S. typhimurium, B. cereus and, *C. albicans* were inhibited by 100 ppm of nanosilver within 2 minutes. *E. coli* did not grow at 10 minutes. *S. aureus* were inhibited at 5 minutes. *A. niger* and *B. subtilis* were not inhibited in the first 10 minutes but lost activity completely after 30 minutes. *P. larvae* were inhibited in the first 30 minutes but it grown 60 minutes and 24 hours. Microorganisms and inhibition periods are shown in Table 2.

Susceptibility to nanosilver is depended on the concentration. *P. larvae* inhibited in the first 30 minutes but it was not inhibited by 30 and 100 ppm of nanosilver solutions.

Table 1: Microorganisms and inhibition periods (30 ppm of nanosilver)

Microorganism	Starting number of microorganism kob/ml	Decrasing values of microorganisms (Kob/ml)						
		0. min Kob/ml	2.min Kob/ml	5. min Kob/ml	10 min Kob/ml	30. min Kob/ml	60. min Kob/ml	24 hours Kob/ml
E.coli ATCC 25922	10^6	$6x10^6$	5.1×10^6	1.2×10^6	No growth	No growth	No growth	No growth
Staphylococcus aureus ATCC 25923	10^6	$6x10^6$	6×10^2	No growth	No growth	No growth	No growth	$6x10^2$
Salmonella typhimurium CCM 5445	10^6	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Enterococcus faecalis ATCC 29212	10^6	$5x10^5$	$1.84 x10^5$	No growth	No growth	No growth	No growth	No growth
Bacillus cereus ATCC 6633	10^6	$8x10^3$	$8.2x10^3$	$9.4x10^3$	$8.5x10^3$	No growth	No growth	No growth
Bacillus subtilis ATCC 6051	10^6	10^9	10^9	10^9	10^9	10^9	10^9	10^9
Paenibacillus larvae ATCC 25747	10^6	No growth	No growth	No growth	No growth	No growth	$6x10^6$	$2x10^4$
Candida albicans ATCC 90028	10^6	$6x10^3$	No growth	No growth	No growth	$2x10^2$	No growth	No growth
Aspergillus niger Clinical isolate	10^6	$6x10^6$	4×10^3	2.1×10^3	1.2×10^3	No growth	No growth	No growth

Table 2: Microorganisms and inhibition periods (100 ppm of nanosilver)

Microorganism	Starting number of microorganism kob/ml	Decrasing values of microorganisms (Kob/ml)						
		0. min Kob/ml	2.min Kob/ml	5. min Kob/ml	10 min Kob/ml	30. min Kob/ml	60. min Kob/ml	2424 hours Kob/ml
Escherichiae coli ATCC 25922	10^6	10^6	$1,2 \times 10^4$	$5,0 \times 10^2$	No growth	No growth	No growth	No growth
Staphylococcus aureus ATCC 25923	10^6	10^6	$4,0 \times 10^2$	No growth	No growth	No growth	No growth	No growth
Salmonella typhimurium CCM 5445	10^6	10^6	No growth	No growth	No growth	No growth	No growth	No growth
Enterococcus faecalis ATCC 29212	10^6	10^6	No growth	No growth	No growth	No growth	No growth	No growth
Bacillus cereus ATCC 6633	10^6	10^6	No growth	No growth	No growth	No growth	No growth	No growth
Bacillus subtilis ATCC 6051	10^6	10^6	2×10^6	$1,7 \times 10^6$	$1,2 \times 10^6$	1×10^6	No growth	No growth
Paenibacillus larvae ATCC 25747	10^6	10^6	No growth	No growth	No growth	No growth	7.2×10^3	1.3×10^2
Candida albicans ATCC 90028	10^6	10^6	No growth	No growth	No growth	No growth	No growth	No growth
Aspergillus niger Clinical isolate	10^6	10^6	$3,2 \times 10^3$	$2,1 \times 10^3$	$1,2 \times 10^3$	No growth	No growth	No growth

Esherichia coli, Staphlococcus aureus, Aspergillus niger, Candida albicans, Enterococcus faecalis and *Bacillus cereus* were inhibited in different time intervals (minutes).

P. larvae inhibited the first 30 minutes but after it was not inhibited by 30 and 100 ppm of nanosilver solutions. Nanosilver was not effective for *P. larvae. E. faecalis, S. typhimurium, B. cereus and, C. albicans* were inhibited by 100 ppm of nanosilver within 2 minutes. We did not detect growth on plates. *E. coli* did not grow at 10 minutes. *S. aureus* were inhibited at 5 minutes. *A. niger* and, *B. subtilis* were not inhibited in first 10 minutes but it lost activity completely after 30 minutes.

The antibacterial activity of 100 ppm of nanoparticles was stronger than the antibacterial activity of 30 ppm of nanoparticles. But *P. larvae* were not inhibited after 60 minutes and 24 hours by both nanoparticle solutions. *B. subtilis* was not inhibited by 30 ppm nanosilver. *S. aureus* was inhibited at 5, 10, 30, 60 minutes but it was grown in 24 hours.

In one study, different concentrations of silver on *E. coli* were investigated in vitro. According to the research, it was determined that nano silver at 10 μg/cm^3 concentration was effective at 70% on 10^5 CFU of *E. coli*. 50-60 μg/cm^3 nanosilver concentration was effective for 100%. In the same study, 20 μg/cm3 of nano silver completely inhibited 10^4 CFU of *E. coli*. As the number of bacteria decreased, nano silver was effective at lower concentrations (Yoon *et al*., 2007). 70 μg/mL concentration of silver nanoparticles were found to be effective on *B. subtilis* and *E. coli. B. subtilis* was found to be more susceptible to silver than *E. coli* (Ki-Young *et al*., 2007).

In another study, antimicrobial effect of a commercial nanosilver product, NanoCidR L2000, against some foodborne pathogens was evaluated. The MIC values of Ag NPs against tested pathogens were in the range of 3.12-6.25 μg/mL. While *Listeria monocytogenes* showed the MIC value of 6.25 μg/mL, *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Vibrio parahaemolyticus* all showed the MIC values of 3.12 μg/mL. However, all the pathogens showed the same MBC value of 6.25 μg/mL (Zarei *et al*., 2014). Sixty-five bacterial isolates were isolated from 40 diabetic patients, *S. aureus* (37%) and *P. aeruginosa* (18.5%) were the predominant isolates in the ulcer samples. Squilla chitosan silver nanoparticles (Sq. Cs-Ag(0)) showed the maximum activity against the resistant bacteria (El-Naggar *et al*., 2016).

Thirty ppm of nanosilver solution inhibited *Salmonella typhimurium* in 2 minutes. These results are similar to our research findings.

Conclusions: The antibacterial activity of 100 ppm of nanoparticles was stronger than the antibacterial activity of 30 ppm of nanoparticles. Nanosilver is very effective to important pathogens. Antimicrobial activity of nanosilver can be used for pathogens.

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