

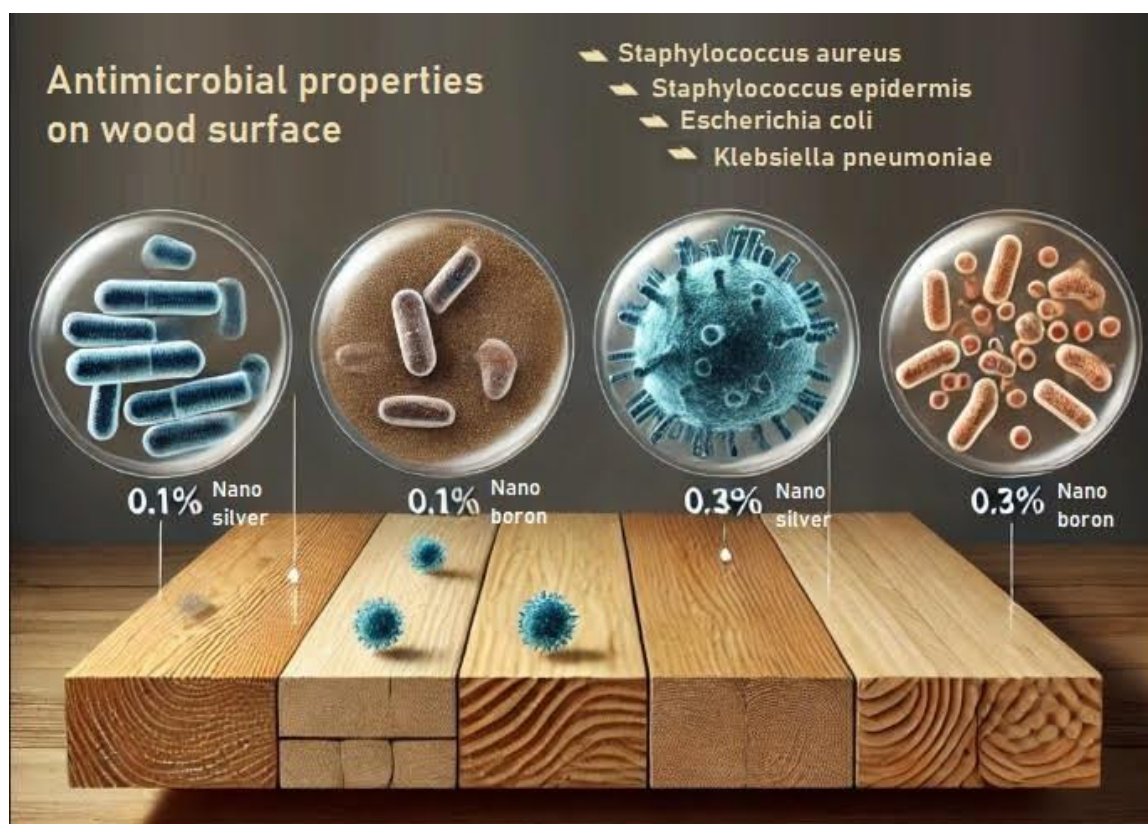
Determination of the Antimicrobial Effect of Varnishes Modified with Nano Particles on the Surface of Wood Materials

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GRAPHICAL ABSTRACT



Determination of the Antimicrobial Effect of Varnishes Modified with Nano Particles on the Surface of Wood Materials

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This study was conducted to determine the effect of modifying some varnishes used in wood materials with different nanomaterials on the antimicrobial properties of wood surfaces. For this purpose, samples prepared from Scots pine (*Pinus sylvestris* L.) Oriental beech (*Fagus orientalis* Lipsky), and sessile oak (*Quercus petraea* Liebl.) were varnished with water-based and synthetic varnish with 0.1% and 0.3% nano boron and nano silver added according to ASTM D3023 (2017). Four different microorganisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) were used to determine the antimicrobial effect on wood surfaces. Among the microorganisms, the highest growth was found in *S. aureus* and the lowest growth was found in *S. epidermidis*. In terms of antimicrobial activity, the lowest growth was found in samples with 0.1% nano boron synthetic varnish and highest growth was found in 0.1% water-based varnish. As a result, it is thought that the use of nano materials together with varnishes applied to the surface of wood materials in areas where antimicrobial properties are desired will be beneficial for human and environmental health.

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Keywords: Wood material; Antimicrobial; Varnish; Nano material; Modification

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INTRODUCTION

Wood is a natural material used since ancient times. Although there are many alternative materials to wood, the natural structure of wood is one of the reasons why wood is preferred. However, due to its natural nature, it needs protection against dust, dirt, and weak acids and bases, as well as mechanical effects such as abrasion and scratching. For this reason, to protect the surfaces of wood based against these effects, various transparent varnishes that are resistant against different effects (dust, dirt, scratches, etc.) are used.

