The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(1): 395-403, 2025 www.thebioscan.com

Enhanced Antimicrobial Effect of Biosurfactant Coated Copper Nanoparticles against Selected Plant Pathogens

Dhanashree Vinayak Deshmukh[1] Hanumantrao Vitthal Deshmukh[2] Masidd Khalate[3]

1. Department of Microbiology, Shivaji University, Kolhapur Maharashtra India, 416004.
2. Department of Microbiology, Yashwantrao Chavan Institute of Science, Satara Maharashtra India, 415001.
3. Infinite Biotech Institute of Research and Analysis, Sangli, Maharashtra, 416415

Corresponding Author Email: masiddkhalate22@gmail.com

DOI: https://doi.org/10.63001/tbs.2025.v20.i01.pp395-403

KEYWORDS
Biosurfactant,
Antimicrobial,
Copper Nanoparticles

Received on:

16-12-2024

Accepted on:

14-01-2025

Published on:

20-02-2025

ABSTRACT

Due to their therapeutic and antibacterial uses, copper nanoparticles have gained popularity in antibacterial research. Copper nanoparticles (CuNPs) are becoming a more accessible and reasonably priced medical tool. When coated with the correct surfactant, copper nanoparticles are recognised for their antibacterial and other therapeutic qualities. Two biosurfactants, which are synthesised using ascorbic acid and obtained from culture broth from specific bacterial isolates A. xylosoxidans (P5C) and P. aeruginosa (P11), were employed in this work as capping agents for the coating of CuNps. The synthesised copper nanoparticles were characterised by UV visible spectroscopy, which showed an absorption peak at 222 nm. FT-IR analysis revealed that copper oxides (CuO) were present in each of the samples that were being examined. The zeta potential of CuO+P5C NPs is -24.1 mV, whereas that of CuO+P11 NPs is -12.1 mV, indicating the stability of the synthesised particles. The TEM and SEM examinations revealed that the irregularly sized particles had a spherical shape. An EDX analysis revealed that the sample had higher oxygen and copper. In a similar vein, morphological analysis was finished. Both types of CuNPs have shown good antimicrobial activity against the selected plant strains with minimum inhibitory concentration (MIC) value of 125 µg/ml concentration.

INTRODUCTION

Nanotechnology is the use of nanoscale materials ranging in size from 1 to 100 nm for specialised physical, chemical, and biological applications to benefit mankind (Kumar, Y et al., 2020). Nanoparticles are made from the earth's most plentiful and affordable metals, which have a wide range of uses and are beneficial to mankind. Nanoparticles have begun to be employed as antimicrobial agents or alternatives to traditional antibiotics because to their various and unique qualities, such as their involvement in energy conversion and storage, catalytic capabilities, and implications in surface treatments (Crisan, M. C et al., 2021). Copper-based bactericides are a novel crop protection weapon that efficiently combats bacterial resistance (Varympopi, A. et al., 2022). For more than a century, copper bactericides and fungicides have been used on a broad scale over the world to protect agricultural yield (La Torre et al). (2018). Currently, nanotechnology is largely focused with the use of nanoscale materials, with diameters ranging from 1 to 100 nm, for particular physical, chemical, and biological applications that will benefit humanity (Kumar, Y et al., 2020). Because nanoparticles are beneficial to humanity and have multiple applications, the most abundant and fairly priced metals on the planet are used to produce them. Nanoparticles are now used as antimicrobial agents or as alternatives to traditional antibiotics due to their multiple unique qualities, which include the capacity to convert and store energy, function as a catalyst, and be applied to surface treatments (Crisan, M. C. et al., 2021). Copper-based nanoparticles (CuNPs), which are now being studied as prospective replacements to the conventional bactericides, have been found by some researchers to have a stronger antibacterial impact due to their smaller size (Strayer-Scherer et al., 2018). Nanoparticles' increased activity is due to their small size and high surface-to-volume ratio, which have been shown to improve membrane penetration and increase metal ion production in solution (Strayer-Scherer et al., 2018). The current work aims to compare the antibacterial properties of copper nanoparticles coated with biosurfactant against gram-negative bacteria. The study has been taken over since numerous recent research have showed good findings. Recent independent research have shown that Cu-based NPs of various sizes reduce bacterial leaf spot more effectively than standard bactericides in the field (Fan, Q. et al., 2021).

Material:

Copper sulphate pentahydrate and Ascorbic acid was purchased from sigma Aldrich. Deionised water was used to prepare plant extract and the copper sulphate solution, Sodium hydroxide, nitric acid and the sodium bicarbonate used in the synthesis process was also obtained from Qualigen Pvt Ltd.

1. Methodology:

1.1 Preparation of copper nanoparticles

Copper nanoparticles were synthesised by using ascorbic acid as the reducing agent in a chemical reduction procedure. 0.1 M copper sulphate pentahydrate solution was prepared, stirred constantly for 30 minutes, and then 50 mL of ascorbic acid solution and 30 mL of 1 M NaOH solution were added. After two hours at 80°C, the reaction mixture's colour changed instantly from yellow

to brown-red. After that, the solution was left to sedimentation for 24 hours.

