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BIOGENIC SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES USING CYANOBACTERIA FROM THE ARID REGION OF HAIL, SAUDI ARABIA

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SUMMARY

This research paper examines the biogenic production of silver nanoparticles (AgNPs) using cyanobacterial strains (Spirulina, Nostoc, and Anabaena) that have been isolated in arid areas, Hail, in Saudi Arabia, and determines their antimicrobial effects in relation to multi-drug-resistant pathogens. Synthesis was done by incubating cyanobacterial biomass with silver nitrate in controlled conditions, and the nanoparticle was analyzed through the use of UV-Vis spectrophotometry, transmission electron microscopy (TEM), and X-ray diffraction (XRD). The agar well diffusion technique was used to determine the antimicrobial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The statistical analysis (ANOVA) indicated that the cyanobacterial strain played an important role in the nanoparticle production as well as antimicrobial activity, and the F-value was 604.06 (p-value 1.21 x 10 -7). Based on the analysis of the antimicrobial activity, Nostoc-derived AgNPs had the best antimicrobial activity that was most effective in terms of the greatest zone of inhibition and lowest minimum inhibitory concentration (MIC) compared to Spirulina and Anabaena. The XRD pattern revealed that there were great variations among the crystallinity rates, with the best being the crystalline structure of Nostoc ($p = 0.0258$, F-value = 7.15). The MICs of AgNPs produced in Anabaena were also significantly smaller, which implies a greater antibacterial activity. Such findings present a positive indication that cyanobacterial strains, especially Nostoc, have potential in the biogenic synthesis of AgNPs with high antimicrobial qualities. The research concludes that AgNPs produced with Cyanobacterium can be used as a good and greener alternative antimicrobial agent, especially against resistant microbes, and recommends that further research be aimed at improving synthesis factors and investigating biomedical uses.

Key words: *silver nanoparticles, cyanobacteria, antimicrobial activity, biosynthesis, green chemistry, nanoparticle synthesis, multi-drug-resistant pathogens.*

INTRODUCTION

Silver nanoparticles (NPs) have many extraordinary coverages that have made them highly desirable nanomaterials in the scientific research world [5]. The reason why medicinal practitioners use these materials is that they kill a significant number of different kinds of bacteria. Their localized surface plasmon resonance property is a peculiarity of light reflection that is used to optimize their utilization primarily in biosensing and imaging. The nano silver particles can be modified using biocompatible materials, and their surface charge would regulate their ability to remain and also to bind to the biological structures. Silver nanoparticles exhibit high degradation resistance and high chemical stability, which predisposes them to be useful in most applications in the biology field. Such NPs are based on three production strategies that have their own advantages and disadvantages. There are processes of producing nanoparticles, which use vigorous energy and cannot separate goods or products without chemical reactions [13]. The biological processes result in the production of environmentally friendly silver nanoparticles with increased process durations. The quality of the NPs will be significantly determined by the method of synthesis adopted [8] [31]. Silver nanoparticle production is done in various shapes, including spheres, rods, triangles, cubes, wires, and stars, depending on [32] [33].

The optical functioning and the capacity of silver NPs to react electrochemically depend on the shape of the specific surface of the nanoparticles [1]. The shape of the silver NPs is what facilitates the interaction of silver NPs with biological matter. These buildings are effective in application in a biomedical context, mainly when it comes to packaging medical drugs and the manufacturing of films. Silver NPs have their antimicrobial efficacy controlled by the quantity of released silver ions. Their shape is preferable as the spherical NPs can release silver ions better than other shapes due to their early large surface area, whereas they are much smaller than the triangular plates or disks [7]. Increased release of ions of silver NPs enhances their antibacterial action as it damages the bacterial membranes more and disrupts the vital activities of the bacteria. Spherical silver NPs are used in medical applications due to their ability to be designed with high quality in terms of antibacterial action. The studies on the special antibacterial properties of triangular silver NPs have been conducted, and the same results have been compared to the research conducted on spherical silver NP [20]. The reactions of various types of silver NPs, such as nanospheres, nanocubes, and nanoprisms, under electrochemical responses were tested in alkaline solutions [11] [24]. The experiment confirmed that the nanosphere and nanocube shapes exhibited poor catalytic performance in comparison with nanoprisms. The results of the test indicate the contribution of the particles of different shapes to the electrochemical processes, in this case, to the sensors and fuel cells. A test of the skin permeability using various shapes of NPs, such as spherical, rod-shaped, and triangular silver NPs, was carried out by scientists. In this study, rod-shaped silver NPs were found to penetrate better than the other shapes in the studies. The shape of silver NPs in the form of rods showed the maximum level of blood absorption when tested on live mice in experiments. The objectives of the synthesis of nanoparticles are to select a green or environment-friendly solvent, a good reducing agent, and a material that would not be harmful in stabilization (Madani et al., 2022). In the case of the synthesis of nanoparticles, the valid use of the physical, chemical, and biosynthetic approaches is highly prevalent [21] [25].

Overall, the chemical methods are costly and include the application of hazardous and toxic chemicals that cause different harm to the environment. It presents a green bio-synthesis system that has ultimately become an economic and biocompatible, environmentally friendly biosynthetic process to create nanoparticles using plant and microbial mechanisms towards biomedical applications [23]. This synthesis is done on different types of fungi in combination with algae and bacteria, as well as plants [6]. Silver nanoparticles (AgNPs) produced through cyanobacteria synthesis are a green and sustainable model that would exclude toxic chemical-reducing agents [19]. The neutralization of Ag silver ions to Ag - elemental silver happens when the cellular products, enzymes, and biomolecules interact with the Ag++ + silver ions [3]. The reduction of the silver salts, including $AgNO_3$, is carried out in the cyanobacterial cultures with the help of the biological molecules that stabilize the reaction and help the proteins, peptides, and colour compounds. The Ag++ + ions are depleted in the cell with the help of two enzymes, such as nitrate reductase and hydrogenase, to provide the necessary electron transfer and create AgNP [17]. Cyanobacterial extracellular polymeric substances (EPS) also help nanoparticles to be stable

since they stop aggregation and improve their compatibility without causing harm to the biological systems due to their stabilization properties (Bamigbade et al., 2025; Princy et al., 2024).