However, products such as varnish may not be able to resist the effects that occur or may occur in an environment of interest. Sometimes modification processes are not required to improve these features. Today, wooden surfaces are modified with nanotechnological materials against various effects.

Nanotechnology and nanomaterials enter our lives in many different fields. These areas are primarily health, communication, space technology, and defense industry (Nanotechnology Strategy Group 2004). The controllability feature allows materials to be

given the desired qualities and to produce high performance materials and systems that can be used in various fields (Kasap 2012).

With nanotechnology, changes can occur in the molecular structure of the material. With the addition of nanomaterials to many paints and varnishes, resistance to light and high protection against gases can be seen (Çeliker 2005). Nanocoatings can be coated with nanoparticles as a layer of paint, varnish, or film on a surface. Nanoparticles can be added to the material mixture where necessary to improve surface properties. Nano material technology, as in many areas, is used in wood materials for protection against fire, moisture, insects, and fungi. In addition, it has been used by adding chemicals and varnishes to increase some properties above the expected level.

Today, one of the main areas of use of nano materials is antimicrobial properties. Wooden products are used in many areas such as shopping malls, schools, *etc.* In these areas, protection is provided by adding nanomaterials to varnishes to improve the antimicrobial properties of wood (Atılğan *et al.* 2022)

In a study on the effect of silver nanoparticles on the spread of *E. coli*, it was reported that silver nanoparticles enter the cell by disrupting the cell membrane structure of *E. coli* and prevent its growth by blocking bacterial respiration (Li *et al.* 2010). Silver nanoparticles in combination with meropenem were found to destroy more than 75% of *E. coli* and *Klebsiella pneumoniae* microorganisms (Gurunathan 2015).

The effects of different protective coatings using some varnishes and paints, bacteria and yeast on the surface of wooden toys were investigated. Accordingly, effective products against bacteria and viruses have emerged (Aykan *et al.* 2022).

Silver and zinc ions were added to nano-SiO₂ and it was determined that the binding amounts and antibacterial properties of the ions showed an inhibitory effect against bacteria (Husbeng 2008). Today, various organic substances, liquid glass, paraffin, water repellent oils, industrial wood preservatives, and varnishes are used against abiotic, biotic, and antibacterial pests that harm the environment on the surfaces and within the wood material (Atılğan *et al.* 2022).

Morphological differences of silver ions on Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *E. coli* bacteria were investigated. They used silver nitrate compound (AgNO₃) as an ion source. Gram-positive *S. aureus* was found to be more resistant to silver ions due to its thick cell wall (Feng *et al.* 2000).

Boron derivatives show biocidal effect on a wide range of microorganisms such as *E. coli*, *Klebsiella spp*, *Acinetobacter calcoaceticus*, *Enterobacter spp*, *Staphylococcus spp*, *Morganella spp*, *Proteus spp*, *Pseudomonas aeruginosa*, *Citrobacter spp* and so on (Meers and Chow 1990).

In a study investigating the effects of boron compounds on the inhibition of biofilms produced by *Pseudomonas aeruginosa* ATCC-27853 and *Escherichia coli* MG1655/K12 in water sources, it was found that the concentration of 25 mg/mL sodium tetraborate decahydrate (STD) and disodium octaborate tetrahydrate (DOT) caused the most effective inhibition on bacterial biofilm formation tested (Ardeşir 2017).

One of the areas of use of nano materials is where antibacterial properties are desired. The usage areas of new materials with antibacterial properties are increasing every day. With the ever-increasing human population, the reproduction of bacteria in common places is also increasing. Therefore, there is a need for the use of nano materials with high antibacterial protection properties in a wide variety of fields (Magana *et al.* 2008).

EXPERIMENTAL

Wood Material

Scots pine (*Pinus sylvestris* L.), Oriental beech (*Fagus orientalis* Lipsky), and sessile oak (*Quercus petraea* Liebl.) woods were selected as experimental materials. Wood materials were obtained from timber enterprises in Ankara in accordance with ISO 3129 (2019) principles.

Varnishes

Water-based and synthetic varnish were used to varnish the samples. The amount of solids and manufacturer's recommendations were taken into consideration in determining the amount of varnish to be applied. The properties of the varnishes used in the experiments are given below.

Water Based Varnish

Water-based varnish, which can be diluted and mixed with water and dissolved in organic solvents, protects the wood material without changing the existing color too much (Atar 1999; Baykan 2000). The conditions of use of the varnish used in the study were as follows: drying was carried out at 20 °C and 40 to 65% relative humidity. Touch drying time was 30 min, drying time between coats was 1.5 to 2 h, and complete drying time was 24 h. The application method was by brush.

