



Synthesis of silver nanoparticles using *Aspergillus niger* Tiegh and its effects on *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT: Silver nanoparticles (AgNPs) have become widely recognized for their potent antibacterial properties. The current study develops an environment-friendly approach using *Aspergillus niger* Tiegh as a reducing agent in the synthesis of AgNPs. Hence, this study aimed to evaluate the nanomaterials synthesized with optimized concentrations of silver nitrate (0.5, 1.0 and 1.5 mM) and assessed its antibacterial activity against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight in rice. Optimizing different concentrations of silver nitrate in the biosynthetic process revealed significant effects on the size of synthesized AgNPs. The nanoparticles were characterized through UV-visible spectroscopy, SEM coupled with EDX and FTIR Spectroscopy. The study concluded that 1.0 mM concentration yields the smallest synthesized nanoparticles sizes ranging from 68-99 nm. *In vitro* assay was done through agar well diffusion method and showed high significance ($p < 0.0000$) against *Xanthomonas oryzae* pv. *oryzae*.

Keywords: synthesis; AgNPs; reducing agent.

Síntese de nanopartículas de prata usando *Aspergillus niger* Tiegh e seus efeitos sobre *Xanthomonas oryzae* pv. *oryzae*

RESUMO: As nanopartículas de prata (AgNPs) tornaram-se amplamente reconhecidas por suas potentes propriedades antibacterianas. O estudo atual desenvolve uma abordagem ecologicamente correta usando *Aspergillus niger* Tiegh como um agente redutor na síntese de AgNPs. Portanto, este estudo teve como objetivo avaliar os nanomateriais sintetizados com concentrações otimizadas de nitrato de prata (0,5, 1,0 e 1,5 mM) e avaliou sua atividade antibacteriana contra *Xanthomonas oryzae* pv. *oryzae*, causadora da queima bacteriana das folhas em arroz. A otimização de diferentes concentrações de nitrato de prata no processo biossintético revelou efeitos significativos no tamanho das AgNPs sintetizadas. As nanopartículas foram caracterizadas por espectroscopia UV-visível, SEM acoplada a espectroscopia EDX e FTIR. O estudo concluiu que a concentração de 1,0 mM produz os melhores tamanhos de nanopartículas sintetizadas, variando de 68 a 99 nm. O ensaio *in vitro* foi feito pelo método de difusão em poço de ágar e mostrou alta significância ($p < 0,0000$) contra *Xanthomonas oryzae* pv. *oryzae*.

Palavras-chave: síntese; AgNPs; agente redutor.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the lifeblood of agriculture in the Philippines, the staple food for most of its population. As the world's population grows, ensuring steady and successful rice production becomes crucial to meet the increasing food demand. However, there are challenges due to various biotic factors, and one of the significant threats to rice cultivation is the infection of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight disease.

Bacterial blight alone can result in yield reductions ranging from 20% to 30%, with losses reaching up to 50% in certain regions (VERDIER et al., 2012). In the Philippines, bacterial leaf blight is the second most important disease next

to rice blast as it significantly affects the yield for about 24.50% in moist to 7.21% in completely dry season in vulnerable crops and 9.50% to 1.08% respectively, in healthy crops (EXCONDE, 1973). It kills two to three weeks upon infection, and plants that survived the infection manifest stunted, reduced tillering, and poor-quality grains.

Widespread pesticide treatment has caused problems in developing resistance to plants and negatively impacted crop physiology by disrupting photosynthetic activity. This can lead to decreased production of photoassimilates, ultimately reducing crop growth and yields (PETIT et al., 2012). Researchers have developed new antimicrobial agents to

address these issues that avoid stimulating resistance and do not disrupt plant photosynthetic activity. One promising approach is the use of nanoscale materials.

Silver nanoparticles (AgNPs) are essentially silver ions that have been reduced to nanoscale dimensions, resulting in the outermost shell being completed, similar to noble metals. Despite their small size, these nanoparticles exhibit remarkable stability and diverse chemical and optical properties (HASSAN et al., 2019). High thermal stability and low toxicity make these features highly attractive across various fields (RHIM; WANG, 2014).