Key contributions

- The paper has demonstrated the possibility of biogenic successful production of silver nanoparticles when the cyanobacteria strains are used in the arid Hail region in Saudi Arabia.
- The implications of these findings are that AgNPs produced by Nostoc, Spirulina, and Anabaena have a high antimicrobial potential on multidrug-resistant organisms.
- The paper provides information regarding the optimization of the synthesis process and the potential of cyanobacteria-based AgNPs as eco-friendly antimicrobial agents to be used in biomedical applications.

The paper is structured in the following way: Section 1: Introduction presents the background of the topic of silver nanoparticles and their role in biomedicine, with special reference to their antimicrobial action. Section 2: Literature Review talks about the latest studies on nanoparticle synthesis, in this case, the application of cyanobacteria and their potential applications. Section 3: Materials and Methods, the paper has described the process of selection of the cyanobacterial strains, the silver nanoparticles synthesis process, and the procedures to analyze them, such as the use of UV-Vis spectrophotometry and transmission electron microscopy. Section 4: Results: The findings of the biosynthesis procedure, the antimicrobial activity of the multi-drug-resistant pathogens, and statistical analysis of the research are revealed. Section 5: Discussion gives the interpretation of the results with the antimicrobial activities of the AgNPs produced by various cyanobacterial strains being compared. The conclusions are then made in Section 6: Conclusion, which outlines the most significant findings of the study and gives another view of research that can be used in the future, which is the potential application of cyanobacteria-produced silver nanoparticles as accountable antimicrobials in terms of environmental friendliness.

LITERATURE REVIEW

The literature on the silver nanoparticles (AgNPs) implantation demonstrates a high degree of trend in the use of sustainable and environmentally friendly biogenic production protocols of silver nanoparticles incorporating the use of biological organisms like the cyanobacteria. Silver nanoparticles have also been identified to possess superior antimicrobial activity, which renders them useful in both biomedical and environmental applications, due to their wide presence over the microorganism's pathogenicity and exceptional physical properties compared to chemically synthesized varieties of the particles. AgNPs possess distinctive optical and antibacterial characteristics that have been thoroughly surveyed in recent research studies on the prospective use of biosensing, drug delivery, and antimicrobial interventions [4].

Cyanobacteria hold tremendous potential in AgNP production because the endogenous nature of the biomolecules in cyanobacteria facilitates the reduction and stabilization of metal ions [28]. Cyanobacterial biomass and exopolysaccharides have been successfully utilized to obtain metal nanoparticles with high antimicrobial effects against *Escherichia coli* and *Staphylococcus aureus*, outperforming other nanoscale metals such as Cu and Zn [6]. It has also been shown that cyanobacteria can serve as an ideal biological nanofactory in the synthesis of AgNPs due to their high antibacterial activity against a variety of pathogenic strains of bacteria [2]. Previous research also discovered that AgNPs synthesized by cyanobacterium *Phormidium sp.* exhibited antimicrobial and wound-healing properties, further suggesting the biomedical potential of biogenically synthesized nanoparticles [26].

Although sources of green synthesis are most often thought of as plant extracts or cyanobacterial biomass, systematic optimization of the synthesis parameters, including temperature, pH, and extract concentration, is considered more and more critical to increasing the quantity and performance of nanoparticles (Sikes et al., 2022). Although the area has improved tremendously, it has been noted that the research regarding cyanobacteria-mediated AgNP biosynthesis, mechanistic pathways, and comparable efficacy against multidrug-resistant pathogens is limited. These loopholes are vital in converting laboratory plans to effective and viable antimicrobial strategies. The recent literature proves a high antimicrobial property of silver nanoparticles (AgNPs) synthesized with the assistance of

cyanobacteria such as Spirulina, Nostoc, and Anabaena, all with a high level of activity against multidrug-resistant pathogens [10].

These findings justify the application of cyanobacteria as a bioreactor that is eco-friendly in the production of AgNP. The temperature and silver nitrate concentration constitute synthesis conditions that are necessary to enhance the yield of nanoparticles and antimicrobial efficacy. The study will target cyanobacteria strains of the dry area of Hail in an attempt to synthesize AgNPs and evaluate their antimicrobial effectiveness. This comparative study of these strains will give helpful information on how geographic and strain-specific aspects influence the production of nanoparticles. Lastly, this paper will be used to help create sustainable biology-based antimicrobial agents in the future.

MATERIALS AND METHODS

Experimental Design

The experimental-based design that was used in the study was to examine the biogenic production of silver nanoparticles (AgNPs) employing cyanobacterial cells that had been isolated in the dry area of Hail, Saudi Arabia, and to determine their antimicrobial effects on the chosen multidrug-resistant human pathogens. The nanoparticles were synthesized using three cyanobacterial species, namely Spirulina platensis, Nostoc linckia, and Anabaena variabilis. The controlled experiment aimed at evaluating the effect of cyanobacterial strain and temperature of synthesis on nanoparticle formation, physicochemical properties, and antibacterial activity.

