Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Antifungal activity of *Pinus elliottii* wood treated with silver nanoparticles

Gabriel Victor Gazoni Ferreira
Professor Mestre, UNOESTE, Brasil
gazoniferreira@gmail.com

Jacqueline Roberta Tamashiro
Professora Doutora, UNOESTE, Brasil.
Arquiteta.jtamashiro@gmail.com

Fábio Friol Guedes de Paiva
Professor Doutor, UNOESTE, Brasil.
Fabio.vha@hotmail.com

Kelly Cristina Barzan Yabunaka
Professora Mestre, UNOESTE, Brasil.
kc-barzan@hotmail.com

Angela Kinoshita
Professora Doutora, UNOESTE, Brasil.
angelamitie@gmail.com

Daniela Vanessa Moris
Professora Doutora, UNOESTE, Brasil.
danimoris@unoeste.br

Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

ABSTRACT

Wood is a versatile and sustainable material that finds extensive use in civil construction, fulfilling both structural and finishing roles. However, it is susceptible to the growth of filamentous fungi, including molds. This study assesses the effectiveness of silver nanoparticles in combating *Aspergillus niger*, a common fungal strain, on *Pinus elliottii* wood. Sterilized specimens were treated with varying concentrations of silver nanoparticles to evaluate their antifungal activity. Both treated and untreated specimens were inoculated with *Aspergillus niger* to promote biofilm formation. The colony-forming units per milliliter of each specimen were quantified, and metabolic activity was assessed. The results demonstrated that all wood specimens treated with silver nanoparticles effectively inhibited the growth of the biofilm and metabolic activity of *Aspergillus niger*. Consequently, it can be concluded that the treatment of wood with silver nanoparticles effectively inhibit the growth of the biofilm and metabolic activity of *Aspergillus niger*. This finding holds significant potential to enhance the quality and durability of wooden structures in construction. Additionally, the prevention of mold formation contributes to improving the quality of life for residents and the environment, as it reduces the need for cleaning products, and minimizes the necessity for building renovations that generate waste in landfills.

PALAVRAS-CHAVE: *Pinus elliottii*. AgNP. Antimicrobial. Antifungal. *Aspergillus niger*.

1 INTRODUCTION

Pinus elliottii is a pine tree species that provides certified wood for use and reforestation. It exhibits rapid growth and ease of handling and serve as crucial sources of timber for construction and furniture industries. Additionally, they are utilized to make many products, like turpentine, rosin, pulp, paper and others. *Pinus elliottii* var. *elliottii*, commonly known as slash pine, is a very significant species for timber and resin production (YANG; LI, 2016).

The emergence of "mass timber" technologies, along with related technical research and regulatory revisions, highlights the potential for engineered timber products and structural systems to serve as viable alternatives to mineral-based materials in urban building construction (CHURKINA et al., 2020). Himes and Busby 2020 replaced conventional building materials with mass timber reduces construction phase emissions by 69%, or an average of 216 $kgCO_2e/m^2$ of floor area. The analyzed studies unanimously show emissions reductions with the use of mass timber. Scaling up its use in 50% of new urban construction could provide up to 9% of the global emissions reduction needed to meet 2030 targets for limiting global warming below 1.5°C (HIMES; BUSBY, 2020).

Although some wood species are less susceptible to spoilage agents, no wood possesses natural resistance to all forms of biodeterioration such as fungi, insects, and marine borers. Wood protection involves the addition of toxic or repellent substances, called preservatives, to increase resistance to decay and improve durability. Preservative treatments can extend the service life of wood, reduce replacement costs, and maximize the utilization of forest resources. Increasing environmental concerns and the desire for sustainable practices have led to pressure to reduce or eliminate the use of traditional preservatives, such as chromated copper arsenate and chromated copper borate, which are effective but contribute to environmental pollution, emphasizing the need for the development of safer products for environmental preservation (BORGES et al., 2018).

Silver nanoparticles have demonstrated exceptional antimicrobial properties, leading to their widespread application in sectors such as biomedicine, pharmaceuticals, cosmetic

industry, and textiles. Compared to other metallic particles, AgNPs exhibit superior antimicrobial efficacy, reduced toxicity, and improved biocompatibility (CRISAN et al., 2021; DE SOUZA; SOUZA; FRANCHI, 2019). AgNPs have a large surface contact area, exhibit high chemical reactivity and antimicrobial action (NAKAMURA et al., 2019). Therefore, it has been applied in medical and biomedical products to reduce infections, prevent bacterial colonization on surfaces.

The possibility of using AgNP in civil construction materials to make them antimicrobial has been demonstrated by (DA SILVA et al., 2019). The authors demonstrated the prevention of *Staphylococcus aureus* biofilm formation in gypsum and mortar composed with AgNP and the efficacy against and *A. Niger*. In Web of Science search, only two papers described the antifungal potential of pine wood treated with AgNP. Paril et al. (2017) evaluated the treatment of *Pinus Sylvestris* L. with silver and copper nanoparticles, testing against brown-rot fungus (*Poria placenta*). The pine wood specimens were vacuum impregnated at 80 kPa for 20 minutes with solutions of 1 and 3 g/L of AgNP and Cu-NP. The mass loss was evaluated after 16 weeks of exposure to the fungus. Although it was not possible to confirm the effectiveness of the treatment with AgNP, the 3 g/L of Cu-NP treatment presented a lower mass loss compared to untreated samples (PAŘIL et al., 2017). Can et al. (2018) also evaluated the weight loss of *Pinus Sylvestris L.,* due to white-rot fungus (*Trametes versicolor*) infection. Samples of 5 x 15 x 30 mm (H x W x L) were treated with autoxidized soybean oil polymer containing Ag nanoparticles (Agsbox) and polystyrene-soybean oil copolymer (AgPSsb). The control specimens showed a weight loss of 29.74%, while the treatment with Agsbox and AgPSsb reduced the weight loss by 90.38% and 97.07%, respectively (CAN; SIVRIKAYA; HAZER, 2018). Studies of antifungal efficiency were carried out with the application of Ag nanoparticle in transparent wood and poplar wood, resulting in the reduction of degradation by fungal attack of the studied woods (KÜNNIGER; HEEB; ARNOLD, 2014; MORADI MALEK et al., 2013). Therefore, the literature is still sparse, indicating need of studies to demonstrate the potential of AgNP as antifungal treatment of pine wood.