Due to its ability to exert multiple inhibitory actions on microorganisms (Clement; Jarrett, 1994), it can be employed for controlling various plant pathogens in a comparatively safer manner than synthetic pesticides (Park et al., 2006) as it attaches to the cell walls and membranes of microorganisms, enabling penetration into the cell interior and leads to the disruption of cellular structures.

Several techniques have been employed to synthesize silver nanoparticles, including physical, chemical, and biological methods. However, physical and chemical approaches are associated with numerous drawbacks, including requiring costly equipment, high heat production, substantial energy consumption, and low production yields (GAHLAWAT; CHOUDHURY, 2019). Therefore, there is a need to implement green/ biosynthesis methods to reduce hazardous and toxic wastes. , the preference for microbial synthesis of silver nanoparticles has grown significantly compared to the chemical method. This shift is driven by its remarkable advantages, including faster and higher production rates, lower costs, and eco-friendliness (ZHANG et al., 2011). Compared to bacteria, fungi have been recognized as having a high tolerance to metals. They are easy to handle and produce substantially larger quantities of proteins, boosting the productivity of silver nanoparticles (SASTRY et al., 2003).

Filamentous fungi like *Aspergillus niger* Tiegh are composed of the cell wall, which is mainly made of polysaccharides such as chitin and β 1,3-glucan, proteins, polyphosphates, polypeptides, and other molecules (Bernard; Latge, 2001) capable of reducing silver ions and has high binding capacity (YADAV et al., 2015). To date, numerous successful studies have focused on synthesizing silver nanoparticles using *Aspergillus niger* Tiegh as a reducing agent. Still, limited research has been done on rice's antibacterial activity against bacterial pathogens. Hence, this study aimed to optimize the different concentrations of AgNO₃ and evaluate the synthesized silver nanoparticles using *Aspergillus niger* Tiegh as a reducing agent against *Xanthomonas oryzae* pv. *oryzae*, isolated from rice.

2. MATERIAL AND METHODS

2.1. Time and Place of the Study

The biosynthesis and the characterization of silver nanoparticles (AgNPs), such as Ultraviolet-visible Spectroscopy and FTIR Analysis, were done at Central Mindanao University – Center for Natural Products Research, Development, and Extension (CPRNDE) and for Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray Spectroscopy Analysis (EDX), samples were sent the Chemistry Analytical and Research Laboratory, Ateneo de Davao University. The *in vitro* assay was conducted at Central Mindanao University- Plant Pathology Department, Musuan, Maramag, Bukidnon. The duration of the study was January- October 2024.

2.1. Isolation of Microorganisms

The inoculum of *Aspergillus niger* Tiegh was obtained from an infected onion bulb and was grown in Potato Dextrose Agar (PDA) and then incubated for 72h under room temperature. The mycelium formed was homogenized using a blender for 20 seconds. For the *Xanthomonas oryzae* pv. *oryzae*, the diseased leaves showing typical symptoms of bacterial leaf blight were cut into pieces and surface sterilized using sodium hypochlorite and washed with sterile distilled water three times. After drying the leaves, it was transferred to the nutrient agar medium and incubated for 48 hours. The pure culture was obtained by sub-culturing the single colony into agar slants.

2.2. Biosynthesis of Silver Nanoparticles

In the synthesis of silver nanoparticles using *Aspergillus niger* Tiegh, the protocol was adapted and modified by Pasha et al. (2022). The homogenized mycelia were grown in Potato Dextrose Broth in a flask and incubated in a shaker at a speed of 200 rpm for 3 days. After incubation, the fungal biomass was separated using Whatman No. 1 filter paper and aseptically washed with sterile distilled water to prevent contamination from the medium. A 20g (wet weight) biomass was mixed with 100 mL distilled water in a 500 mL Erlenmeyer flask and incubated in a shaker (150 rpm) for 2 days. After two days of incubation, the cell suspension was again filtered with Whatman No. 1 filter paper, and the supernatant was collected to synthesize nanoparticles. Equal quantities (1:1) of both filtered supernatant and AgNO₃ were used. A 50 mL supernatant of cell filtrate was added to 50 mL AgNO₃ in a 200 mL Erlenmeyer flask and kept in a shaker at 200 rpm at 30°C.

2.3. Optimization of the Biosynthesized Silver Nanoparticles

The optimization of silver nanoparticles used three different concentrations of silver nitrate (AgNO₃): 0.5, 1.0, and 1.5 mM solutions. The solution was maintained at room temperature, and the pH level was 8. The optimal concentration that produced the most favorable size nanoparticles was selected for subsequent assays.