Synthetic Varnish

Synthetic varnish is resistant to water and moisture. Its viscosity is low in spray gun applications and high in brush applications (Temiz *et al.* 2008). It consists of a mixture of synthetic resins and is usually formulated as solvent-based. The conditions of use of the varnish used in the study are: complete drying 48 h, with an application temperature between 5 and 35 °C.

Nano Materials

Nano boron

Boron is not found freely in nature. Artificial boron is produced in amorphous and crystalline structures. Amorphous boron is initially black and matte, but when heated to 1260 °C, it partially becomes red and becomes transparent. Crystal boron is brittle, rigid, and black in color. Boron minerals are used in many areas, such as the glass and wood industry, in the production of flame retardant or flame retardant materials (State Planning Organization 2001).

Nano silver

Silver is used in many fields as an antimicrobial agent with its antibacterial and antifungal properties. Metal ions, such as zinc, copper, titanium, *etc.*, also have antimicrobial properties, but it is known that silver shows the best activity against microorganisms (Rai *et al.* 2009). Silver, which has high antibacterial properties, is used for coating on surfaces where undesirable microorganisms are found (Cansız 1997).

Microorganisms Used in the Study

Staphylococcus aureus ATCC 25923

S. aureus is a bacterial species belonging to the family Micrococcaceae family of the order Eubacterials, which causes infection in humans. It is very abundant in nature and is resistant to environmental conditions (Hacıbektaşoğlu *et al.* 1993). *S. aureus* bacteria is also an infection. The transmission of this infection mostly from places where human crowds are dense such as hospitals, schools, and social facilities.

Staphylococcus epidermidis ATCC 35984

Staphylococcus epidermidis belongs to the Micrococcaceae family and is frequently seen in the external parts and mucous membranes of the human body (Otto 2009; Raue *et al.* 2020).

Escherichia coli ATCC 25922

E. coli belongs to the bacterial family Enterobacteriaceae (Allocati *et al.* 2013). *Escherichia coli* (*E. coli*) was first found by bacteriologist Thedor Escherich in 1885. Since then, it has been frequently used in research and experiments. Their optimum growth temperature is 37 °C (Betlejewska *et al.* 2001).

Klebsiella pneumoniae ATCC 13883

Klebsiella pneumoniae belongs to the Enterobacteriaceae family with a length of 1-2 µm and a width of 0.5 to 0.8 µm. Although they are resistant to cold, they are not resistant to heat (Kıraç 2011).

Preparation of Experimental Samples

Test samples were prepared from the sapwood parts of wood materials with uniform fibre, free of knots and cracks, tulle formation, resin, growth defects, and reaction wood, without colour and density difference, without fungal and insect damage.

To air dry the rough cut samples, the test samples were kept in an air conditioning room at with a temperature of 20 ± 2 °C and a relative humidity $65 \pm 5\%$ until their weight became constant. The air-dried test samples were cut to $100 \times 100 \times 10$ mm dimensions.

Varnishing of Test Samples

Before the application, the sample surfaces were sanded to remove fibre blisters, dusted, and varnished. The varnishing process was carried out in accordance with the principles specified in ASTM D3023 (2017). Nanomaterials were added into the water-based and synthetic varnish applied to the sample surface at the levels of 0.1% and 0.3% separately. The weight percentages of the nanomaterials (0.1 and 0.3) are relative to the mass of the varnish. The nano materials added into the varnish with a special equipment were mixed for 30 min to ensure complete dissolution and homogeneous mixing. Water-based varnish 160 g/m² and synthetic varnish 120 g/m² were applied in 3 layers with a brush. The density of nano materials per square metre does not vary according to the varnish type. Varnishing was carried out under 20 ± 2 °C temperature and $65 \pm 3\%$ relative humidity conditions. Fig. 1. shows the varnishing of the test specimens.