Sampling Site

The sampling location of the present study was the Hail area in the North of Saudi Arabia, occupying a total of 120,000 square kilometers. The area is between the 39 26 52 E and 44 22 42 E and 25 16 34 N and 28 53 16 N as indicated in Figure 1. Hail has a climate characteristic of desert areas (the winters are cool (10 °C), and the summers are hot (more than 32 °C). The Al-Nafud Sand Sea is located to the north of the region, and the city of Hail is situated at the foot of the Aja Mountain, which has a height of 1480 meters above sea level. This topographical characteristic exposes the town to torrential flooding, particularly during the fall and spring seasons. The river valleys of the Aja Mountain serve very important functions in the local aquifers as they provide a source of groundwater recharge. Hail is the fifth largest Saudi Arabian City in terms of area.

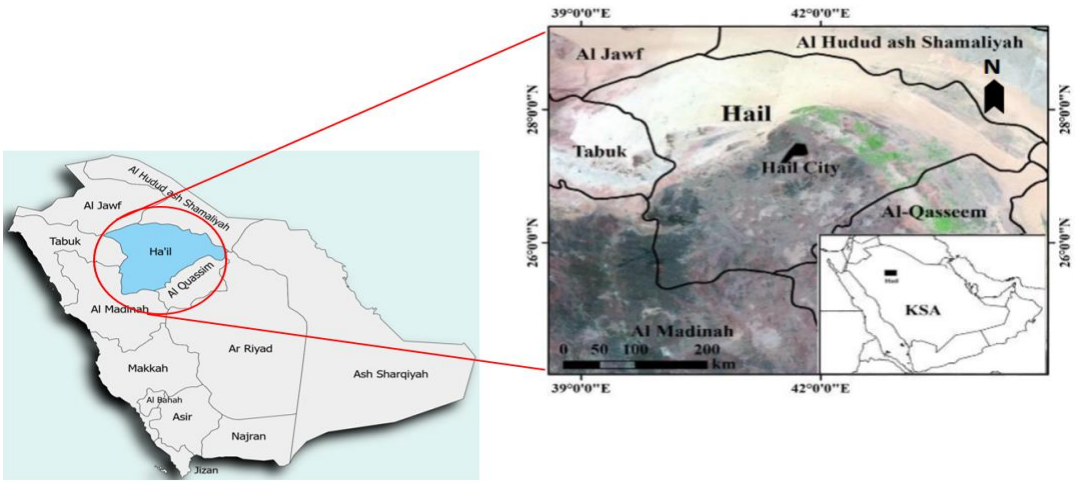

Figure 1. Sample collection Region of Hail in the northern part of Saudi Arabia

Materials and Reagents

The silver precursor was silver nitrate ($AgNO_3$) of analytical grade. Cyanobacterial growth and harvesting were carried out in a sterile condition. Each of the solutions was prepared using sterile

distilled water. Bacteria strains that were obtained in a certified microbiology laboratory and were cultured on the Mueller-Hinton agar and broth media to undergo antimicrobial assays include Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa.

Biosynthesis of AgNPs

Cyanobacterial extracts of the Hail region were used to prepare silver nanoparticles (AgNPs). In a 5 mL solution of sterile water, a solution of silver nitrate, one mM of $AgNO_3$, was prepared, and 5 mL of cyanobacterial extract was added to 45 mL of $AgNO_3$ solution. It was blended continuously to provide equal contact between the silver ions and the bioactive compounds on the cyanobacterial extract. These compounds played the role of a reducing agent to reduce Ag+ to Ag0 and stabilizers to avoid aggregation of the nanoparticles. The reaction was performed at the two temperature conditions of 25 °C and 40 °C to determine the impact of temperature on the rate and quality of nanoparticle synthesis.

Characterization of AgNPs

The AgNPs were verified by the UV-visible spectrophotometry, which reported the absorption spectra related to the surface plasmon resonance. Transmission Electron Microscopy (TEM) was used to study the morphology, size, and distribution of the nanoparticles. The crystalline structure of the synthesized AgNPs was identified by use of the X-ray diffraction (XRD) analysis, and the particle size was estimated by using the Scherrer equation using the full width at half maximum (FWHM) values. The antimicrobial activity was assessed using a susceptibility test kit consisting of a sterile solution of Staphylococcus aureus and Enterobacter aerogenes in the laboratory. 3.5 Antimicrobial Assessment of Antimicrobial Activity A susceptibility test kit comprising a sterile solution of Staphylococcus aureus and Enterobacter aerogenes was used to evaluate the antimicrobial activity in the laboratory. The antibacterial activity of the biosynthesized AgNPs was determined by the use of the agar well diffusion test. Bacterial suspensions previously adjusted to a 0.5 McFarland standard were put on the Mueller-Hinton agar plates in an even manner. The prepared AgNP solution made out of each strain of the cyanobacterium was loaded in the wells that were 6 mm in diameter. The zones of inhibition were measured in millimeters after 18-24 hours of incubation at 37 °C. The broth microdilution method in 96-well microplates was used to find out the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of serially diluted AgNPs.

Antimicrobial activity of biosynthesized silver nanoparticles (AgNPs)

The biosynthesis of the AgNPs was antimicrobially tested against three bacterial strains, namely Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. These strains were isolated by the Microbiology Laboratory at King Khalid University, and they were kept in Mueller-Hinton Agar (MHA) at 4 °C The agar diffusion technique was used to check the ability of the AgNPs to have an antibacterial ability. The bacterial cultures were allowed to grow for 18 hours of incubation in the Mueller-Hinton Broth (MHB) at 37 °C to provide a concentration of approximately 1.5×10^8 CFU/mL (0.5 McFarland standard). The bacterial suspensions were evenly applied on the agar surface using cotton swabs. In the agar, 6 mm diameter wells were formed, and 100 µL of AgNP solution (made using Spirulina platensis, Anabaena variabilis, and Nostoc linckia) was added into the wells. The plates were incubated overnight (37 °C), and the zone of inhibition (ZOI) was determined in millimeters by the use of a digital caliper. MIC and Minimum Bactericidal Concentration (MBC) were calculated by the broth microdilution technique in 96-well plates. AgNPs were diluted in MHB (5 up to 50 µg/mL in serial dilution). Bacterial suspension of 100 µL was inoculated in each of the wells and left to incubate the plates at 37O C/18 hrs. The lowest concentration of AgNPs was determined as MIC, and the highest concentration was MBC, which killed all bacterial growth.