2.4. Characterization of Silver Nanoparticles

The morphological and elemental compositions of three different concentrations (0.5 mM, 1.0 mM, & 1.5 mM) of silver nitrate synthesized with *A. niger* Tiegh were determined using Scanning Electron Microscopy coupled with Energy Dispersive X-ray Analysis (EDX). Fourier Transform Infrared Spectroscopy Analysis was employed to determine the presence of possible functional groups responsible for the formation of silver nanoparticles. The absorption spectrum of silver nanoparticles was measured using Ultraviolet-visible Spectroscopy, indicating the presence of silver nanoparticles and providing information on their size and shape.

2.5. Experimental Design and Treatments

The *in vitro* study was laid out using a Complete Randomized Design (CRD) with seven treatments replicated three times and sub-replicated five times. The treatments that were used were the following:

- Treatment 1 - Negative Control (Water only)
- Treatment 2- Positive Control (Bactericide)
- Treatment 3- 100ppm of synthesized AgNPs
- Treatment 4- 200ppm of synthesized AgNPs
- Treatment 5 -300ppm of synthesized AgNPs

Treatment 6- 400ppm of synthesized AgNPs
 Treatment 7- 500ppm of synthesized AgNPs

2.6. *In vitro* Assay of Silver Nanoparticles Against *Xanthomonas oryzae* pv. *oryzae*

The antibacterial activity of biosynthesized silver nanoparticles was done through the agar well diffusion method. The assay was conducted using nutrient agar plates inoculated with bacterial suspension and spread on plates using a sterile cotton swab. Wells were made using a 6mm cork borer, and different concentrations of silver nanoparticles were added to the wells. Sterile distilled was used for the negative control, and commercial bactericide was used for the positive control. The plates were incubated at room temperature, and the zone of inhibition was measured after 24 hours of incubation.

2.7 Statistical Analysis

The data obtained from the experiment were analyzed using Analysis of Variance (ANOVA). Significant differences between the means of different treatments were further examined using Tukey's Honestly Significant Difference (HSD) test at a significance level of 5%.

3. RESULTS

3.1. Optimization of Silver Nitrate Concentration

Based on these reactions (Figure 1), the different concentrations of silver nitrate used in this study confirm the presence of silver nanoparticles as the solution changes its color from light to dark reaction. The 0.5mM concentration of silver nitrate produces a slightly dark color compared to 1mM and 1.5 mM concentrations. In contrast, the 1.5mM concentration produces a dark color after 4 days of incubation, while the cell-free fungal filtrate does not change color.



Figure 1. (a) Control sample of the cell-free fungal filtrate of the *A. niger*; (b) Formation of silver nanoparticles after 4 days of incubation of the different concentrations of AgNO₃.

3.2. UV-Visible Spectroscopy Analysis

Silver nanoparticle production was monitored by the change in color from light to dark reaction (Figure 1). Figure 2 shows the UV-visible spectroscopy of the three concentrations of silver nitrate (0.5mM, 1.0mM, and 1.5mM) mediated with *Aspergillus niger*. The UV-visible spectra at 300-800nm wavelength of the different concentrations (0.5m, 1.0 mM, and 1.5 mM) showed a characteristic surface absorption band at 412nm, 410nm, and 416nm, respectively, after 4 days of incubation.

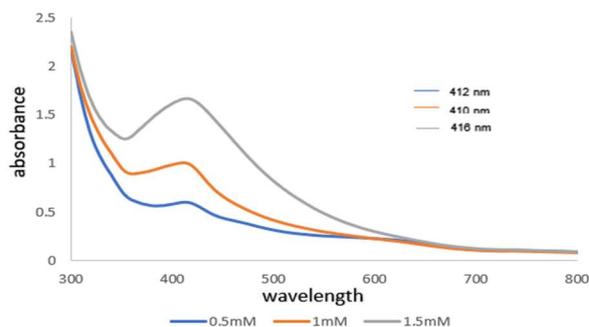


Figure 2. UV spectrum of AgNPs biosynthesis using *A. niger*.
 Figura 2. Espectro UV da biossíntese de AgNPs usando *A. niger*

3.3. Scanning Electron Microscopy- Elemental Dispersive X-ray Analysis

Scanning Electron Microscopy (SEM) is a valuable tool that provides insights into materials' morphology, composition, topography, grain orientation, and crystallographic information (AKHTAR et al., 2018). This study employs SEM-EDX to obtain silver nanoparticles' elemental composition and morphological structure. The analysis results provide insights into the optimal concentration of silver nitrate used in this study.