Production of Biosurfactants from Microorganisms

A volume of 100 ml of Mineral Salt Medium (MSM) containing 2 % glucose and adjusted to a pH of 7.0 was prepared within a 250 ml Erlenmeyer flask. This medium was then sterilized using an autoclave. The next step involved the inoculation of 5.0 ml of culture broth from selected bacterial isolates *A. xylosoxidans* (P5c) and *P. aeruginosa* (P11), with a cell concentration of 1×10^9 cells per ml. To this, 2 ml of petrol was added. The flasks containing the mixture were placed in an orbital rotary shaker set to 120 revolutions per minute and were incubated at a temperature of 35°C for a duration of 3 days.

Following the incubation period, the culture broth underwent centrifugation at 15000 rpm for a duration of 10 minutes, all carried out at a temperature of 4°C to create a cell free supernatant, which was subsequently filtered through a Whatman filter paper with a pore size of 0.2 µm. The filtered supernatant was then used for the biosurfactant extraction process, as detailed in Table 1. This filtrate was acidified with 6N HCl to pH 2 and equal volumes of solvents. The mixture was then placed in a separating funnel and the organic phase was collected. This phase was later evaporated at 45 °C and the remaining solutes were redissolved in solvent. The stock of this crude biosurfactant was maintained at a concentration of 1mg/ml. This was used for synthesis of biosurfactant mediated nanoparticles.

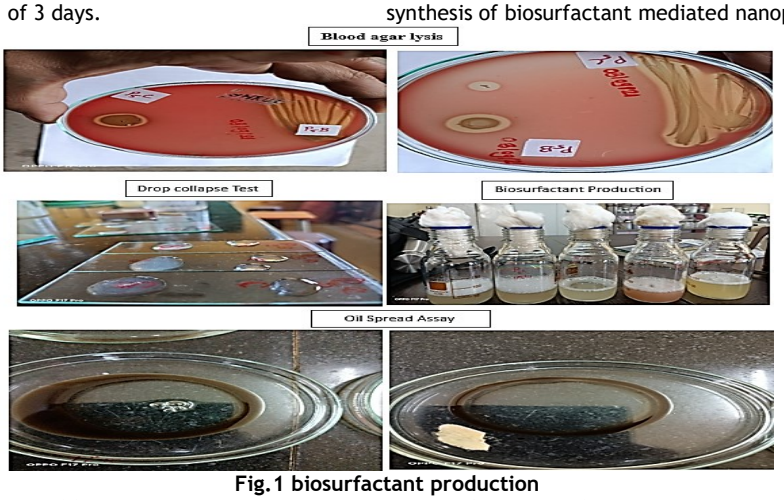


Fig.1 biosurfactant production

Table 1 : Extraction of Biosurfactant using different solvents

Methanol: Chloroform (2:1 ratio)	Equal volume
	Acidification using 6N HCl to pH 2 and added with equal volume of Methanol: Chloroform

3.3 Characterisation of biosurfactant

Biosurfactants produced were characterised by using FTIR analysis and LCMS analysis by using conventional method.

3.4 Coating of copper nanoparticle with biosurfactants

Copper nanoparticles are susceptible to the fast oxidation and are highly reactive which affects the ability of CuNps and to avoid that coating was done. Pre-synthesised nanoparticle solution was placed in the magnetic stirrer after dissolving in 10 ml of water. The uncoated nanoparticles were then kept under stirring condition at 200 ppm and 2M nitric acid as added dropwise to the solution. The solution was taken out and the pH was adjusted. The biosurfactants (P11 and P5C) was dispersed in 50 ml sodium bicarbonate. The surface charged NPs were then treated with solution of biosurfactant in 1:1 ratio. The reaction mixture was incubated at 37ºC for 48 hours. After which the nanoparticles were obtained by centrifuging the mixture at 2500 rpm for 15 mins. The particles were washed in deionised water and ethanol and stored at 4ºC.

3.5 Characterization of copper nanoparticles

3.5.1 UV Analysis

UV spectroscopy was used to determine the size and shape of the nanoparticles as the Nanoparticles exhibit unique interactions with ultraviolet (UV) light based on their size and shape. The suspension was made by using suitable solvent and then the absorbance was measured.

3.5.2 FT-IR analysis

The sample was subjected to FTIR analysis to determine the nanoparticle composition. The minimum quantity of sample was loaded onto the sampler.

3.5.3 Zeta potential

Deionized water was used to make the sample suspension, and the sample was injected into sample holders to prevent the production of air bubbles. After that, the sample holder was retained inside the apparatus to use the electrophonic light scattering method to analyse the particles' zeta potential.

3.5.4 SEM and TEM analysis

The morphology of the nanoparticles was examined using a transmission electron microscope (TEM), and their size and shape were determined using a scanning electron microscope (SEM). Copper nanoparticles were dissolved in appropriate solvents to create the sample, which was then sonicated for one minute. the sample drop was placed on the conductive substrate. Once the material had dried, the samples were analysed using a TEM instrument set to 80–200 keV and SEM apparatus set to 10–20 keV.

3.5.5 Energy dispersive X-ray spectroscopy (EDX)Analysis

The Energy dispersive X-ray spectroscopy was done to identify the elemental composition of the given nanoparticles. The samples of copper nanoparticles were dispersed in a suitable solvent and then deposited on the carbon film. The film was set to dry until the complete evaporation of the solvent. Then the film was analysed under microscope.