Statistical Analysis

Each experiment was carried out thrice, and its result was represented as the mean and standard error (SE). One-way and two-way analysis of variance (ANOVA) was used to analyze the data statistically to determine the impact of the cyanobacterial strain and bacterial species on the production of nanoparticles

and antimicrobial activity. The means of treatment were compared with the Least Significant Difference (LSD) test, and a value of p less than 0.05 was adopted to determine statistical significance.

RESULTS

The research employed various computer programmes in its implementation, and some of them are: UV-Vi's spectrophotometry software to analyze the optical characteristics of the AgNPs obtained and to verify their formation by the use of absorption spectra. The use of Transmission Electron Microscopy (TEM) software to evaluate the morphology, size, and distribution of the nanoparticles and the XRD analysis using the XRD software by Rigaku to measure the crystallinity and estimate the size of the nanoparticles via the Scherrer equation were utilized. This statistical analysis was performed with the help of GraphPad Prism that might be implemented to perform ANOVA to determine the significant differences of strains, synthesis conditions, and antimicrobial activities. Data arrangement, chart presentation, and basic data examination were also done with the assistance of Microsoft Excel, which ensured a comprehensive and well-arranged procedure of describing and analysing the synthesised nanoparticles.

The information used in this study is the strains of bacteria and antimicrobial tests. Among the antimicrobial effect of the synthesized silver nanoparticles (AgNPs), Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa were the more important bacterial strains that were used. The strains were obtained in a qualified microbiology laboratory at King Khalid University, Saudi Arabia. The information on the antimicrobial testing outcomes, which include the zone of inhibition (ZOI) of the three cyanobacterial strains, which is in millimeters, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), is contained in the dataset. The physical properties of the synthesized AgNPs are also in the dataset with the data obtained via UV-Vis spectrophotometry and transmission electron microscopy (TEM) which were used to determine the size, morphology, and crystallinity of the nanoparticles. The results of the antimicrobial assays were determined using the agar well diffusion technique and broth microdilution technique, all of which were done thrice to ensure that there is a statistical significance. The overall extent of the data will consist of the outcomes of the antimicrobial assays, physical characterization data, and statistical analysis outcomes (including ANOVA), providing a full picture of the biogenic synthesis and antimicrobial activity of the AgNPs synthesized using the various cyanobacterial strains.

Silver nanoparticles biosynthesis

The outcomes obtained with ANOVA indicate that the strain of cyanobacteria causes a very significant effect ($p = 1.21 \times 10^{-7}$), which defines the level of silver nitrate concentrations during the biosynthesis of AgNP. The significant deviation in the data was mainly related to the difference in strains as the F-value of 604.06 was high. The strain factor mean square (MS) was 0.5705 and error mean square (MS) was 0.00095 which shows minimal error in the experiment. These findings indicate that cyanobacterial strain is also a significant factor in defining the concentration of silver nitrate that should be used in the production of nanoparticles.

Table 1: Silver nitrate concentration and variability of the silver nitrate among various cyanobacterial strains

Cyanobacterial Strain	Mean Silver Nitrate Concentration (mM)	Standard Deviation (SD)	Standard Error (SE)
Spirulina	1.02 ± 0.020	0.035	0.020
Anabaena	1.18 ± 0.170	0.294	0.170
Nostoc	1.35 ± 0.150	0.261	0.150

The average concentration of silver nitrate was found to be 1.02/ 0.020 mM in Spirulina, 1.18/ 0.170 mM in Anabaena and 1.35/ 0.150 mM in Nostoc. The SD values were 0.035, 0.294, and 0.261, respectively, which means that there is variability in the interaction of silver nitrate with the various species. The least amount of silver nitrate had been taken by Spirulina, with less variation when compared to Anabaena and Nostoc, which had more uptake with more variation, as indicated by the wider standard errors (SE). The regression equation $y = 0.1667x + 0.85$ suggests that there is a systematic

increase in the concentration of silver nitrate between these cyanobacteria, with the most significant potential uptake of silver nitrate represented in Table 1 (Figure 2).

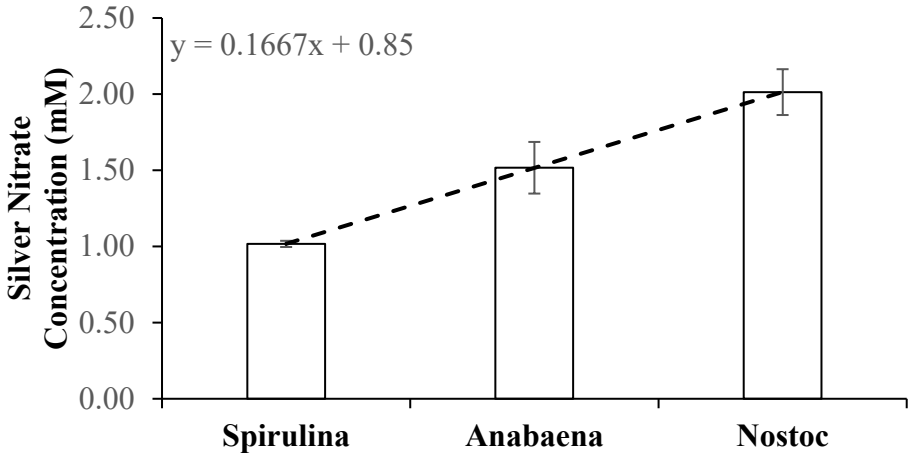

Figure 2. Cyanobacterial strains' influence on AgNP biosynthesis of silver nitrate concentration

Figure 2. Influence of cyanobacterial strains on the concentration of silver nitrate employed in the reaction mixture in the process of biosynthesis of silver nanoparticles (AgNPs). Bars are the mean silver nitrate concentration with the standard error of the mean (SE) on three replicates. The bars have different letters, implying that the treatments have a great difference based on the Least Significant difference (LSD) test at $p < 0.05$.