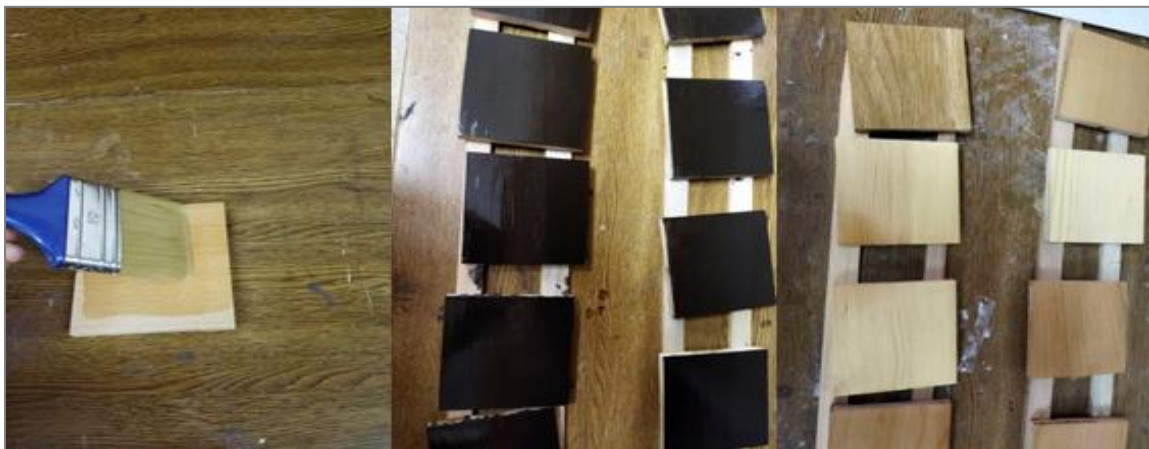


Fig. 1. Varnishing of test samples (Varnishing of nano silver and nano boron samples)

Methods

In this study, nano silver and nano boron, which are nano materials with antimicrobial properties, were mixed with varnishes and applied to wooden surfaces. On each of the surfaces to be planted with microorganisms, five 1 cm² areas were formed and sterilized (with 70% ethyl alcohol and UV). Microorganisms were prepared at a turbidity of 0.5 McFarland (1.5×10^8) (standard used as a reference to adjust the turbidity of bacterial suspensions). Ten surfaces and four microorganism strains were studied at a time. A total of 10 μ L of bacteria were dropped onto the surfaces and kept under aseptic conditions at room temperature for 30 min. Saline physiological solution was used as a negative control.



Fig. 2. Sections taken from sample surfaces and developing growths for microorganism growth

Samples showing antimicrobial activity were artificially contaminated with microorganisms (contamination) and dried under normal air conditions. After the bacterial suspension was dried, it was thoroughly mixed with saline physiological for 30 s and 10

μL of each was inoculated onto Brain Heart Infusion Agar (BHI). Colony counts were determined and the colony forming unit (CFU) per millilitre was multiplied by the dilution factor and the efficacy of the disinfectant was calculated by the following formula according to the reduction factor.

$$\text{Reduction factor} = (\text{Log}_{10} (\text{premicroorganisms count}) - \text{Log}_{10} \text{microorganisms count})$$

where the log reduction factor is the difference between the logarithms of the number of microorganisms surviving on the untreated control surface and the treated test surfaces (Reybrouck 1975). The sections taken from the sample surfaces for microorganism growth and the appearance of the microorganisms formed are given in Fig. 2.

RESULTS

In the study, control samples were compared with the experimental samples. In the evaluation, the number determined as a constant (8.17) was subtracted from the number obtained from the experimental samples with bacteria or and if the number found is above 5, the activity is considered sufficient. These numbers are the accepted standard value.

Colony Count (Log₁₀)

The graph of colony count for water-based and synthetic varnish control samples are shown in Fig. 3.

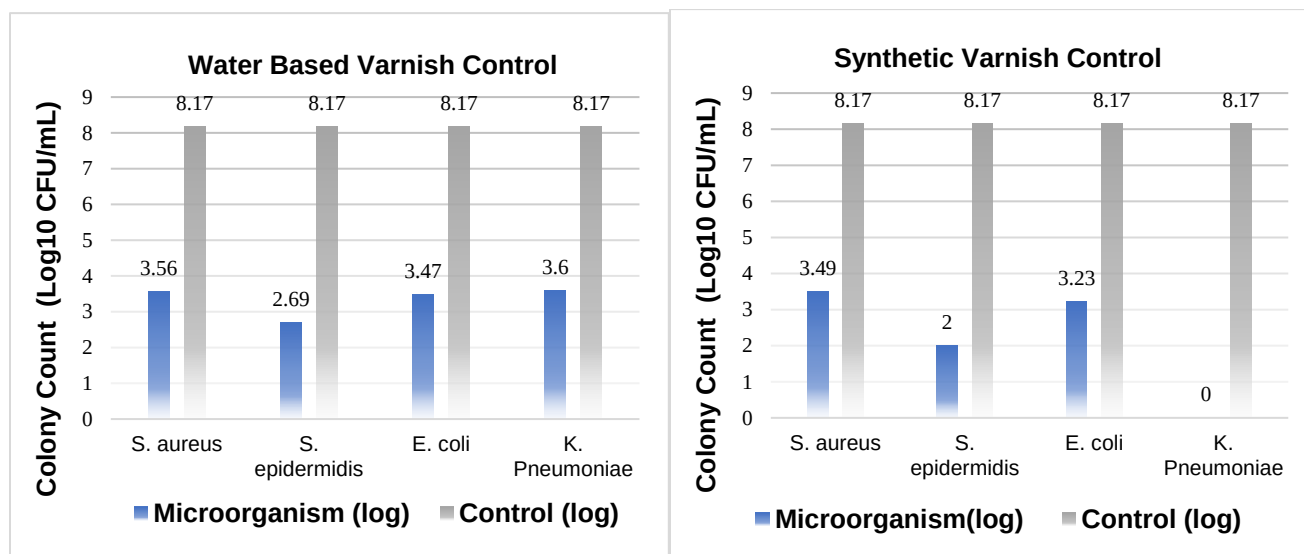


Fig. 3. Antimicrobial activity of water-based and synthetic varnish control samples