3.6 Antimicrobial activity

Selection and Culturing of bacterial strains

The Antimicrobial activity was performed against different bacterial strains named as *xanthomonas axonopodis, xanthomonas citri, ralstonia solanacearum, and Erwinia cartovora,*. these bacterial strains were procured from CSIR-NCIM, Pune, India and were inoculated onto nutrient agar plates at 37^0C, which support the growth of bacteria.

Antimicrobial activity by well diffusion method

The inoculum of the microorganism was prepared from the bacterial cultures. 15ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify. 100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Wells of 6mm in diameter were bored using a sterile cork borer. Solutions of the compounds (100µl/ml) were prepared in DMSO and 100µl of prepared test solutions and standard was added to the wells. The petri plates incubated at 37ºC for 24 h. Streptomycin (1mg/ml) was prepared as a positive control and DMSO was taken as negative control. Antibacterial activity was

evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.

3.7 Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of the copper nanoparticles was determined against four different plant pathogens which are *Erwinia cartovora, ralstonia solanacearum, Xanthomonas axonopodis and xanthomonas citri* by using 96 well plate method. All these are gram-negative, rod-shaped bacterium which caused the many plants pathogenic disease. They can cause leaf spots, cankers, wilting, stunting, and fruit blemishes depending on the host plant and specific strain. the method used was resazurin assay method (Sarker et al. 2007; Valsalam et al. 2019a, b). A resazurin-based assay was used to determine the minimum inhibitory concentration (MIC) of samples. 270 mg of resazurin was dissolved in sterile distilled water, and a 96-well plate was prepared. 100 µL of different sample concentrations were dispensed into designated wells, followed by 50 µL of nutrient broth, ten microliters of resazurin indicator solution, and 10 µL of a standardized fungal or bacterial suspension. Streptomycin was used as the positive control. The plate was incubated at 37˚C for 18-24 hours, and the MIC was determined by visual observation of color change from blue to pink. The percentage of inhibition was calculated using the formula:

% Inhibition = (Control - Test)/Control * 100

3.8 Cytotoxicity analysis over normal human fibroblast cell lines

Normal human fibroblast cell lines (L929 cells) were seeded into a 96-well plate at an appropriate density ($1x10^4$) to ensure even distribution and were incubated at 37˚C with 5% CO2 to allow for cell attachment. After 24 hours, the cells are treated with CuO+P5C and CuOLL+P11 (Nanoparticles with surfactant) and incubated for 24 h at 37˚C with 5% CO_2. Subsequently, 10 µL of MTT solution (5 mg/mL in PBS) is added to each well and incubated for 3-4 h at 37˚C. After incubation, the medium is carefully removed, and 100 µL of DMSO is added to dissolve the formazan crystals. The absorbance is then measured at 570 nm using a microplate reader, with the color intensity correlating to the number of viable cells. The percentage of viability was calculated by using following formula,

% of Viability= OD of Control-OD of Test/OD of Control x100

4 Results:

4.1 Characterization of biosurfactants

FT-IR spectra of both the biosurfactant showing the identical peaks of protein at 1656.24 nm, carbohydrate at 1110.42 and 1018.53 nm. For fatty acid the peak can be seen at 2832.58 nm and 2945 nm. One broad -OH peak was seen at 3334.35 nm. FTIR interferogram is shown in the Figure 1. LCMS analysis was done and the peaks are as shown in Figure 2 which confirms the presence of *Achromobacter xylosoxidans, Pseudomonas aeruginosa.*