The Scanning Electron Microscopy of silver nanoparticles at 5,000x and 70,000x magnification of the three different concentrations of silver nitrate: a) 0.5mM, b) 1.0mM, and c) 1.5mM concentration (Figure 3).

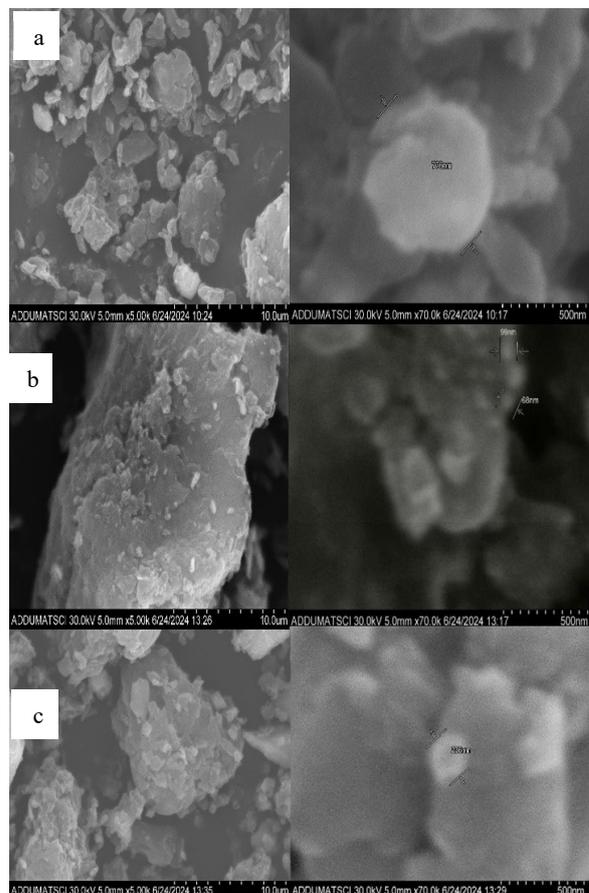


Figure 3. Scanning Electron Microscopy (left) at 5,000x and (right) 70,000x magnification of the three different conc. of silver nitrate synthesized with *A. niger*. a) 0.5mM, b) 1mM and c) 1.5mM AgNO₃ solution.

Figura 3. Microscopia Eletrônica de Varredura (esquerda) com ampliação de 5.000x e (direita) com ampliação de 70.000x das três diferentes concentrações de nitrato de prata sintetizadas com *A. niger*. a) 0,5 mM, b) 1 mM e c) solução de AgNO₃ 1,5 mM.

A nanoparticle ranges in size from 1 nm to 100 nm. Comparing the results through Scanning Electron Microscopy, the 1.0mM concentration revealed the smallest size range from 68nm to 99nm, producing finer silver nanoparticles among the optimized concentrations. On the other hand, concentrations of 0.5mM and 1.5mM obtained a larger particle size of around 776nm and 236 nm, respectively.

Figure 4 shows the results of the Elemental Dispersive X-ray Analysis of the three concentrations of silver nitrate synthesized with *Aspergillus niger* forming silver nanoparticles. EDX was carried out to determine the elemental

composition of the samples. The spectra confirm the presence of carbon (C), oxygen (O), silver (Ag), nitrogen (N) and Chlorine (Cl). The gold (Au) and Palladium (Pd) in the spectra could be accounted for by the coating used to analyze the particles. The EDX profile revealed the following weight percentages of silver in each concentration synthesized. The 1.5mM concentration obtained the highest weight percentage of 38.04, followed by 1.0 mM with 22.24% weight and the lowest weight percentage obtained by 0.5mM with 9.99 %.

The result suggests that the higher the metal precursor concentration, the higher the weight percentage of silver it produces. The elements like carbon (C), oxygen (O), nitrogen (N) and Chlorine (Cl) in the synthesized AgNP indicate the presence of biomolecules from *Aspergillus niger* Tiegh fungal filtrate that are responsible for the reduction of silver ions and have capped the silver nanoparticles.