Treatments with nanoparticles present alternative methods for wood protection and are crucial to reduce environmental impacts. This study evaluated the antifungal action against *Aspergillus niger* of *Pinus elliottii* wood samples treated with silver nanoparticles (AgNPs).

2 MATERIAL AND METHODS

2.1 Synthesis of AgNP

The AgNPs were synthesized through the chemical reduction of silver nitrate ($AgNO_3$) using sodium borohydride ($NaBH_4$) (GUIDELLI et al., 2013). A solution of 8 mM $AgNO_3$ was added to a solution of 16 mM $NaBH_4$. The entire system was kept under agitation at 200 rpm for 6 hours at 21ºC to ensure complete reduction of the silver.

During the synthesis, $NaBH_4$ is added in excess compared to $AgNO_3$, as it acts as a reducing agent for silver ions and is responsible for stabilizing the nanoparticles. The $NaBH_4$ adsorb onto the nanoparticle surface, enveloping them and creating charges on their surfaces,

which generate electrostatic repulsion between them, preventing agglomeration (MELO JR. et al., 2012)

2.2 Minimum inhibitory concentration (MIC) of AgNP against *Aspergillus niger*

The experiment was performed to determine a breakpoint for the antifungal action of AgNPs. *Aspergillus niger* (CCCD-AA 001) was used in all experiments. The final concentration of the inoculum ranged from 1.0 x 10^5 to 2.5 x 10^5 CFU/mL, using the conidial suspension method prepared following the guidelines of "The European Committee on Antimicrobial Susceptibility Testing" with modifications proposed by "The Brazilian Committee on Antimicrobial Susceptibility Testing" (BRCAST) (MELETIADIS et al., 2020).

The isolates were cultured on potato dextrose agar and incubated at 35ºC. Inoculum suspensions were prepared from cultures after 5 days of incubation. The 5-day culture of *Aspergillus niger* (CCCD-AA 001) was covered with approximately 5 mL of sterile distilled water supplemented with 0.1% Tween 20. Subsequently, the conidia were carefully scraped with a sterile swab, and the material was transferred with a sterile pipette to a sterile Falcon tube. The suspension was homogenized for 15 seconds on a vortex mixer at approximately 2000 rpm. Then, 5 mL of the suspension was filtered through a sterile filter with 11 µm diameter pores. This step removed the hyphae to obtain a suspension composed of conidia. The suspension was adjusted to a concentration of 2-5 x 10^6 conidia/mL by counting in a hemocytometer (Neubauer chamber). Then, a 1:10 dilution was made in RPMI 1640 medium (with L-glutamine and pH indicator without bicarbonate) supplemented with glucose to a final concentration of 2% (RPMI 2% G), sterile, to obtain the working concentration of the inoculum, 2-5 x 10^5 CFU/mL.

Plates with 96 wells with a capacity of approximately 300 µL per well were used. From wells 2 to 11, in each column, 100 µL of the corresponding concentration of AgNP was dispensed. Serial dilutions (1:2) of AgNPs solution were made in RPMI culture medium with 2% glucose (MELETIADIS et al., 2020). The initial concentration of AgNPs was 216 µg/mL, and the final concentration was 0.42 µg/mL.

In column 01, 200 µL of RPMI 2% glucose medium was dispensed as a sterility control, and in column 12, 100 µL of RPMI 2% glucose medium plus 100 µL of *Aspergillus niger* inoculum was dispensed, serving as the growth control.

The microdilution plates were inoculated within 30 minutes of preparing the inoculum suspension to maintain the concentration of viable conidia. The conidial suspension at 2-5 x 10^5 CFU/mL was agitated and inoculated into each well of the microdilution plate, without touching the well content, using a multichannel pipette in 100 µL, in a horizontal direction - rows "A, B, C, D, E, F, G, and H" in wells 2 to 12 of each row. After this procedure, the required concentration of AgNP and the density of the inoculum was obtained.

The microdilution plates were incubated without agitation at 37°C for 48 ± 2 hours in atmospheric air. Viability counting was performed for quality control and confirmation that the test wells contained between 1-2.5 x 10^5 CFU/mL.

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

The MIC (Minimum Inhibitory Concentration) for AgNP is defined as the lowest concentration that visually inhibits fungal growth in the well, compared to the control growth (column 12). In addition to direct visual reading, another test was performed using resazurin, a redox indicator. Initially, resazurin has a blue color, but upon entering the cells and in response to the metabolic activity of viable cells, it is reduced to resorufin, which has a pink color and is fluorescent. Therefore, after the incubation period and visual reading to determine the MIC of AgNP, 20 µL of the resazurin solution (2 mg/mL) was added, and the test was revealed after an additional two-hour incubation period.

2.3 Wood specimen and treatments

Wood specimens measuring 5 x 5 x 5 mm of *Pinus elliottii* were used. The size of the samples allowed the use of a 24-well plate for the microbiology experiments. The wood specimens were cut using a table saw with a 10-inch blade and a miter saw with the same size blade. They were subsequently sterilized in an autoclave.