While 8.17 log₁₀ cfu/mL growth was detected in the control strain obtained from the water-based varnish surface, *S. aureus* 3.56 log₁₀ cfu/mL, *S. epidermidis* 2.69 log₁₀ cfu/mL, *E. coli* 3.47 log₁₀ cfu/mL, and *K. pneumoniae* growth of 3.6 log₁₀ cfu/mL was observed in the test group. In contrast, *S. aureus* 3.49 log₁₀ cfu/mL, *S. epidermidis* 2 log₁₀ cfu/mL, *E. coli* 3.23 log₁₀ cfu/mL, and *K. pneumoniae* < 2 log₁₀ cfu/mL growth was determined from synthetic-based varnish surface (Fig. 3).

It was determined that the water-based varnish control sample reduced the growth of microorganisms *S. epidermidis* (2.69 log), the synthetic varnish control sample reduced the growth of microorganisms *S. epidermidis* (2 log) and there was no growth of *K. pneumoniae* (0).

The graph of colony counts for synthetic varnish + 0.1% nano boron and water-based varnish + 0.1% nano boron samples are shown in Fig. 4.

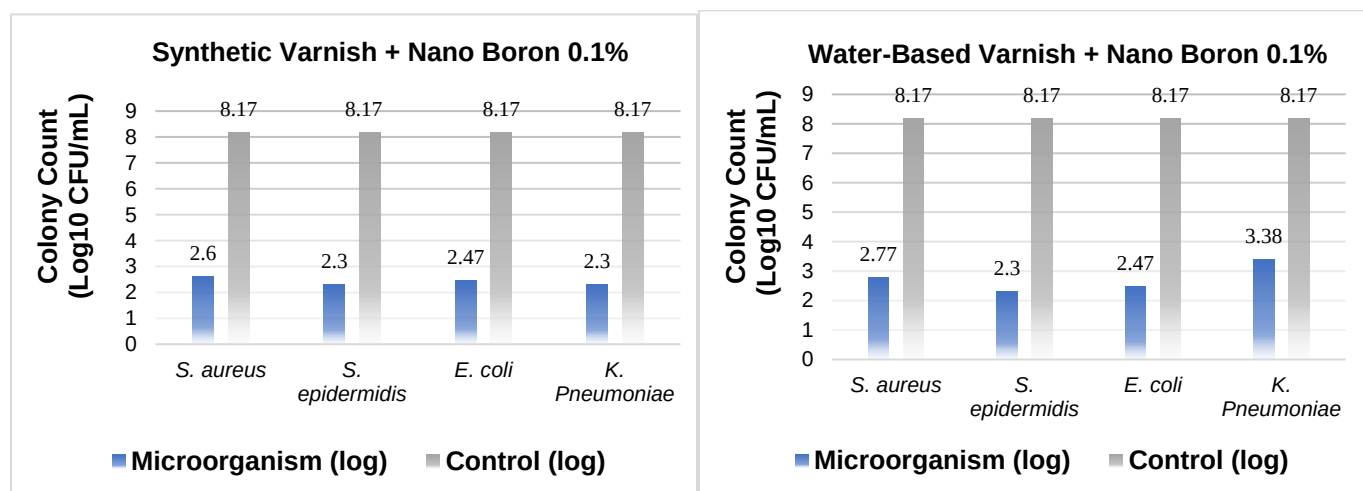


Fig. 4. Antimicrobial activity of synthetic varnish and water-based varnish with 0.1% nano boron

While 8.17 log₁₀ cfu/mL growth was detected in the control sample obtained from synthetic varnish + 0.1% nano boron surface, *S. aureus* 2.6 log₁₀ cfu/mL, *S. epidermidis* 2.3 log₁₀ cfu/mL, *E. coli* 2.47 log₁₀ cfu/mL, and *K. pneumoniae* 2.3 log₁₀ cfu/mL growth was observed in the test group. On the surface with water-based varnish + 0.1% nano boron, *S. aureus* 2.77 log₁₀ cfu/mL, *S. epidermidis* 2.3 log₁₀ cfu/mL, *E. coli* 2.47 log₁₀ cfu/mL, and *K. pneumoniae* 3.38 log₁₀ cfu/mL (Fig. 4) were observed.