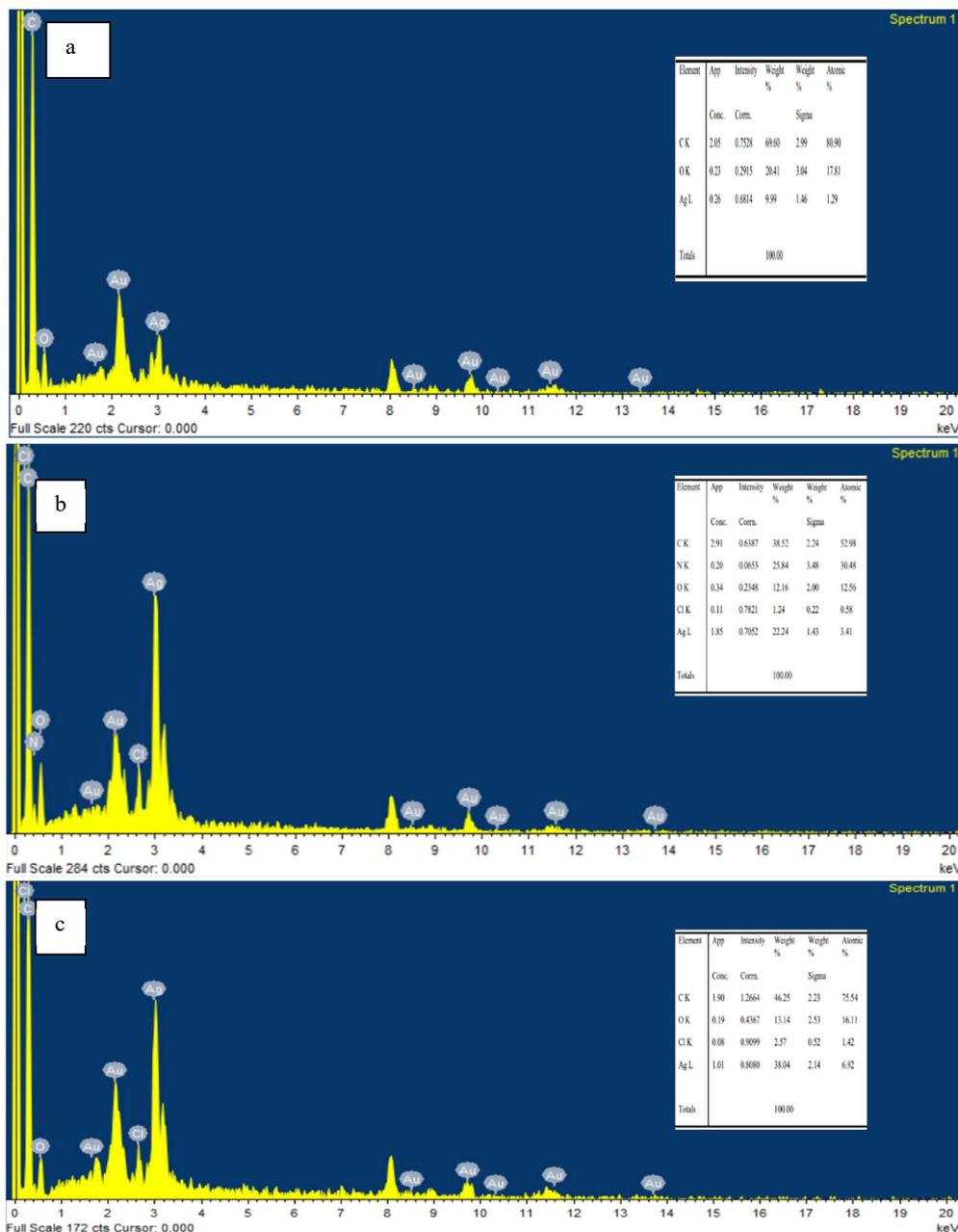


Figure 4. Energy Dispersive X-ray Analysis of biosynthesized silver nanoparticles a) 0.5mM, b) 1.0mM & c) 1.5mM.

Figura 4. Análise de energia dispersiva de raios X de nanopartículas de prata biossintetizadas a) 0,5 mM, b) 1,0 mM e c) 1,5 mM.