The treatment with AgNPs was performed by immersing the specimens in AgNP solutions at 3 concentrations: 432 µg/mL, 216 µg/mL, and 108 µg/mL. Three Falcon tubes were used, with 40 mL of each solution and 12 specimens. The tubes with the samples were placed in an orbital shaker for a period of 60 minutes. After an interval of 60 minutes, they were agitated again for 60 minutes. After this 3-hour immersion process, the samples were removed using sterile forceps and placed in Petri dishes with sufficient spacing to dry in an oven at 37°C for 24 hours. It was observed that each specimen has the capacity to absorb 0.125 mL of nanoparticle solution, and the residual solution maintained the same coloration, demonstrating the stability of the nanoparticles.

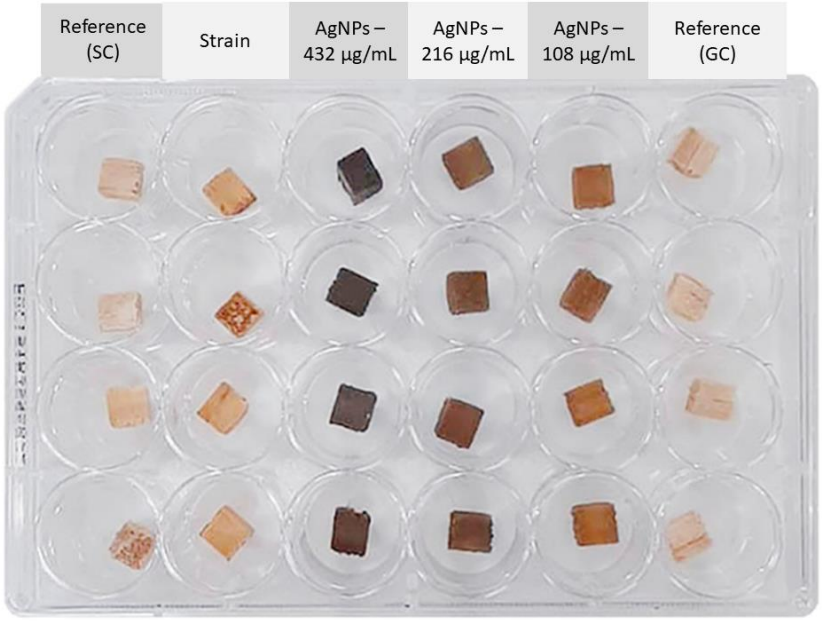
As a reference, 4 wood specimens were treated with Osmocolor® preservative stain. According to the manufacturer (Montana® Química LTDA, São Paulo, Brazil), this product is composed of 0.25% IPBC and 99.75% resins, pigments, mineral fillers, and solvent. When applied, it provides to the wood high resistance to ultraviolet rays and fungi infestation, as well as a hydrophobic function. However, it is not recommended for containers in contact food, water, or soil. For this treatment, the wood specimen, previously sterilized, were immersed in the product for 1 hour. They were then removed and dried in an oven at a controlled temperature of 37°C. All specimens were autoclaved after treatment to ensure the sterility of samples.

2.4 Microbiological evaluation of *P. elliottii* treatment

To evaluate the efficacy of the treatments, a biofilm experiment was conducted with *A. niger*. Four wood specimens of each type, treated with AgNPs at the 3 concentrations, four treated with stain, and eight untreated specimens, were subjected to the fungal biofilm experiment. The specimens were positioned in a 24-well plate (Fig. 1).

Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"
ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Figure 1 - *Pinus elliottii* wood specimens. Reference (untreated) and treated with silver nanoparticles (AgNPs) and Stain. In the first column, the reference material was kept without Biofilm, for sterility control (SC). In the last column, the reference was exposed to the biofilm for growth control (GC)



Source: prepared by the authors.

Wells containing only culture medium were included in the assay as a control for reaction sterility, as well as wells with inoculum in RPMI medium as growth controls (100% cell viability). Four untreated specimens were kept without contact with the fungus (negative control) and four untreated specimens were placed in contact with fungus (positive control).

The fungal suspension prepared according to previously described, with the inoculum concentration of 2-5 x 10^6 CFU/mL. The fungal suspension at 2-5 x 10^6 CFU/mL was agitated, and 2.0 mL were inoculated into corresponding well without touching the specimen, using a 5.0 mL automated pipette.

Then, the plate was incubated at 37°C for 4 hours for the cell adhesion phase. After the incubation period, the supernatant was aspirated, and 2.0 mL of RPMI were added to each well containing the wood specimen, followed by further incubation for a total of 72 hours (PIERCE et al., 2008; SEIDLER; SALVENMOSER; MÜLLER, 2008).

The results of biofilm formation were evaluated through Colony Forming Units (CFU) counting method (2 specimens), metabolic activity (MTT) (1 specimen), and Field Emission Scanning Electron Microscopy (FE-SEM) imaging (1 specimen).

2.4.1 Evaluation of biofilm mass by Colony Forming Units (CFU) counting

After the incubation period, the wood specimen was washed with sterile phosphate-buffered saline (PBS) three times and transferred to 10 mL of PBS. It was then agitated on a vortex mixer for 5 minutes. Decimal dilutions were made in PBS, and the antifungal activity of each specimen was evaluated by plating 100 µL on Sabouraud Dextrose Agar (SDA) and incubating it at 30°C for up to 7 days.

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

The number of Colony Forming Units (CFU) per milliliter (mL) was calculated based on the number of colonies formed multiplied by the dilution factor (WILSON et al., 2017). Fungicidal activity was considered when there was a reduction equal to or greater than 3 Log_{10} CFU/mL compared to the initial inoculum, resulting in a reduction of 99.99% or more of CFU/mL, while fungistatic activity was considered when there was a growth reduction of less than 99.9% or <3 Log_{10} CFU/mL of the initial inoculum (CANTÓN et al., 2003).

Each sample was tested individually, and the mean of the triplicate CFU/mL values was used for comparative analysis. A sample from the dilution sequence and controls was reserved for subsequent electron microscopy analysis. The following formula was used to determine CFU/mL:

$$CFU/mL = \frac{\text{number of colonies} \times 10^{n}}{q}$$

Where *n* corresponds to the dilution value and *q* is the volume pipetted in mL on each Petri dish containing Sabouraud agar.