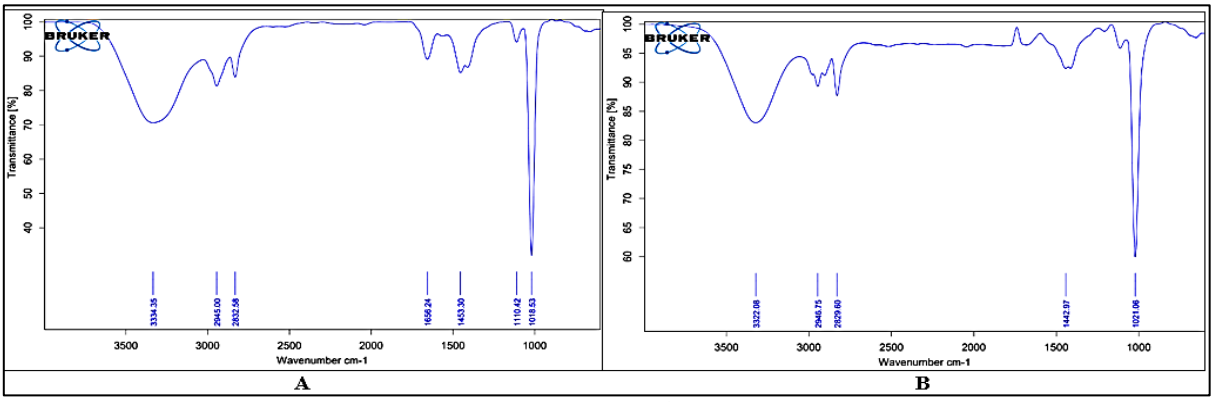


Figure 1 (A) FTIR spectra of P5C and (B) FTIR spectra of P11

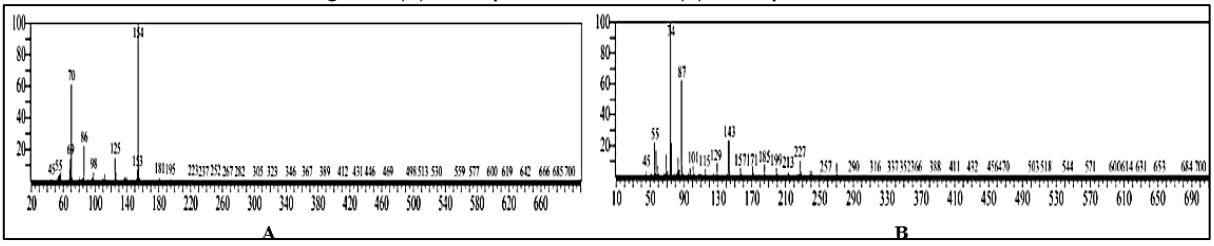


Figure 2 (A) LCMS spectra of P5C and (B) LCMS spectra of P11

4.2 Characterization of Copper Nanoparticles (UV-Vis Spectroscopy)

The maximum absorbance was observed at 222 and 225 nm wavelength. The maximum absorbance at 350 nm indicating the presence of biosynthesized CuNPs in the reaction mixture as presented in Figure 3.

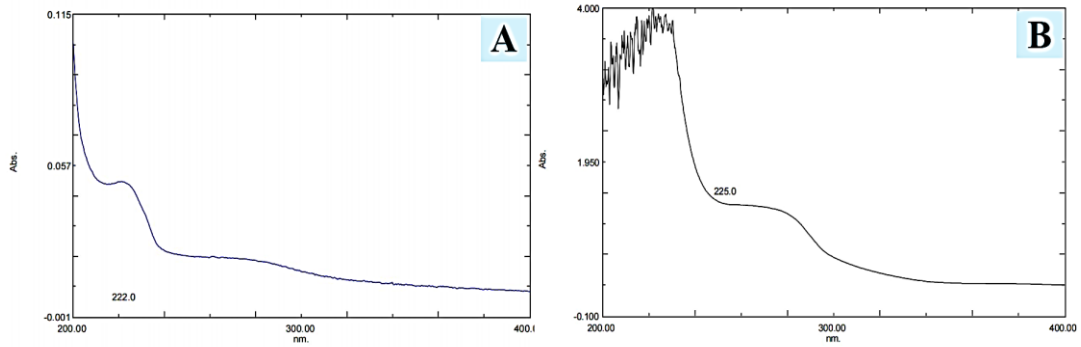
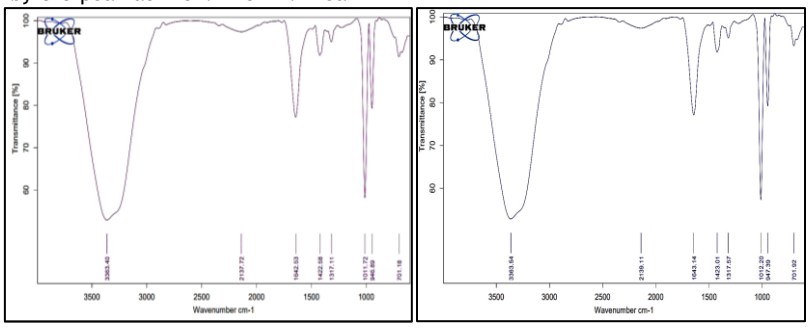


Figure 3: Uv spectrum of A) Cuo+P5C B) Cuo+P11

4.3 FTIR analysis for copper nanoparticles

The FTIR spectrum of the both the samples of nanoparticles are same and showing same peaks. The peak at 701 cm^{-1} showing the cu-o stretching. Which is the identical peak of copper oxide. the peak at 3363.40 cm^{-1} is the identical peak of O-H stretching. C≡C-Cu Stretching was identified by the peak at 2137.72 cm^{-1}. Peak at 1643 assigned to C=O stretching vibrations from carbonyl groups like in capping agents like citrate, or conjugated C=C stretching from aromatic compounds. Peaks at 1422 cm^{-1} showing the C-H bending vibrations. In all FT-IR results showing the presence of desired stretching and bending frequencies (Figure 4).



(A) **(B)**

Fig. 4 (A) FTIR spectra of CuO+P5C NPs and (B) is FTIR spectra of CuO+P11 NP

4.4 Zeta potential to check the stability of nanoparticles

The morphological characteristics of the copper nanoparticles were investigated using zeta potential analysis. It has been demonstrated in the past that the copper nanoparticle's zeta potential ranges from -24 to -45 mV, depending on the pH. The zeta potential of CuO+P5C NPs is determined to be -24.1 mV, whereas that of CuO+P11 NPs is determined to be -12.1 Mv, based on the results of the two samples analysed. While examining the material's biological effects, the zeta potential value also indicates that the copper nanoparticles have a moderately negative charge on them, suggesting reduced toxicity. These results lend credence to copper nanoparticles' antibacterial properties (Figure 5 and 6).