3.4. FTIR Spectroscopy

FTIR analysis can detect biomolecules responsible for capping and stabilizing AgNPs and reducing silver (Ag⁺) ions (JALALI et al., 2020). The band spectra analysis of biosynthesized silver nanoparticles mediated with *Aspergillus*

niger Tiegh at 1.0mM concentration is presented in Figure 5. The FTIR analysis of biosynthesized silver nanoparticles showed absorption peaks at 3518.16 cm⁻¹ and 1625.99 cm⁻¹, which were interpreted to identify the functional group responsible for reducing and capping AgNPs.

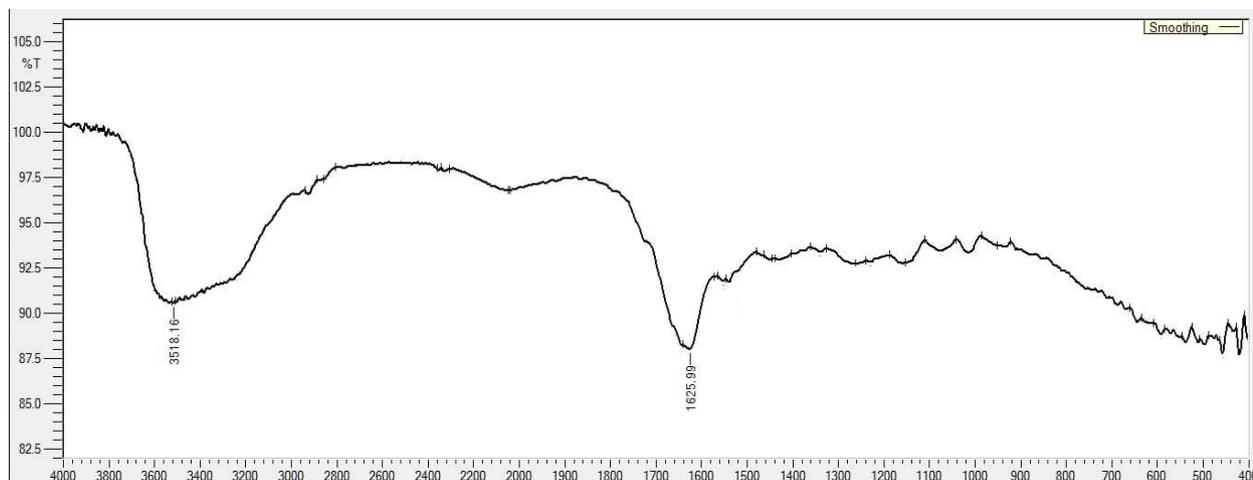


Figure 5. FTIR spectra of the biosynthesized AgNPs.

Figura 5. Espectros de FTIR dos AgNPs biossintetizados.

3.5. In vitro assay of biosynthesized silver nanoparticles against *Xanthomonas oryzae* pv. *oryzae*

Figure 6 shows the inhibitory activity of silver nanoparticles synthesized with *Aspergillus niger* Tiegh against *Xanthomonas oryzae* pv. *oryzae*. The *in vitro* assay used the agar well diffusion method on Nutrient Agar. The zone of inhibition was observed after 24 hours of incubation.

inhibition was recorded in Treatment 2 with a mean of 15.1 mm (+), comparable to Treatments 7, 5, 4, and 6 with 14.9 mm, 13.1 mm, 12.6 mm, and 12.1 mm, respectively. However, it differs significantly from Treatment 3 with 11.4 mm.



Figure 6. Inhibitory activity of silver nanoparticles against *Xanthomonas oryzae* pv. *oryzae*.

Figura 6. Atividade inibitória de nanopartículas de prata contra *Xanthomonas oryzae* pv. *oryzae*.

Table 1 summarizes the antimicrobial activity of silver nanoparticles against *X. oryzae* pv. *oryzae*. The results showed significant variations in treatment means. The largest zone of

inhibition was recorded in Treatment 2 with a mean of 15.1 mm (+), comparable to Treatments 7, 5, 4, and 6 with 14.9 mm, 13.1 mm, 12.6 mm, and 12.1 mm, respectively.

However, it differs significantly from Treatment 3 with 11.4 mm.