2.4.2 MTT assay and biofilm metabolic activity

The evaluation of the metabolic activity of the biofilms was performed through the colorimetric assay of salt reduction using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide). The water-soluble MTT is converted by viable fungal cells into insoluble formazan crystals, which are purple. When these crystals are solubilized with isopropyl alcohol, their concentration can be determined by optical density (spectrophotometry).

After the biofilm incubation time, the test specimen was washed with sterile PBS three times and transferred to 10 mL of PBS. It was sonicated for 5 minutes in a vortex. In a 96-well plate, an aliquot of 100 µL was placed for the assessment of metabolic activity. Then, 100 µL of MTT solution (5 mg/mL) was added to each well. The microplates were incubated in the dark at 37°C for 3 hours. After incubation, the MTT solution was removed from all wells. Then, 100 µL of 0.5% dimethyl sulfoxide (DMSO) was added to each well to dissolve the MTT formazan crystals. The microplates were shaken for 10 minutes on an orbital shaker. Subsequently, the DMSO content from each well was transferred to another 96-well plate, and the absorbance was read using a microplate reader (Mindray, model MR 96-A, USA) at a wavelength of 490 nanometers (OD 490 nm). The percentage (%) of biofilm reduction, based on the metabolic activity of the fungus, using the following formula:

$$\%\ biofilm\ reduction = \frac{positiveC\ -\ test\ average}{positiveC}$$

Where PositiveC represents the mean of the positive control, and test average represents the mean of the treatment groups.

2.4.3 FE-SEM images of specimens

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

One sample from each treatment group and one reference was prepared for imaging by Field Emission Scanning Electron Microscopy (FE-SEM). The samples were washed three times with PBS, following the same technique for both treated and untreated biofilms. For fixation of the biofilm, 1 mL of 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M potassium phosphate buffer at pH 7.4 were added to each well. Subsequently, the samples underwent six washes with pure PBS solution at 15-minute intervals, followed by post-fixation with 1% osmium tetroxide for 16 hours. After the fixation period, the material was subjected to six washes with the same buffer solution and dehydrated using increasing concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 95%, 100%) (RAMAGE et al., 2012).

The images were obtained to investigate the surface of the material and the action of AgNPs on *A. niger* biofilms by Field Emission Scanning Electron Microscopy (FE-SEM), Jeol, model JSM-7500F, software PC-SEM v.2.1. 0.3. The samples were coated with ultrathin gold by a sputter coater (Edwards Model T-Station 75) to avoid any artifacts on images.

2.5 Statistical analysis

All assays were performed in triplicates. The data of metabolic activity were analyzed using ANOVA test followed by Tukey *post hoc* test. The UFC/mL data were subjected to Kruskal-Wallis test, followed by Dunn's *post hoc* test. For all tests, differences were considered statistically significant when $p<0.05$.

3 RESULTS AND DISCUSSION

3.1 Silver nanoparticles synthesis (AgNP)

The colloidal solution synthesized by the chemical reduction method, with a silver concentration of 432 µg/mL of Ag, exhibited a brown coloration. The confirmation of nanoparticle formation was achieved by the presence of a plasmonic peak at 400 nm, obtained through UV-Vis spectrophotometry. The absorption spectrum with a resonant plasmon band in this region indicates that the silver nanoparticles have a spherical shape (ISLAM; JACOB; ANTUNES, 2021). Variations in coloration can occur due to the extinction of incident light (absorption and/or scattering) at specific wavelengths, which strongly depend on the size, morphology, and chemical environment of the nanoparticles (KHAN; SAEED; KHAN, 2019). The stability of the system is because the formed nanoparticles remain in suspension and do not aggregate to form larger particles (MELO JR. et al., 2012)

3.2 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of AgNP, that is, the breakpoint of *Aspergillus niger* (CCCD-AA001) growth, found is 27 µg/mL, characterized by the of fungal growth in the wells of column 4, also confirmed by the reduction of resazurin, which has a blue color and is reduced to resorufin, which has a pink color, upon contact with viable cells.

The antimicrobial properties of AgNPs are already known against microorganisms such as Gram-positive and Gram-negative bacteria, fungi, and viruses. The effect occurs when nanoparticles encounter the cell membrane of these microorganisms, causing damage to the cellular respiration and permeability processes. They also bind to sulfur and phosphorus present in DNA, inhibiting cell division (ISLAM; JACOB; ANTUNES, 2021; WAHAB; MAMUN, 2020).

Assis da Silva et al. (2022) demonstrated inhibition against different tested pathogens, The author showed that AgNPs at concentrations ranging from 80 to 300 µg/mL inhibited the growth of *Aspergillus niger, Penicillium funiculosum, Fusarium oxysporum, Aspergillus fumigatus, Aspergillus parasiticus,* and *Trichophyton mentagrophytes*, similar to the results found in this workGutarowska et al. (2012) also found the effectiveness of AgNPs against 32 strains of bacteria and fungi belonging to 12 genera, responsible for the contamination of museums and archives. The authors tested two strains of *Aspergillus niger*, one from the ATCC, which showed an MIC of 22.5 ppm, and another provided by the Central Tissue Museum of Poland, with an MIC of 45.00 ppm. It was revealed that a concentration of 90 ppm was effective against all microorganisms tested, while a concentration of 45 ppm was effective against 94% of them. Thus, it can be observed that there are variations in the MIC results depending on the type of microorganism, and even when dealing with the same fungus, there are variations in the MIC depending on its origin. The result of this study are in agreement within the range of values found in the literature Assis da Silva et al. (2022) and Gutarowska et al. (2012) indicates that the treatment of wood with AgNPs may be effective against other microorganisms.