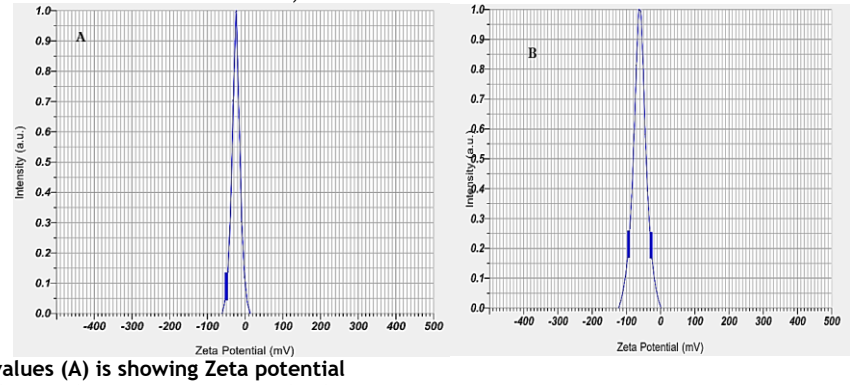


Figure 5: Zeta potential values (A) is showing Zeta potential value of CuO+P5C NPs and (B) is showing Zeta potential value of CuO+P11 NPs

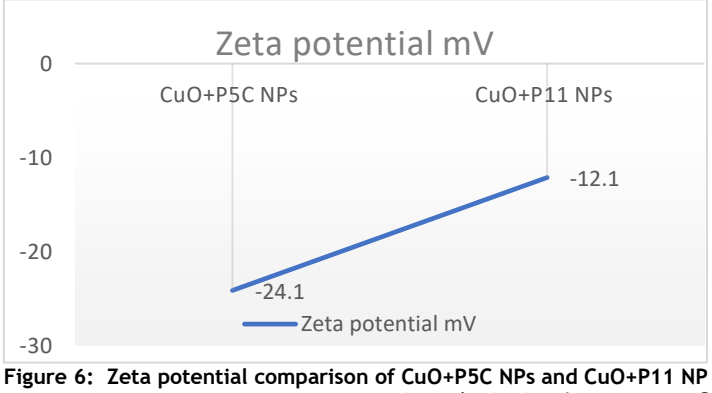


Figure 6: Zeta potential comparison of CuO+P5C NPs and CuO+P11 NP

4.4 SEM Analysis

The morphology of the copper nanoparticles was examined using SEM analysis, and it was found that the particles are round and irregular in size. In contrast to CuO+P5C NPs, CuO+P11 NPs exhibit more granular and spherical characteristics. As shown in Figure 7.

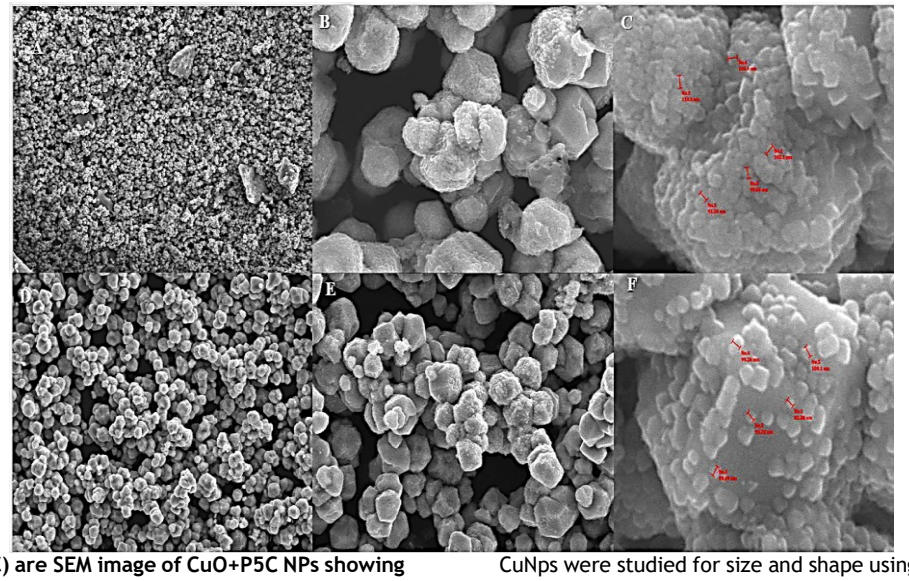


Figure 7 (A), (B) and (C) are SEM image of CuO+P5C NPs showing the spherical particle, and (D), (E), (F) are SEM images of CuO+P11 NPs showing regular spherical and granular particles

4.5 Morphological analysis by TEM

CuNps were studied for size and shape using transmission electron microscopy (TEM) pictures, which revealed that the particles are spherical in shape and vary in size. The diameter of the particle is 90-100 nm. The spherical form and irregular size of all the particle images are displayed as in Figure 8 and 9.