Table 1. Analysis of the antibacterial activity of silver nanoparticles synthesized with *Aspergillus niger* Tiegh.

Tabela 1. Análise da atividade antibacteriana de nanopartículas de prata sintetizadas com *Aspergillus niger* Tiegh.

Treatment	Zone of Inhibition diameter (mm)
	Mean
Treatment 1- Negative control	0.0 ^c
Treatment 2- Positive control	15.1 ^a
Treatment 3- 100 ppm AgNPs	11.4 ^b
Treatment 4- 200ppmAgNPs	12.6 ^{ab}
Treatment 5- 300 ppm AgNPs	13.1 ^{ab}
Treatment 6- 400ppm AgNPs	12.1 ^{ab}
Treatment 7- 500ppmAgNPs	14.9 ^{ab}
Ftest	**
CV%	11.7

This means a column with the same letter is not significantly different at a 5% level based on Tukey's HSD test. ** - highly significant.

Isso significa que uma coluna com a mesma letra não é significativamente diferente no nível de 5% com base no teste HSD de Tukey. ** - altamente significativo.

4. DISCUSSION

The synthesis of silver nanoparticles (AgNPs) using fungi presents several benefits compared to plant-based biosynthesis. It produces large quantities of efficient proteins and enzymes for the rapid and sustainable synthesis of AgNPs (VAHABI et al., 2011; ALGHUTHAYMI et al., 2015). Unlike chemical methods, the biological approach is non-toxic, cost-effective, and stable.

Optimization of the different concentrations of metal salt is essential in enhancing the yield and quality of silver nanoparticle synthesis, as it is dependent on substrate concentration. The current result shows that the higher the

concentration of silver nitrate, the darker the color it produces. The present study is consistent with Othman et al. (2019), where a 1.5 mM concentration of silver nitrate produces a dark color solution, while a 0.5 mM concentration produces a less dark color solution. Similarly, as the concentration of the metal precursor increases, so does the intensity of the dispersion's color (GUILGER et al., 2019). Additionally, a higher concentration of AgNO₃ may lead to greater toxicity for fungi (BALAKUMARAN et al., 2015).

The characterization of silver nanoparticles mediated with *Aspergillus niger* Tiegh, UV-visible spectroscopy, Scanning Electron Microscopy coupled with Elemental Dispersive X-ray and Fourier Transmission Infrared Analysis was employed. Uv-visible spectroscopy revealed that 0.5 mM, 1.0 mM, and 1.5 mM peaks at 412 nm, 410 nm, and 416 nm, respectively. Due to the surface plasmon resonance (SPR) occurring from the interaction between electrons and electromagnetic radiation in the conduction band around nanoparticles, an optical absorption band with a specific λ max value is typically observed. This characteristic absorption band indicates the presence of metallic silver nanoparticles (AgNPs) in the solutions (BILAL et al., 2017). As reported in previous studies by Tsuji et al. (2002), small silver nanoparticles exhibit a Surface Plasmon Resonance (SPR) band that spans the range of 350–500 nm, with a peak position around 410 nm. Ider et al. 2016 confirmed the presence of narrow resonance absorption peaks at wavelengths of 405–415 nm is attributed to the excitation of surface plasmon vibrations in the silver nanoparticles.

Figure 3 shows the results of Scanning Electron Microscopy of silver nanoparticles at 5,000x and 70,000x magnification of the three different concentrations of silver nitrate (a) 0.5 mM, b) 1.0 mM, and c) 1.5 mM concentration, respectively. The irregular and amorphous shape of the nanoparticles is influenced by their interactions with stabilizers and inductors, as well as their preparation method (HARUTA, 2004).

The optimization of the different concentrations in this study aligns with the findings of Echavarría et al. (2021), who investigated the efficacy of three concentrations (0.5, 1.0, and 1.5 mM) of AgNO₃ suitable to yield quality silver nanoparticles. The results showed that a 1.0 mM concentration of AgNO₃ gives off the smallest size of silver nanoparticles, ranges 70-90 nm, mediated with white rot fungus *Bjerkandera* sp. Balashanmugam et al. (2016) also confirmed that among the different concentrations of AgNO₃ used, 1mM concentration showed the best quality silver nanoparticles synthesized with *C. roxburghii* at the pH of 7. One crucial factor to consider when synthesizing silver nanoparticles is their particle size. It has been reported that smaller nanoparticles exhibit more effective antimicrobial activity against pathogens. However, its antimicrobial effects diminish as the nanoparticle size increases (MORONES et al., 2005). Smaller nanoparticles are more likely to penetrate cell membranes than larger ones (CONTINI et al., 2020).