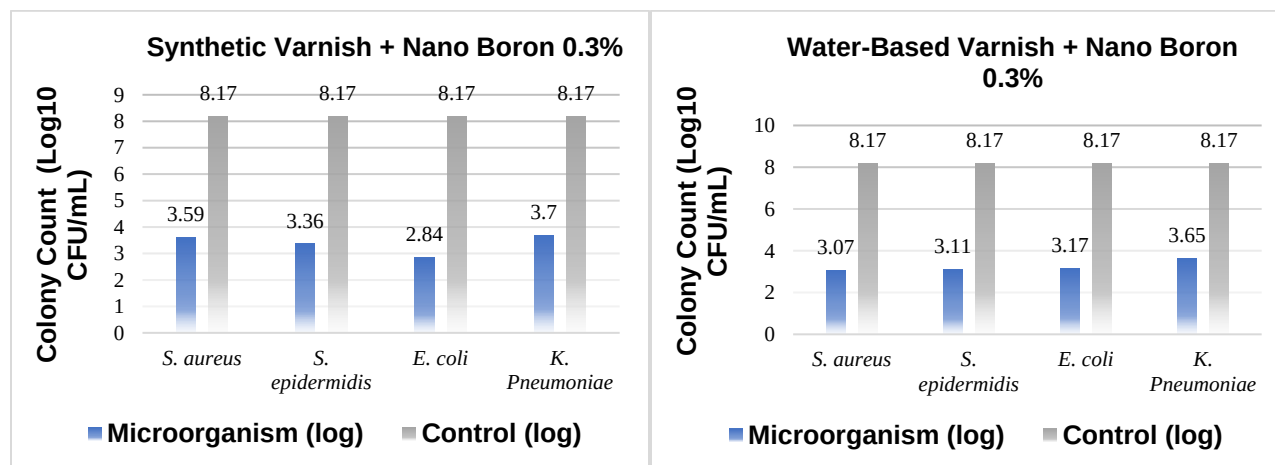


Fig. 5. Antimicrobial activity of synthetic varnish and water-based varnish with 0.3% nano boron

In the synthetic varnish + 0.1% nano boron sample, *S. aureus* (2.6 log), *S. epidermidis* (2.3 log), *E. coli* (2.47 log), *K. pneumoniae* (2.3 log) and in the water-based

varnish + 0.1% nano boron sample, *S. aureus* (2.77 log), *S. epidermidis* (2.3 log) and *E. coli* (2.47 log) showed antimicrobial properties and reduced the growth of microorganisms.

Colony counts of synthetic varnish + 0.3% nano boron and water-based varnish + 0.3% nano boron samples are shown in Fig. 5.

While 8.17 log₁₀ cfu/mL growth was detected in the control strain obtained from synthetic varnish + 0.3% nano boron surface, the following growth was detected in the test group: *S. aureus* 3.59 log₁₀ cfu/mL, *S. epidermidis* 3.36 log₁₀ cfu/mL, *E. coli* 2.84 log₁₀ cfu/mL, and *K. pneumoniae* 3.7 log₁₀ cfu/mL. On the surface with water-based varnish + 0.3% nano boron surface, *S. aureus* 3.07 log₁₀ cfu/mL, *S. epidermidis* 3.11 log₁₀ cfu/mL, *E. coli* 3.17 log₁₀ cfu/mL, and *K. pneumoniae* 3.65 log₁₀ cfu/mL growth was observed. A growth of 0.65 log₁₀ cfu/mL was determined (Fig. 5).

In the synthetic varnish + 0.3% nano boron sample, *E. coli* (2.84 log) and in the water-based varnish + 0.3% nano boron sample, *S. aureus* (3.07 log), *S. epidermidis* (3.11 log) showed antimicrobial properties.

The colony count graph of synthetic varnish + 0.1% nano silver and water-based varnish + 0.1% nano silver samples are shown in Fig. 6.