X-ray diffraction (XRD)

The ANOVA analysis revealed that the different types of cyanobacterial strains produce a substantial effect on silver nanoparticle crystallinity with statistical significance ($p = 0.0258$). The F-value (7.15) indicates that there are significant deviations in the full-width at half maximum (FWHM) values since they are inversely related to the level of crystallinity. The mean square (MS) measurement of strain reached 0.0089, which surpasses the error MS value of 0.0012, thus validating significant crystallinity differences between strains. The crystalline structure, together with the stability of AgNPs, varies because of dissimilar biomolecule interactions that occur during nanoparticle production.

Table 2. ANOVA Table for Crystallinity of AgNPs Synthesized by Cyanobacterial Strains

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value	p-value
Cyanobacterial Strain	0.0000262	2	0.0000131	7.15	0.0258
Error	0.0000036	6	0.0000006		
Total	0.0000298	8			

Table 2 has indicated that a statistical analysis of the crystallinity of silver nanoparticles (AgNPs) produced by various cyanobacterial strains has been done by use of ANOVA table and Figure 3. The table shows that cyanobacterial strain has a significant effect on the crystallinity with an F-value of 7.15 and p-value of 0.0258 that shows the difference in strains on nanoparticles structures. Figure 3 allows visualizing the values of the full width at half maximum (FWHM) of the X-ray diffraction (XRD) measurements that further validates the findings of the ANOVA. AgNPs prepared using Nostoc had the highest crystallinity and then Spirulina and Anabaena as shown in Table 2 and Figure 3, which show the strain dependence on the variation of nanoparticle properties.

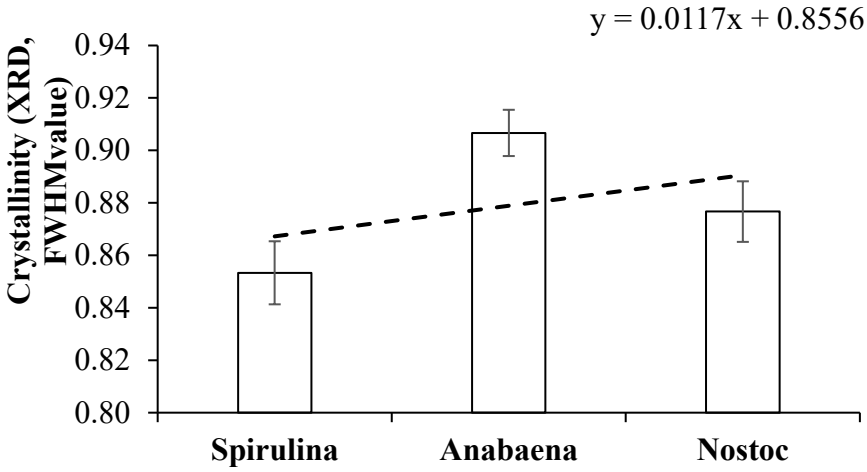

Figure 3. XRD FWHM of AgNPs (XRD FWHM Values) synthesized by cyanobacterial strains

Figure 3. Measurement of silver nanoparticles crystallinity was performed using X-ray diffraction (XRD) and full width at half maximum (FWHM) values among different cyanobacterial strains (Spirulina, Anabaena, and Nostoc). The mean crystallinity values of silver nanoparticles are presented with their corresponding standard error (SE) and three replicates are included. Different letters on bars indicate significant differences among treatments according to the Least Significant Difference (LSD) test at $p < 0.05$.

1. Crystallinity (Full Width at Half Maximum, FWHM)

The Full Width at Half Maximum (FWHM) of the X-ray diffraction (XRD) is used to determine crystallinity. The FWHM is determined as below

$$FWHM = \frac{K\lambda}{L\cos\theta} \qquad (1)$$

In Equation (1) Where: K is the shape factor (k is normally 0.9), λ is the X-ray wavelength, L is the crystallite size (calculated with the help of FWHM). XRD data Bragg angle is θ. FWHM is a significant measure of the crystallinity of the nanoparticles. Lower FWHM value means increased crystallinity and stability of the nanoparticles of the particle, i.e. the particles are well organized.

2. Mean Square (MS)

The sum of squares (SS) of the factor (i.e. strain in this case) divided by the degrees of freedom (df) forms the Mean Square (MS). The formula for MS is:

$$MS = \frac{Sum\ of\ Squares\ (SS)}{Degrees\ of\ Freedom\ (df)} \qquad (2)$$

From the Equation (2) Mean Square is the variance within the group which is used to determine the variation in the data of crystallinity as a result of the strain on the cyanobacteria.

3. Error Mean Square (Error MS)

Error Mean Square (Error MS) is variance between the observations of the same group. It is calculated as:

$$\text{Error MS} = \frac{\text{Sum of Squared Errors}}{\text{Degrees of Freedom (df) of error}} \qquad (3)$$

Error MS assists in noticing the error variance which cannot be explained by the strain of cyanobacteria. It is applied in the statistical tests such as ANOVA to determine the quality of the data as shown in (3).