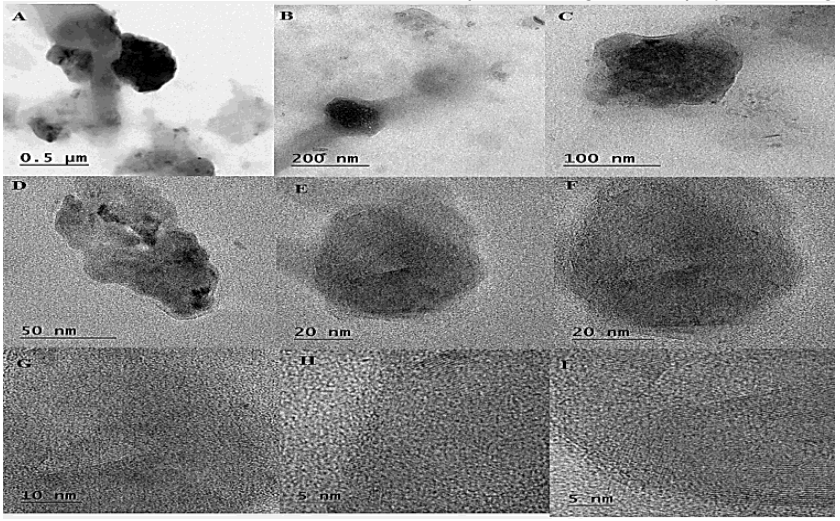


Figure 8: TEM images of CuO+P11 NPs (A) TEM image at 0.5 µm showing the spherical particle (B) is showing the particles at 200 nm and the same zoomed image at 100nm is shown in (C), (D) showing the image at 50 nm, (E and F) showing clear spherical image at 20nm, (G, H, And I) showing the inner zoomed core of the same particle at 10 and 5 nm respectively.

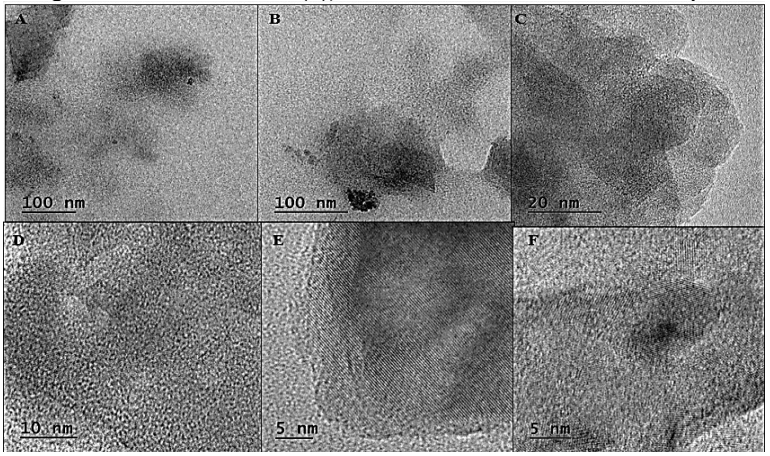


Figure 9: TEM images of CuO+P5C NPs (A) TEM image at 100 µm showing the spherical particle (B) is showing the particles at 100 nm and the same zoomed image. (C), (D) showing the image at 20 And 10 nm, (E and F) showing clear spherical image at 5nm.

4.6 Determination of the composition of nanoparticles by EDX analysis

95.51 ± 3.33 percent of the total weight was determined to be copper in the CuO+P5C NP sample. Additionally, it was discovered that the weight percentage of copper in CuO+P11 NPs was 95.44 ± 3.20 percent. The prominent peak at 8.04 keV in both the

graphs, is evidence of the successful production of copper nanoparticles, and is demonstrated to suggest the high copper content in the sample under study based on the EDX analysis (Figure 10 and 11).

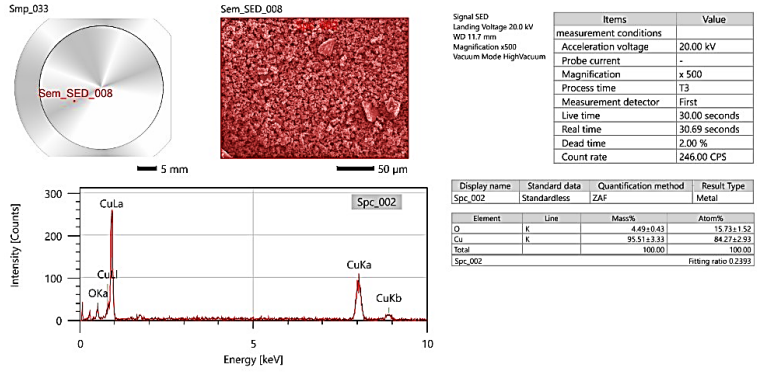


Figure 10 showing EDX analysis of CuO+P5C

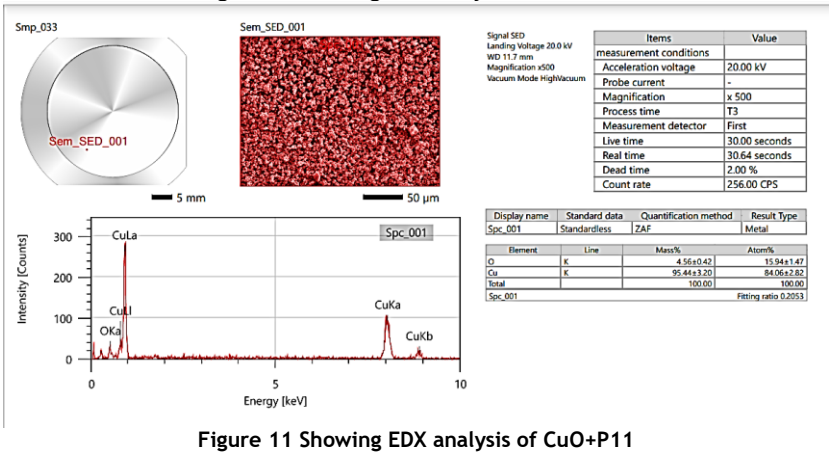


Figure 11 Showing EDX analysis of CuO+P11

4.7 Antimicrobial activity of CuNPs

The antimicrobial activity was performed against different bacterial strains named as *Erwinia cartovora, ralstonia solanacearum, Xanthomonas axonopodis and xanthomonas citri* the results are shown in terms of diameters as shown in the Table 2. The overall antimicrobial activity of CuNps is found good as compared to standard streptomycin. CuO+P5C and CuO+P11 have shown the comparable activity against all microbial strains under study. all the results are summarised in the Table 2 and images are shown below in Figure 12. *Erwinia cartovora* has shown the highest sensitivity towards the nanoparticles as compared to the other strains. Thus, the biosurfactant coated copper nanoparticles have shown the comparable and at the same time best antimicrobial activity (Figure 12).