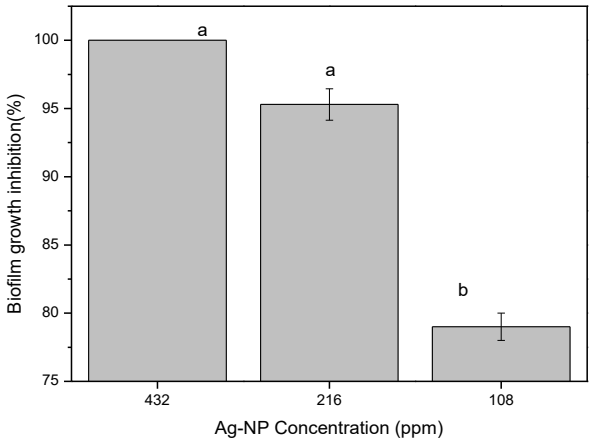
Falcão et al. (2022) studied the use of silver nanoparticles associated with fluconazole, found that AgNPs exhibited fungistatic and fungicidal activity at low concentrations (between 4 to 8 ppm and 8 to 16 ppm, respectively). When AgNPs were used in combination with fluconazole, it was possible to reverse the resistance of *Candida albicans* strains. The MIC reduction of fluconazole changed from 87.5 to 99.8%. All associations were synergistic. Even at subinhibitory concentrations, such as 2 ppm (50% of MIC), silver nanoparticles were able to inhibit germ tube formation. The results demonstrated fungistatic and fungicidal activity at concentrations lower than those obtained in this work, however, these results strengthen the demonstration that AgNPs to promote fungistatic and fungicidal action against different species of microorganisms.

3.3 Evaluation of *Aspergillus niger* biofilm growth inhibition

The evaluation of colony-forming units per mL (CFU/mL) of the biofilms revealed inhibition of *Aspergillus niger* (CCCD-AA001) growth at all tested concentrations compared to the fungal viability control (growth control) ($p<0.05$) (Fig. 2).

Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"
ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Figure 2 - Percentage of growth inhibition of Aspergillus niger (CCCD-AA001) on wood treated with silver nanoparticles at different concentrations (µg/mL). Different letters indicate significant differences determined by Kruskal Wallis and Dunn post hoc test ($p<0.05$)



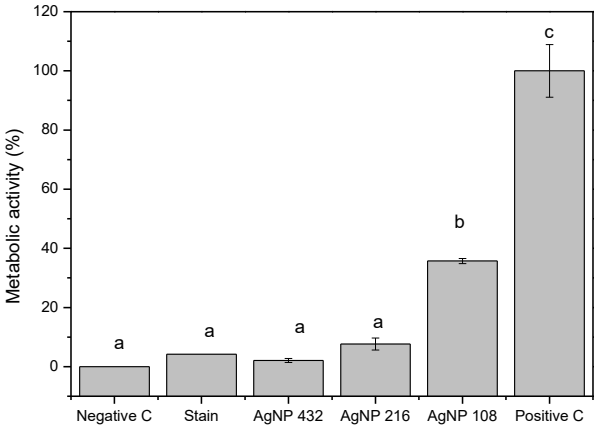
Source: prepared by the authors.

There was fungicidal activity at a concentration of 432 µg/mL, with a 100% reduction compared to the initial inoculum, and fungistatic activity was observed at a concentration of 108 µg/mL, with an 80% reduction in growth compared to the initial inoculum (CANTÓN et al., 2003).

3.4 Reduction of biofilm metabolic activity

Regarding the metabolic activity of viable cells in the biofilm, higher metabolic activity of *Aspergillus niger* (CCCD-AA 001) was observed in the untreated group (growth control group) compared to the other treated groups. The reduction in metabolic activity of *Aspergillus niger* was 95.8%, 97.9%, 92.3% in the Stain, AgNP 432, and 216 µg/mL specimens, respectively, and a 64.3% reduction in metabolic activity in the 108 µg/mL group (Fig. 3)

Figure 3 - Prevalence of metabolic activity of Aspergillus niger (CCCD - AA 001) due to treatment with silver nanoparticles at different concentrations and controls. Different letters indicate significant differences determined by One way ANOVA test followed by Tukey post hoc test ($p<0.05$).



Source: prepared by the authors.