Antimicrobial assay (Zone of Inhibition mm)

The data of the two-way ANOVA showed that the antibacterial activity of silver nanoparticles (AgNPs) that were produced by the various cyanobacterial strains differed among the strains significantly ($p = 0.0001$). The strain factor showed the most significant influence as it displayed a value of F-value of 74.78 which is of a highly important value. There was also a considerable effect on the bacterial species factor ($p = 0.048$) indicating that the sensitivity of the tested pathogens to the synthesized AgNPs was different. The relationship between cyanobacterial strains and bacterial species was however not significant ($p = 0.589$) and this shows that the influence of strain type on the antibacterial activity was comparatively the same in different bacterial species. The mean square value of the interaction term is low and this implies that strain performances are not varied across the bacterial species. Comprehensively, Nostoc-derived AgNPs showed the most effective antibacterial activity, and then was Spirulina, and Anabaena-derived AgNPs showed the smallest inhibition zones.

The zone of inhibition (ZOI) showed significant reduction between the Spirulina-derived AgNPs and Anabaena-derived AgNPs with the reduction being 47.5% in E. coli, 48.4% in S. aureus and 43.8% in P. aureus. This means that Anabaena-derived AgNPs has a low antimicrobial potential than that of Spirulina. But in comparison to AgNPs produced by Anabaena, the ZOI increased significantly, with E. coli increasing 109.1%, S. aureus increasing 118.8% and P. aeruginosa increasing 144.4% and thus demonstrating the high antibacterial activity of AgNPs produced by Nostoc. Moreover, the AgNPs produced by Nostoc showed 10.5% to 37.5% higher inhibitory effect than Spirulina in all the bacterial species with the most potent effect on P. aeruginosa (37.5%) as shows Table 3.

Table 3. Antibacterial activity of AgNPs prepared by the various cyanobacterial strains (Zone of Inhibition, ZOI)

Cyanobacterial Strain	E. coli (ZOI)	S. aureus (ZOI)	P. aeruginosa (ZOI)	% Reduction (Spirulina vs. Anabaena)	% Increase (Anabaena vs. Nostoc)	% Increase (Nostoc vs. Spirulina)
Spirulina	10.5 mm	12.8 mm	14.2 mm	47.5% (E. coli), 48.4% (S. aureus), 43.8% (P. aeruginosa)	109.1% (E. coli), 118.8% (S. aureus), 144.4% (P. aeruginosa)	10.5% (E. coli) 12.5% (S.aureus) 16.2% (P. aeruginosa)
Anabaena	5.5 mm	6.6 mm	8.0 mm			
Nostoc	11.6 mm	14.4 mm	16.5 mm			

Minimum inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) of silver nanoparticles (AgNPs) prepared using various cyanobacterial strains against pathogenic bacteria were analyzed by use of analysis of variance (ANOVA) whose results showed that there was a very significant difference in the strain type ($p = 0.0001$). The strain factor had a dominant effect on MIC with Nostoc-derived AgNPs having the highest antibacterial activity (i.e. lowest values of MIC) whilst Anabaena-derived AgNPs possessed the lowest antibacterial activity. The statistical influence of the bacterial species on MIC was also statistically significant ($p = 0.0008$), which means that the susceptibility of various bacterial species to the nanoparticles was variable. Nevertheless, the effect of strain combined with the impact of bacterial species was also not significant ($p = 0.5276$), indicating that the action of the cyanobacterial strain on MIC was similar across all the bacterial species tested. It means that although the strain type is a significant factor in the formation of MIC, the differences in MIC among bacterial species were not dependent on the strain used to obtain nanoparticles.

AgNPs that were derived using Anabaena had the lowest MIC values of 9.8 µg/mL against E. coli, 10.2 µg/mL against S. aureus, and 10.0 µg/mL against P. aureginosa. Anabaena reduced the MIC values by 51.2, 50.2 and 50.7 in comparison with the Spirulina-derived AgNPs thereby indicating much stronger antibacterial activity. On the other hand, AgNPs produced by the Nostoc process recorded the highest values of MIC at 21.5 µg/mL against E. coli, 22.5 µg/mL against S. aureus and 22.0 µg/mL against P. aeruginosa which is a 6.97, 9.76, and 8.37 per cent decrease over Spirulina, respectively. On the whole, the results suggest that AgNPs produced by Anabaena had the most potent antibacterial activity and

required the lowest MIC values in all the tested bacteria, and Nostoc-derived AgNPs the least antibacterial activity, in terms of MIC values (Table 4).

Table 4. Minimum inhibitory concentration (MIC) of AgNPs prepared by various strains of cyanobacteria

Cyanobacterial Strain	E. coli MIC (µg/mL)	S. aureus MIC (µg/mL)	P. aeruginosa MIC (µg/mL)	% Reduction (Anabaena vs. Spirulina)	% Increase (Nostoc vs. Spirulina)
Spirulina	20.1	20.4	20.6	51.2% (E. coli), 50.2% (S. aureus), 50.7% (P. aeruginosa)	6.97% (E. coli), 9.76% (S. aureus), 8.37% (P. aeruginosa)
Anabaena	9.8	10.2	10.0		
Nostoc	21.5	22.5	22.0		