Table.1 Antimicrobial activity of biosurfactant coated CuNps

Sample	Concentration µg/ml	*Zone of inhibition against Erwinia cartovora*	*Zone of inhibition against ralstonia solanacearum*	*Zone of inhibition against Xanthomonas axonopodis*	*Zone of inhibition against xanthomonas citri*
Standard	1000	29	29	29	29
CuO+P5C	250	21	18	16	16
	500	22	21	21	19
	1000	**24**	**23**	**23**	**21**
CuO+P11	250	17	14	19	12
	500	23	16	22	18
	1000	**25**	22	**24**	**21**

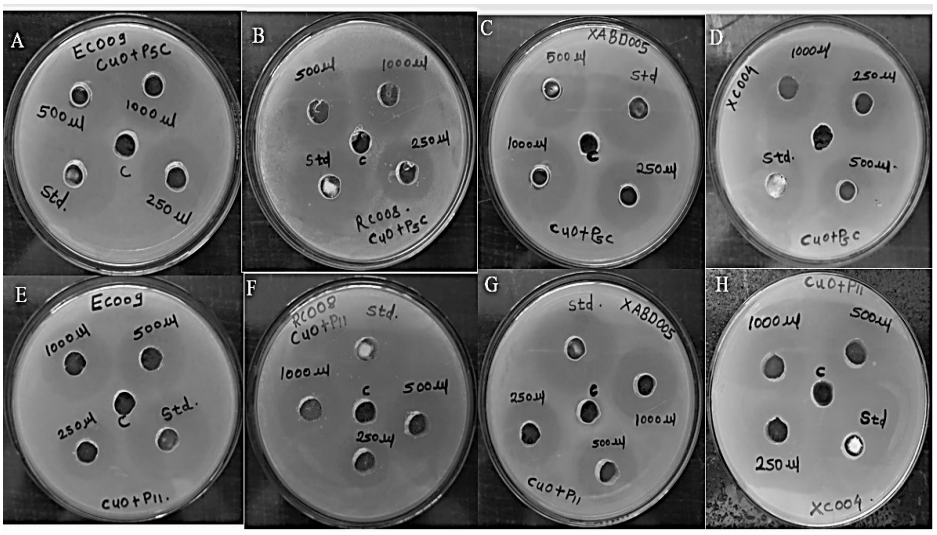


Figure 12 Antimicrobial activity (A, B,C,D) showing the activity of CuO+P5C and (E, F,G,H) showing the activity of CuO+P11.

4.7.1 Minimum Inhibitory Concentration

The ELISA test reader absorbance was measured at 600nm and both the samples, CuO+P5C NPs and CuO+P11 NPS the lowest growth inhibitory concentration was found to 125 µg/ml as compared to the streptomycin. Results are summarised in the Table 2 showing the MIC of the copper nanoparticles under study. the minimum inhibitory concentration of both the samples was found to be 125 µg/ml towards all micro-organisms under study. Biosurfactant coated copper nanoparticles have shown the better inhibition activity.

Table.1 MIC Values of copper nanoparticles.

SAMPLE	CONCENTRATION G/ML	%GROWTH INHIBITION AGAINST *Erwinia cartovora*	%GROWTH INHIBITION AGAINST *ralstonia solanacearum*	%GROWTH INHIBITION AGAINST *Xanthomonas axonopodis*	%GROWTH INHIBITION AGAINST *xanthomonas citri*
Standard	7.8	-	-	-	-
	15.6	-	-	-	-
	31.2	11.35	11.35	11.35	11.35
	62.5	12.85	12.85	12.85	12.85
	125	47.97	47.97	47.97	47.97
	250	61.88	61.88	61.88	61.88
	500	66.87	66.87	66.87	66.87
	1000	73.67	73.67	73.67	73.67
CuO+P5C	7.8	-	-	-	-
	15.6	-	-	-	-
	31.2	-	-	-	-
	62.5	-	-	-	-
	125	1.31	2.99	4.55	2.81
	250	40.48	41.11	40.11	42.73
	500	54.33	55.08	53.71	55.39
	1000	59.76	59.51	59.38	60.94
CuO+P11	7.8	-	-	-	-
	15.6	-	-	-	-
	31.2	-	-	-	-
	62.5	-	-	-	-
	125	1.43	3.93	1.43	9.73
	250	41.98	42.42	40.73	48.03
	500	55.08	55.02	54.46	59.13
	1000	60.07	61.32	59.51	64.31

Cytotoxicity

Copper Nanoparticles (CuO+P5C and CuO+P11) was tested for five concentrations (20, 40, 60, 80, 100µg/ml) by MTT assay to check its toxicity. CuNps revealed good cyto-protective activity (78-80%-cell viability) against normal fibroblast cell line as compared to the positive control 70% Ethanol (Figure 13).