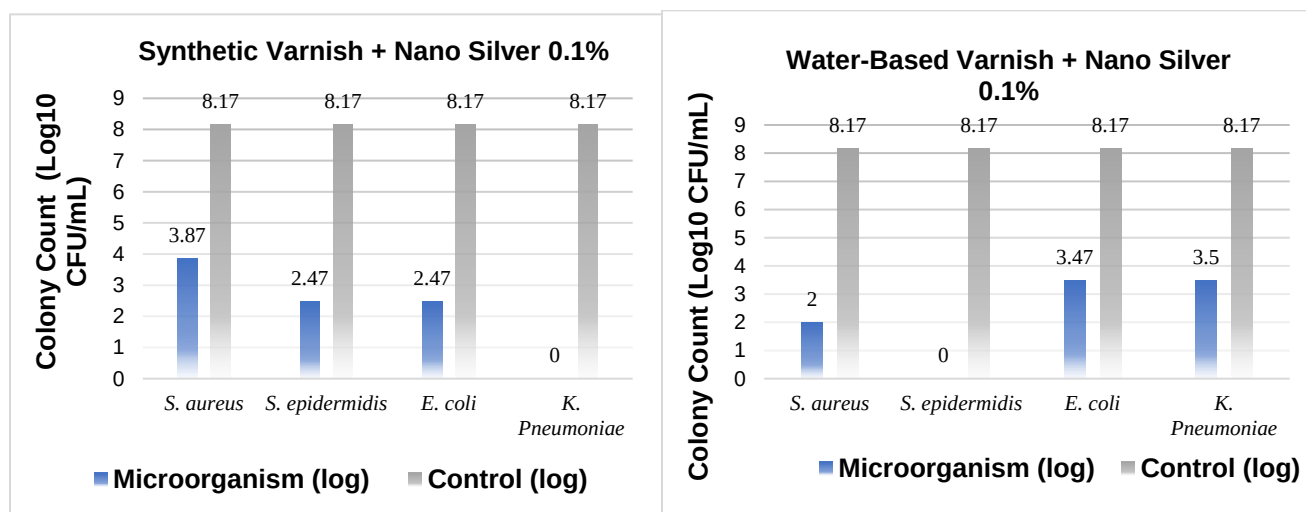


Fig. 6. Antimicrobial activity of synthetic varnish and water-based varnish with 0.1% nano silver

While 8.17 log₁₀ cfu/mL growth was detected in the control strain obtained from synthetic varnish + 0.1% nano silver surface, the following growth was obtained: *S. aureus* 3.87 log₁₀ cfu/mL, *S. epidermidis* 2.47 log₁₀ cfu/mL, *E. coli* 2.47 log₁₀ cfu/mL, and *K. pneumoniae* < 2.47 log₁₀ cfu/mL. On the water-based varnish + 0.1% nano silver surface (Fig. 6), the following growth was obtained: *S. aureus* 2 log₁₀ cfu/mL, *S. epidermidis* < 2 log₁₀ cfu/mL, *E. coli* 3.47 log₁₀ cfu/mL, and *K. pneumoniae* 3.5 log₁₀ cfu/mL.

In the synthetic varnish+0.1% nano silver sample, *S. epidermidis* and *E. coli* showed 2.47 log antimicrobial properties, while there was no growth in *K. pneumoniae*. In the water-based varnish + 0.1% nano silver sample, *S. aureus* (2 log) showed antimicrobial properties, while there was no growth in *S. epidermidis*.

Colony count of synthetic varnish + 0.3% nano silver and water-based varnish + 0.3% nano silver samples are shown in Fig. 7.

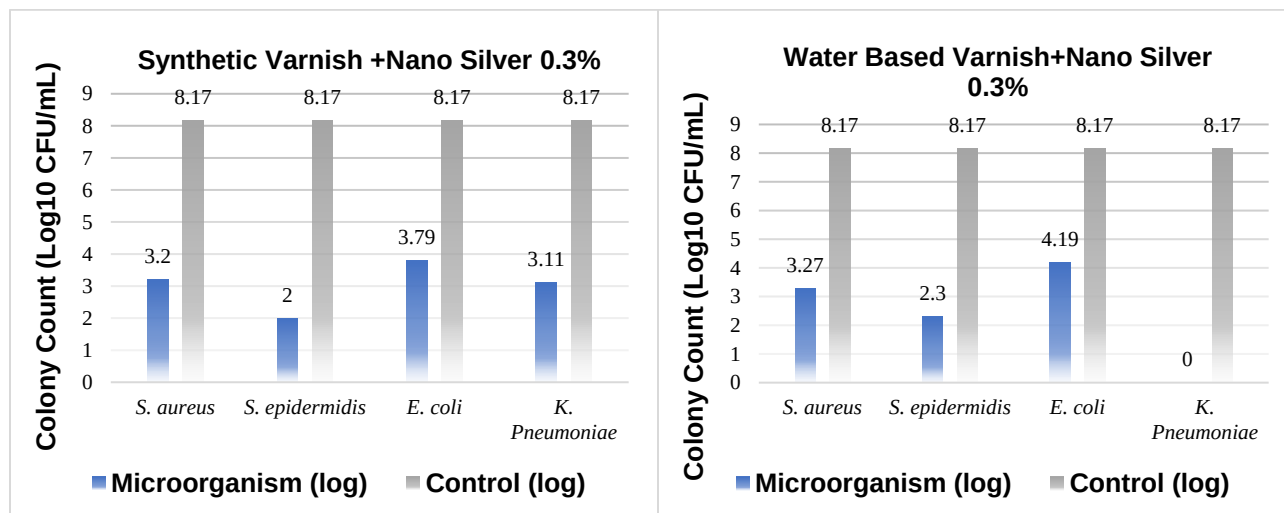


Fig. 7. Antimicrobial activity of synthetic varnish and water-based varnish with nano silver at 0.3% concentration