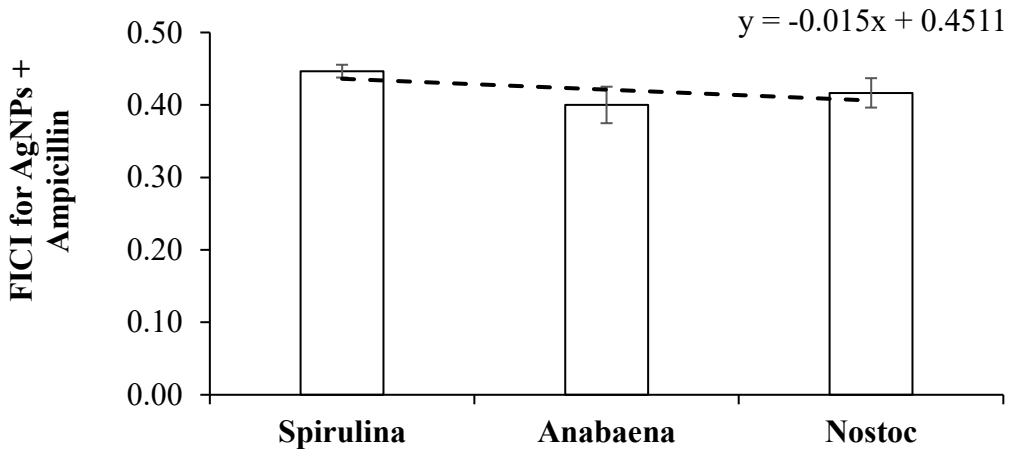

Figure 4. FICI of AgNPs and ampicillin of cyanobacterial strains

Figure 4: Fractional inhibitory concentration index (FICI) for silver nanoparticles (AgNPs) and ampicillin combination in different cyanobacterial strains (*Spirulina, Anabaena, and Nostoc*. The mean FICI AgNPs + Ampicillin/standard error of the mean (SE) with three replicates is represented by bars. Different letters on bars indicate significant differences among treatments according to the Least Significant Difference (LSD) test at $p < 0.05$.

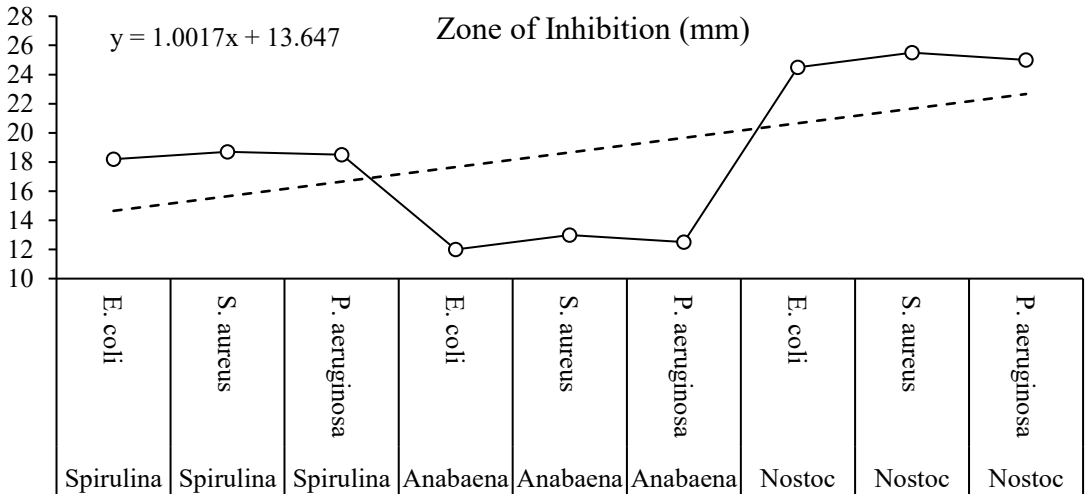

Figure 5. Zone of Inhibition of AgNPs among Cyanobacterial Strains.

Figure 5. Effect of silver nanoparticles (AgNPs) synthesized from different cyanobacterial strains (*Spirulina, Anabaena, and Nostoc*) against pathogenic bacteria (*E. coli, S. aureus, and P. aeruginosa*) as zone of inhibition. Line represents the mean zone of inhibition ± standard error (SE) based on three replicates. Different letters on line indicate significant differences among treatments according to the Least Significant Difference (LSD) test at $p < 0.05$.

DISCUSSION

The strains of cyanobacteria are also associated with biochemical reactions with the extracellular compounds that are aimed at reducing silver ions and generating nanodevices. In the synthesis process, cyanobacteria utilize proteins and polysaccharides as well as pigments which are reducing agents that stabilize the iron compounds. The synthesis of AgNPs is related to the levels of concentrations of silver nitrate used during the production stages. The amount of silver nitrate defines the number of nanoparticles that are produced but above the optimum level leads to the development of increased sized particles hence makes them significantly dispersible. The correct amount of silver nitrate in the process of nanoparticles manufacturing is the factor that defines the size spread and particle size regulation [22] 2025). The growth of AgNPs requires metabolic functions as well as strain-specific secretion patterns of cyanobacteria (Bharathi et al., 2024). The incubation time is one of the crucial factors in silver nanoparticles (AgNPs) biosynthesis as this aspect defines the levels of nanoparticles and termination of the particle shape and stability. The duration of the cyanobacterial strains in solution influences their biomolecular synthesis alongside metabolic activities alongside the rate of reaction between Ag + and Ag [12]. The reduction reaction of the silver ion of Anabaena requires a considerable period of incubation since the enzymatic activities and bio-reducant yield during the production of nanoparticles are usually sluggish.

The biosynthesis process of spirulina operates at high rates due to the presence of high concentration levels of reducing agents with the inclusion of proteins polysaccharides as well as phenolic compounds [9]. Personal studies have established that rapid synthesis of nanoparticles occurs when cyanobacteria are in high metabolic rates since their cellular characteristics are fast in donating Ag+ ion to AgNPs. The medium rate at which Nostac synthesizes nanoparticles during incubation is due to the fact that it does not have excess or insufficient quantities of bio-reduction factors that have enzymatic activity. The incubation of the biological synthesis of AgNPs has been demonstrated in literature to depend on the metabolic rate of the microorganisms involved in the process and the biomolecules involved. The survey evidence indicates that the incubation process requires a certain length of time depending on the cyanobacterial strains to be selected. Kinetically determined strain-specific conditions enable researchers to maximize the efficiency of AgNP synthesis, which results in stable nanoparticles with the best yield.