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

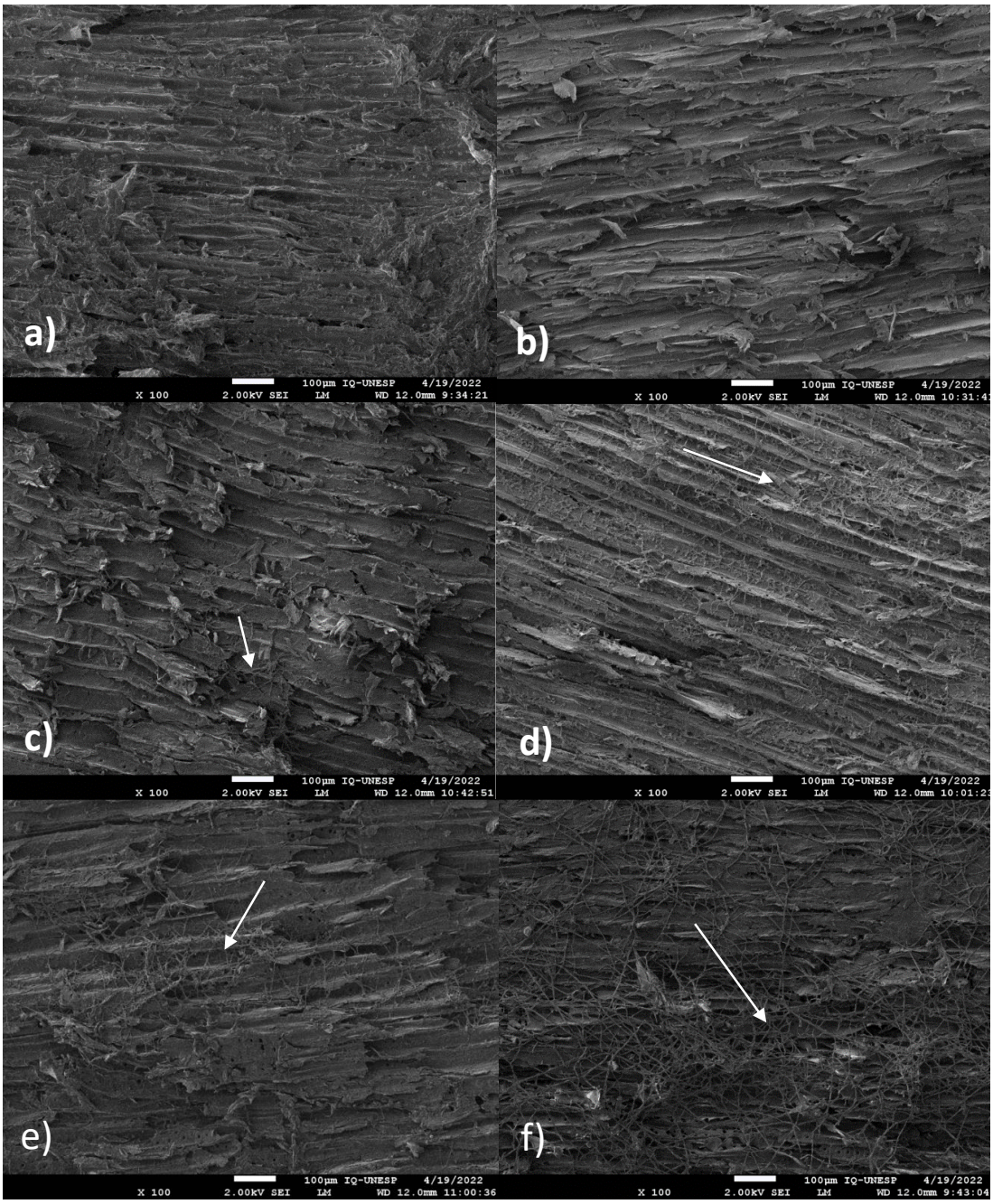
3.5 Field Emission Scanning Electron Microscopy (FE-SEM) and Energy-Dispersive X-ray Spectroscopy (EDS)

Fig.4 show FE-SEM images of the reference specimens (negative and positive control), treated with different concentrations of AgNPs and with Stain. In comparison to the image of Negative Control (4a), it is observed that the Stain (4b) treatment prevents the attachment of *Aspergillus niger* biofilm. This is probably due to the hydrophobic nature of the material, as specified by the manufacturer. In the samples treated with AgNPs (4c, 4d e 4e), it can be observed, at a magnification of 100x, the presence of, but in much lower quantities than in the positive control (4f), which refers to the untreated specimen that was in contact with the fungus. In higher magnification (Fig. 5) it is possible to observe the structure of the *Aspergillus niger* biofilm. In Fig. 5a, of positive control specimen, mature biofilm revealed a microarchitectural biofilm, consisting of clusters of compact cells, connected by extracellular matrix (ECM) mainly composed of galactomannan, galactosaminogalactan, α-1,3-glucan and melanin. Additionally, in the Fig.5b of AgNP treated specimen (432 µg/mL) it can be observed that the fungus present in the samples is inactivated, characterized by a depletion in the number of cells and a decrease in extracellular material, loss of structural organization in addition to morphological changes in cells that were collapsed.

Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

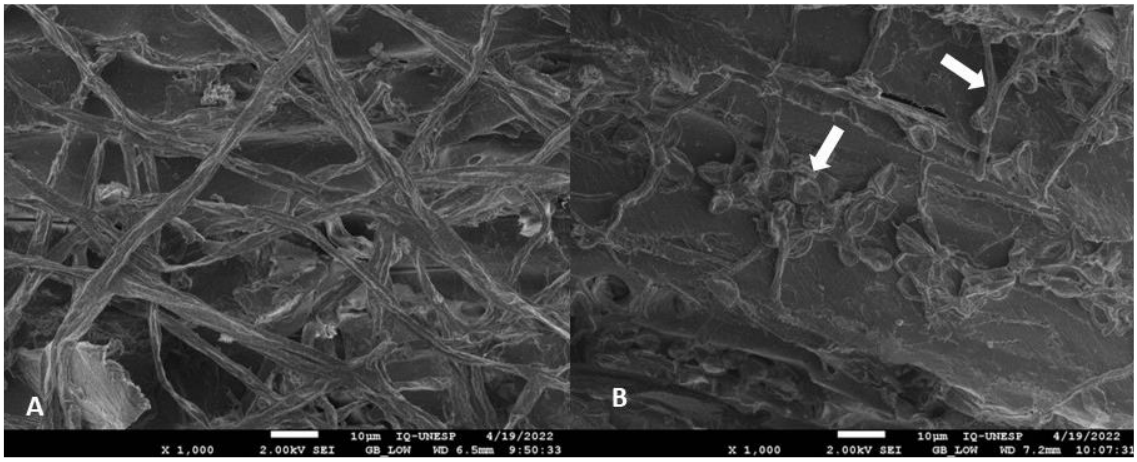
Figure 4 - Scanning electron microscopy (FE-SEM) of P. elliottii samples (Mag 100x) - (a) Negative Control; (b) Stain; samples treated with AgNPs: (c) Ag-NPs 432 µg/mL, (d) Ag-NPs 216 µg/mL, (e) Ag-NPs 108 µg/mL and (f) Positive Control (without treatment). White arrows indicate the A. Niger biofilm.



Source: prepared by the authors.