While 8.17 log₁₀ cfu/mL growth was detected in the control strain obtained from the synthetic varnish + 0.3% nano silver surface, the following growth was obtained in each strain: *S. aureus* 3.2 log₁₀ cfu/mL, *S. epidermidis* 2 log₁₀ cfu/mL, *E. coli* 3.79 log₁₀ cfu/mL, and *K. pneumoniae* 3.11 log₁₀ cfu/mL. For the water-based varnish + 0.3% nano silver surface (Fig. 7), the following growth was obtained: *S. aureus* 3.27 log₁₀ cfu/mL, and *S. epidermidis* 2.3 log₁₀ cfu/mL, *E. coli* 4.19 log₁₀ cfu/mL, and *K. pneumoniae* < 2.3 log₁₀ cfu/mL.

In the synthetic varnish + 0.3% nano silver sample, *S. epidermidis* (2 log), *K. pneumoniae* (3.11 log), and in the water-based varnish+0.3% nano silver sample, *S. aureus* (2.3 log) showed antimicrobial properties, while there was no growth in *K. pneumoniae*.

DISCUSSION

Escherichia coli, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* microorganisms were grown on the varnished samples. The highest growth was observed in the samples varnished with water-based varnish 0.3% nano silver in which *E. coli* microorganisms were cultivated. The least growth was found in *K. pneumoniae* (0) in samples varnished with synthetic varnish control, synthetic varnish + 0.1% nano silver, water-based varnish + 0.3% nano silver. Similar results were found in *S. epidermidis* (0) samples varnished with water-based varnish + 0.1% nano silver.

Among the microorganisms, the lowest growth occurred in *S. epidermidis* throughout the sample groups and it was determined that synthetic varnish + 0.1% nano boron samples showed antimicrobial effects on four types of including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. In terms of antimicrobial activity, nano boron + 0.1% synthetic varnish was found to inhibit the spread of bacteria. As a matter of fact, it is seen that it provides a significant effect when compared with control samples.

Today, silver is used in many places where antibacterial properties are required. In daily life, nano silver coatings are used in dishwashers and washing machines,

refrigerators, and toilet bowls. It is also used in shoe sprays to prevent bad odors in shoes (Silver *et al.* 2006).

The antibacterial properties of *E. coli* bacteria on chipboards coated with impregnated paper were investigated. As a result of the research, it was stated that the material showed antibacterial properties (Cabbar 2019).

In the antibacterial and anti-mould study of ZnO nanoparticles on melamine laminated surfaces of particle boards, ZnO nanoparticles were applied to the surface structures to improve their antibacterial and anti-mould properties. For impregnation of white decor paper, melamine-formaldehyde resin was modified with ZnO at 0.1%, 0.3%, 0.6%, and 1% by weight and applied to particle boards. On melamine laminated surfaces, ZnO slightly increased the resistance against the Gram-positive bacteria *Staphylococcus aureus* (20.7% or 9.5%). However, improvement was observed against the Gram-negative bacteria *Escherichia coli* (65 or 46.8%) (Nosál and Reinprecht 2017).

In the study on the effect of different nanoparticles on wood decay resistance, five different nanoparticles (zinc-oxide, zinc-borate, silver, copper, and copper-borate) were applied at different concentrations to provide protection against *Coniophora puteana* and *Coriolus versicolor*. One of the investigated fungi showed tolerance to the nanoparticles (zinc oxide, silver nanocubes, and copper) in some cases. The most effective nanoparticles are those containing borate (Bak and Nemeth 2018).

In the study, it was shown that nano silver and nano boron added to wood varnishes had antimicrobial effect and suppress the growth of microorganisms on surfaces. These data are in agreement with the literature.

CONCLUSIONS

1. It was determined that the nano materials applied to the surface together with the varnish types used on the upper surface of the wood material had antimicrobial spread-inhibiting properties and had a positive effect on the wood material.
2. The use of nanomaterials in wood materials where antimicrobial properties are required may provide benefits for human and environmental health. It is known that the prevalence of bacteria increases in crowded places, such as furniture, wooden toys, hospitals, schools, playgrounds, terminals-airports. It is recommended to be used in places where antimicrobial properties are desired, such as social living areas.
3. Today, silver is used in many places where antibacterial properties are required. In this study, it can be said that nano boron is also effective in addition to nano silver.

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