The EDX profiles of the three different concentrations of silver nanoparticles show strong signals of silver (Ag) formation at approximately 3 keV, confirming the formation of silver nanoparticles, which corresponds to the Kapoor et al. (2022) result. The EDX profile revealed the following weight percentages of silver in each concentration synthesized. The elements like carbon (C), oxygen (O), nitrogen (N) and Chlorine (Cl) in the synthesized AgNP indicate the presence of biomolecules from *Aspergillus niger*

Tiegh fungal filtrate that are responsible for the reduction of silver ions and have capped the silver nanoparticles.

FTIR was used to identify potential functional groups involved in the reduction process and subsequent formation of AgNPs. FTIR shows a broad peak at 3518.16 cm⁻¹, which indicates the presence of an amine (N-H) group due to the proteins of *Aspergillus niger* Tiegh. The proteins in the cell-free extract of *Aspergillus niger* may function as a capping and/or stability agent in the biosynthesis and production of AgNPs during the reduction process through the amine groups. This result is consistent with the study of (HASHM et al. 2022; PASHA et al., 2022), which reported that N-H stretching and C-N stretching of primary aliphatic and aromatic amines assist the proteins in the synthesis of AgNPs and 1625.99 cm⁻¹ corresponds to C=C stretching of cyclic alkene. The stretching of C=C alkene was also found in Ninganagouda et al. (2014) at 1613. 81 cm⁻¹ and Lofty et al. (2021) peaks at 1633.96 cm⁻¹ where *A. niger* Tiegh was utilized as a reducing agent (EL-ANSARY et al., 2023), peaking at 1634.21, where *Fusarium nygamai* was the reducing agent. The extracellular proteins in the fungal filtrate can bind AgNPs, acting as capping agents and stabilizing the Ag nanoparticles (CHAN; DON, 2013; AL-ZUBAIDI et al., 2019).

Silver nanoparticles (AgNPs) have demonstrated strong antibacterial activity against a wide range of bacterial species, attracting the attention of many researchers who are evaluating their potential for controlling various diseases, including those affecting crops. The biosynthesized silver nanoparticles showed potential antagonistic activity against *Xanthomonas oryzae* pv. *oryzae*. The extremely small size of these particles can be effectively used to control microbes without promoting the development of resistant strains (ZHANG et al., 2016). Due to its electrostatic attraction and affinity for sulfur-containing proteins, silver ions can bind to the cell wall and cytoplasmic membrane. Once attached, these ions increase the cytoplasmic membrane's permeability, ultimately disrupting the bacterial envelope (KHORRAMI et al., 2018). Once free silver ions are absorbed into cells, they can deactivate respiratory enzymes, generating reactive oxygen species and disrupting the production of adenosine triphosphate (ATP) (RAMKUMAR et al., 2017). Reactive oxygen species can play a key role in causing disruption of the cell membrane and modifying deoxyribonucleic acid (DNA). Since sulfur and phosphorus are essential components of DNA, the interaction of silver ions with these elements can interfere with DNA replication, hinder cell reproduction, or even lead to the death of microorganisms. Additionally, silver ions can inhibit protein synthesis by denaturing ribosomes in the cytoplasm (DURAN et al., 2016).

5. CONCLUSIONS

The effect of silver nitrate (AgNO₃) concentrations in the synthesis of silver nanoparticles mediated with *Aspergillus niger* Tiegh was evaluated, and it established that this factor greatly affects the reaction.

The best-synthesized silver nanoparticles were observed in 1mM AgNO₃, which yielded the smallest particle size of 68-99nm compared to the other concentrations.

The antibacterial potential of biosynthesized silver nanoparticles was explored, showing a promising result against *Xanthomonas oryzae* pv. *oryzae*. Further, an *in vivo* study is recommended to validate the efficacy and to provide a thorough understanding of the antibacterial mechanism of AgNPs.

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Data availability: Study data can be e-mail from the corresponding author or the second author upon request. It is not available on the website as the research project is still under development.

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