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

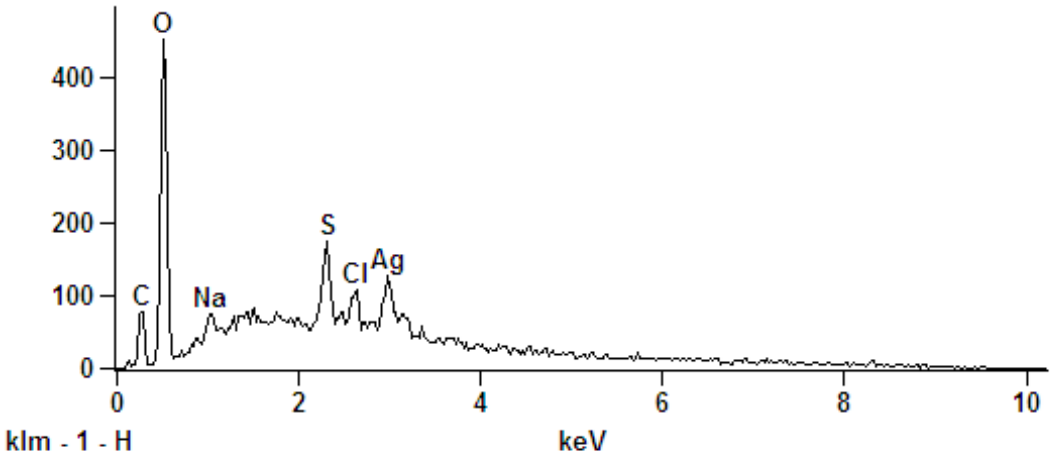
Figure 5 - Scanning electron microscopy (FE-SEM) of P. elliottii samples, (a) mature biofilm in untreated specimen (positive control) showed clusters of compact cells, connected by extracellular matrix (ECM) and (b) mature biofilm on AgNP treated specimen, showing morphologically altered cells (arrows), and fragmented structure of the biofilm.



Source: prepared by the authors.

Fig. 6 shows the EDS spectrum of sample *P. elliottii* treated with AgNP 432 µg/mL, showing the peak of Ag, demonstrating the efficacy of the procedure to impregnate the Ag in the specimen.

Figure 6 - EDS spectrum of sample P. elliottii treated with AgNP 432 µg/mL. Accelerating Voltage: 10.0 kV



Source: prepared by the authors.

Melo Jr. et al. (2012) identified that the stability of the system comes from the fact that the formed nanoparticles remain in suspension and do not aggregate into larger particles. It can be noted that achieving stable synthesis can be a challenge for the industrial-scale production of wood treated with AgNPs. However, the satisfactory results of this work can corroborate the applicability of the suggested treatment, that can be expanded in further scientific research.

FE-SEM analyzes of *Aspergillus niger* biofilms (CCCD-AA 001) shows an ECM involving cells within the biofilm structure. This matrix is an important factor responsible for the resistance of the biofilm to environmental stress and chemical and physical aggression (RAMAGE et al., 2012). Also, from the analysis of the FE-SEM images, it is possible to observe the structure of the *Aspergillus niger* biofilm (CCCD-AA 001) as a set of compact cells, connected by an extracellular matrix (BEAUVAIS et al., 2007) and that silver nanoparticles were able to fragment and disrupt the biofilm, causing loss of structure.

Mohajerani et al. (2019) state that in the industrial sector, nanoparticles provide various benefits, such as new production possibilities that allow for technological, health, and environmental advances. The data obtained in this study also indicate that the treatment of wood with silver nanoparticles proposed is viable and effective for application in various market sectors. Another interesting fact is that the typical dark color of AgNPs can provide intere sting aesthetic characteristics to distinguish between untreated and AgNPs treated wood. It is known that there are different types of treatments that alter the color of wood, such as the treatment with chromated copper arsenate, which gives it a greenish color, or the treatment through surface carbonization of wood, which assign a dark color, similar to AgNPs applied at higher concentrations. Therefore, in addition to the gain from the attribution of antimicrobial activity, the treatment also provides visual appearance that can become a valuable aesthetic feature.
However, despite the numerous demonstrated benefits, it is important to emphasize that further research is needed to ensure the safety use of products with AgNPs, including their potential effects on the environment and human health.

4 CONCLUSIONS

This work evaluated the antifungal action against *Aspergillus niger* in *Pinus elliottii* wood samples treated with silver nanoparticles (AgNPs). It was observed that wood treated with AgNPs resulted in a reduction in the mass of adhered biofilm (100%), and reduction of the metabolic activity of the biofilm (76.1), demonstrating the fungicidal (432 µg/mL) and fungistatic (108 µg/mL) action of the new material.

Thus, it can be concluded that the *Pinus elliottii* wood treated with silver nanoparticles presents antimicrobial activity.

Acknowledgments: This study was financed in part by the Coordination for the Improvement of Higher Education Personnel (CAPES)- Brazil - Finance Code 001 and National Council for Scientific and Technological Development (CNPq grants 309186/2020–0). The authors are grateful to PPGMADRE of University of Western São Paulo.

REFERENCES

ASSIS DA SILVA, C. et al. Effects of mycogenic silver nanoparticles on organisms of different trophic levels. **Chemosphere**, v. 308, p. 136540, 2022.

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

BEAUVAIS, A. et al. An extracellular matrix glues together the aerial-grown hyphae of Aspergillus fumigatus. **Cellular Microbiology**, v. 9, n. 6, p. 1588–1600, 2007.

BORGES, C. C. et al. NANOPARTICLES-BASED WOOD PRESERVATIVES: THE NEXT GENERATION OF WOOD PROTECTION? **CERNE**, v. 24, n. 4, p. 397–407, 2018.

CAN, A.; SIVRIKAYA, H.; HAZER, B. Fungal inhibition and chemical characterization of wood treated with novel polystyrene-soybean oil copolymer containing silver nanoparticles. **International Biodeterioration & Biodegradation**, v. 133, p. 210–215, 2018.