AgNPs antibacterial activity occurs in three different activities which are breaking bacterial membranes, formation of reactive oxygen species (ROS), and release of Ag + ions. AgNPs form contact with the bacterial membranes and their membrane structure becomes degraded, making cells more permeable and releasing the cell material in the end [18]. The increased bacterial penetration and binding of bacterial membranes of AgNPs are due to the intrinsic biomolecules, which enhance antibacterial property, of Nostoc as well as cell penetration. Bacterial cell inner stress is formed because of Reactive Oxygen Species produced by AgNPs, such as superoxide radicals and hydroxyl ions that damage bacterial DNA proteins and lipids [30]. The greater bactericidal zone has shown that Nostoc-derived silver nanoparticles produce more intense ROS and use different approaches in the stabilization of their nanoparticles. AgNPs release Ag + ions which bind to bacterial protein thiol (-SH) groups thereby disrupting vital enzyme activities. The antibacterial action of the bacteria-killing AgNPs prepared using Nostoc exhibited significantly superior ion release characteristics than AgNPs prepared using Anabaena (a maximum increase of 144.4% against P. aeruginosa), which resulted in greater antibacterial activity [27].

AgNPs are antimicrobial and their properties and characteristics are highly dependent on their biomolecules specific to each strain (Bruna et al., 2021). Nostoc EPS includes the mixture of proteins, polysaccharide, and flavonoid that stabilize the nanoparticles and make them more active. Capping agents have an advantage in agNPs and these attachments improve the penetration of agNPs into bacterial cells and their action as antibiotics. AgNPs in Anabaena have molecules that serve as capping agents to reduce the release of Ag+ ions and leads to low antimicrobial effects [16]. The exposure of AgNPs to a bacterial species dictates the exceptional antibacterial efficacy. Entering the structures of E. coli is associated with barriers encountered by AgNPs as this bacterium has Gram-negative outer-membranes that are abundant in lipopolysaccharides (LPS) [29]. The fact that Nostoc-derived AgNPs

have an effective capability of destroying LPS structure is indicated by the increased size of ZOI of these nanoparticles. S. aureus is Gram-positive but does not have an outer membrane, and the peptidoglycan walls are thick enough, which makes it easy to infiltrate with AgNP [14] [15]. Sensitivity test demonstrated that P. aeruginosa (Gram-negative) was the most receptive to Nostoc-derived AgNPs due to its ability to inhibit to the extent of 37.5 percent as compared to Spirulina-derived AgNPs.

The different cyanobacterial strains exhibit values of MIC with the changes in nanoparticle size, zeta potential, and variations in biomolecular capping agents that impact responses by the bacteria [15]. The high antimicrobial effect is observed because of the increased cell contact of bacteria that are a result of the large surface area which occur in the small nanoparticles from Anabaena. The Ag+ ion stability and bioavailability are also influenced by different biomolecular caps which may be the explanation of the differences in the recorded MIC value. The study shows that AgNPs that are obtained using Anabaena have the highest antibacterial effect at the lowest MIC requirement, when analyzed against different bacterial strains, but AgNPs of Nostoc present less effective antibacterial result, which requires higher MIC levels.

CONCLUSION

The paper has managed to illustrate the biogenic production of silver nanoparticles (AgNPs) using cyanobacterial strains (Spirulina, Anabaena and Nostoc) of Saudi Arabia arid Hail region. The produced AgNPs had specific antibacterial actions with significant variation in the effectiveness of the nanoparticles in the various cyanobacterial strains. The antimicrobial potential of the Nostoc-derived AgNPs was highest, as it had the highest zone of inhibition (ZOI) and lowest Minimum Inhibitory Concentration (MIC) values, especially in P. aeruginosa. Conversely, AgNPs obtained through the process of Anabaena exhibited the lowest antimicrobial efficacy indicating the highest MIC. AgNPs prepared using spirulina exhibited a medium effect as compared to the two. Statistical analysis (ANOVA) was used to determine that the cyanobacterial strain of choice has a significant impact on AgNPs production and antimicrobial action, and the differences in the MIC value ($p = 0.0001$) and the ZOI ($p = 0.0001$) were highly significant. The analysis of crystallinity provided p-value of 0.0258 which supported again the importance of strain-selective variations in the structural characteristics of the AgNPs. Nostac showed a high antimicrobial activity and therefore it is a promising source that can be used to manufacture AgNPs of good antibacterial activity.

Further the paper revealed that various species of bacteria such as the E. coli strain, S. aureus and P. aeruginosa were found to exhibit a varying susceptibility to the AgNPs, as well as significant variation in the patterns of inhibition. Further studies in this field ought to aim at optimization of the conditions of synthesis including temperature, pH and concentration of silver nitrate to boost the yield and performance of AgNPs. The relative stability and probable toxicity of the synthesized AgNPs are to be studied to determine their safety when used in biomedicine. Additionally, the mechanism of antimicrobial effect, such as the involvement of biomolecules in the synthesis process, and further exploration of the biological interactions that specify AgNP activity may be more informative on the biological interactions that govern the activity of AgNP. Lastly, future studies of this research to other cyanobacterial strains and their possible uses in the environment including wastewater treatment and environmental clean-ups would help in creating sustainable and greener nanomaterials.

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Contribution of Authors

S.A.: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft, Writing - Review & Editing, Validation, Data Curation, Supervision. **A.A.**: Methodology, Investigation, Writing - Original Draft. **F.A.**: Software, Validation, Investigation, Writing - Review & Editing.

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All the data are included in the manuscript.

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This article does not contain any studies with human participants or animals performed by any of the authors.

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The authors declare that they have no known conflict of interest**.**

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