CANTÓN, E. et al. Minimum fungicidal concentrations of amphotericin B for bloodstream Candida species. **Diagnostic Microbiology and Infectious Disease**, v. 45, n. 3, p. 203–206, 2003.

CHURKINA, G. et al. Buildings as a global carbon sink. **Nature Sustainability**, v. 3, n. 4, p. 269–276, 2020.

CRISAN, C. M. et al. Review on Silver Nanoparticles as a Novel Class of Antibacterial Solutions. **Applied Sciences**, v. 11, n. 3, p. 1120, 2021.

DA SILVA, G. D. et al. Silver nanoparticles in building materials for environment protection against microorganisms. **International Journal of Environmental Science and Technology**, v. 16, n. 3, p. 1239–1248, 2019.

DE FILPO, G. et al. Preventing fungal growth in wood by titanium dioxide nanoparticles. **International Biodeterioration & Biodegradation**, v. 85, p. 217–222, 2013.

DE PERES, M. L. et al. Zinc oxide nanoparticles from microwave-assisted solvothermal process: Photocatalytic performance and use for wood protection against xylophagous fungus. **Nanomaterials and Nanotechnology**, v. 9, p. 184798041987620, 2019.

DE SOUZA, T. A. J.; SOUZA, L. R. R.; FRANCHI, L. P. Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity. **Ecotoxicology and Environmental Safety**, v. 171, p. 691–700, 2019.

FALCÃO, C. M. C. et al. Activity of poly(methacrylic acid)-silver nanoparticles on fluconazole-resistant Candida albicans strains: Synergistic and cytotoxic effects. **Journal of Applied Microbiology**, v. 132, n. 6, p. 4300–4309, 2022.

GUIDELLI, É. J. et al. Silver nanoparticles delivery system based on natural rubber latex membranes. **Journal of Nanoparticle Research**, v. 15, n. 4, 2013.

GUTAROWSKA, B. et al. Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles. **International Biodeterioration & Biodegradation**, v. 68, p. 7–17, 2012.

HARANDI, D.; AHMADI, H.; MOHAMMADI ACHACHLUEI, M. Comparison of TiO2 and ZnO nanoparticles for the improvement of consolidated wood with polyvinyl butyral against white rot. **International Biodeterioration & Biodegradation**, v. 108, p. 142–148, 2016.

HIMES, A.; BUSBY, G. Wood buildings as a climate solution. **Developments in the Built Environment**, v. 4, p. 100030, 2020.

HOLY, S. et al. Physical properties, thermal and fungal resistance of Scots pine wood treated with nano-clay and several metal-oxides nanoparticles. **Wood Material Science & Engineering**, v. 17, n. 3, p. 176–185, 2022.

ISLAM, M. A.; JACOB, M. V.; ANTUNES, E. A critical review on silver nanoparticles: From synthesis and applications to its mitigation through low-cost adsorption by biochar. **Journal of Environmental Management**, v. 281, n. January, p. 111918, 2021.

KHAN, I.; SAEED, K.; KHAN, I. Nanoparticles : Properties , applications and toxicities. **Arabian Journal of Chemistry**, 2019.

KÜNNIGER, T.; HEEB, M.; ARNOLD, M. Antimicrobial efficacy of silver nanoparticles in transparent wood coatings. **European Journal of Wood and Wood Products**, v. 72, n. 2, p. 285–288, 2014.

MELETIADIS, J. et al. E.DEF. 7.3.2- Método para determinação de concentração inibitória mínima em caldo dos agentes antifúngicos para leveduras. **BrCAST, Brazilian Committe on Antimicrobial Susceptibility Testing**, p. 1–19, 2020.

MELO JR., M. A. et al. Preparação de nanopartículas de prata e ouro: um método simples para a introdução da nanociência em laboratório de ensino. **Química Nova**, v. 35, n. 9, p. 1872–1878, 2012.

MOHAJERANI, A. et al. Nanoparticles in construction materials and other applications , and implications of nanoparticle use. **Materials**, v. 12, p. 1–25, 2019.

MORADI MALEK, B. et al. Effects of silver nanoparticles and fungal degradation on density and chemical composition of heat-treated poplar wood (Populous euroamerica). **European Journal of Wood and Wood Products**, v. 71, n. 4, p. 491–495, 2013.

NAKAMURA, S. et al. Synthesis and Application of Silver Nanoparticles (Ag NPs) for the Prevention of Infection in Healthcare Workers. **International Journal of Molecular Sciences**, v. 20, n. 15, p. 3620, 2019.

PAŘIL, P. et al. Antifungal effects of copper and silver nanoparticles against white and brown-rot fungi. **Journal of Materials Science**, v. 52, n. 5, p. 2720–2729, 2017.

PIERCE, C. G. et al. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. **Nature Protocols**, v. 3, n. 9, p. 1494–1500, 2008.

RAMAGE, G. et al. Fungal Biofilm Resistance. **International Journal of Microbiology**, v. 2012, p. 1–14, 2012.

SEIDLER, M. J.; SALVENMOSER, S.; MÜLLER, F.-M. C. Aspergillus fumigatus Forms Biofilms with Reduced Antifungal Drug Susceptibility on Bronchial Epithelial Cells. **Antimicrobial Agents and Chemotherapy**, v. 52, n. 11, p. 4130–4136, 2008.

WAHAB, J. A.; MAMUN, S. AL. Polyacrylonitrile nanofiber mats containing titania/AgNP composite nanoparticles for antibacterial applications. **Materials Research Express**, v. 7, n. 1, p. 015416, 2020.

WILSON, C. et al. Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review Christina. **Res Rev J Eng Technol**, v. 6, n. 4, 2017.

YANG, Y.; LI, C. Photosynthesis and growth adaptation of Pterocarya stenoptera and Pinus elliottii seedlings to submergence and drought. **Photosynthetica**, v. 54, n. 1, p. 120–129, 2016.