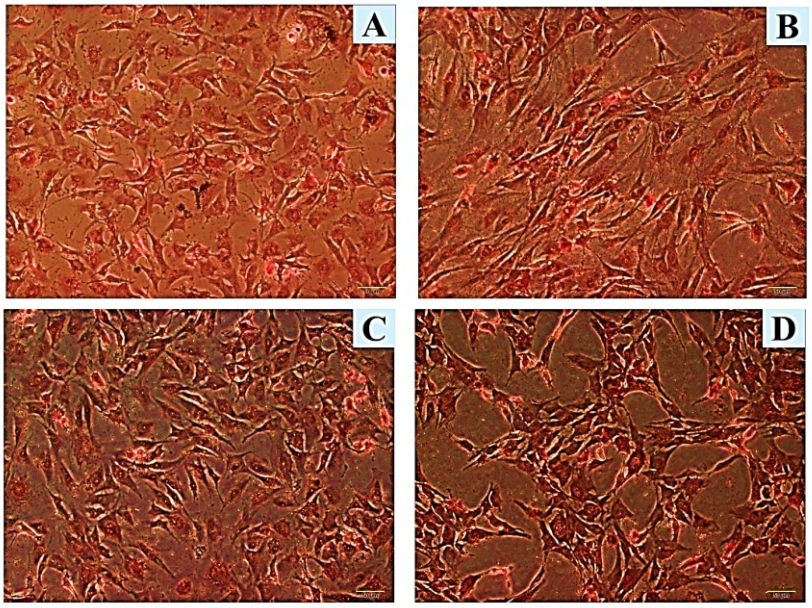


Figure 4.13 A) Cytotoxicity of negative control on fibroblast cell lines (L929), B) L929 cell lines treated with positive control (70% Ethanol) , C) L929 cell lines treated with copper nanoparticles CuO+P5C , and D) L929 cell lines treated with copper nanoparticles CuO+P11

DISCUSSION

Copper nanoparticle synthesis is a highly emphasized field of study due to its low cost and various characteristics. However, copper nanoparticle manufacturing is difficult since it easily transforms into copper oxide, necessitating the use of a stabilizer. Biosurfactants, produced by microorganisms like bacteria, yeast, and fungi, are cost-effective, eco-friendly alternatives for green nanomaterials, suitable for various industries like agriculture, petroleum refining, cosmetics, food, detergents, and medicine [17]. The application of a biosurfactant layer on nanoparticles plays a crucial role in their stabilization, inhibition of aggregation, and serves as an environmentally friendly substitute for synthetic stabilizing agents, owing to their organic source and wide range of advantages [18].

CuNps coated with biosurfactants have demonstrated potential as effective antimicrobials. Several studies have emphasized the effective creation of copper nanoparticles through environmentally friendly techniques that involve the use of biosurfactants [19]. The nanoparticles have shown strong antimicrobial effects against various pathogens, such as Klebsiella oxytoca, Escherichia coli, Staphylococcus aureus, and Bacillus cereus [20]. The effectiveness of the biosurfactant-coated copper nanoparticles in preventing microbial growth was demonstrated by their low minimum inhibitory concentrations (MIC) against these pathogens [21]. Furthermore, the utilization of biosurfactants during the synthesis process plays a significant role in promoting the environmental friendliness and sustainability of the nanoparticles [22]. When biosurfactants and copper nanoparticles are combined, they offer a promising method for developing antimicrobial agents that could have diverse applications in areas like medicine and agriculture. Characterization of nanoparticles establishes their form, size, stability, and coating.

The synthesis methods used in different investigations cause variances in the diameter of copper nanoparticles. Researchers used various ways to produce copper nanoparticles with diameters ranging from 2.47 nm to 400 nm. [23] [24]. In addition, it has been reported by other studies that the average size of synthesized copper nanoparticles is roughly 120 nm [25], with a size range spanning from 5 to 9 nm [26]. These variations in size can be attributed to the different synthesis conditions, compositions of the reaction medium, and methods employed in each study. The size of copper nanoparticles can span from a few nanometers to several hundred nanometers, illustrating the flexibility in manipulating their dimensions for diverse uses in fields like catalysis, biomedicine, and bioremediation [27]. The current study shows that the diameter of the nanoparticles was found to be in the range of 90-100 nm which is in agreement with the literature. When it comes to the shape of the copper nanoparticles, they can exhibit various shapes such as cubes, hemispheres, agglomerates, spheres, nanorods, triangles, and nano disks, depending on the synthesis method, conditions and capping agents [28]. The current investigation shows that the copper nanoparticles synthesised and coated with biosurfactant has spherical shape and they look granular these results are also in agreement with the previous research. The current study mainly deals with the changing effects of the copper nanoparticles when there is change in the coating agent. Both the type of nanoparticles are effective against the gram negative bacteria at MIC of 125 µg/ml. CuO+P5C have shown the best results against XCBD005 with 4.55 % growth inhibition. While CuO+P11 have shown the best results against RC008 with 3.93 % growth inhibition. Many past studies have revealed that copper nanoparticles are effective against gram positive bacteria at lower MIC value and for gram negative bacteria higher concentration is required [29]. Biosurfactants isolated from *Achromobacter xylosoxidans* and *Pseudomonas aeruginosa* bacterium were not specifically used before for the synthesis of copper nanoparticles, but the synthesized nanoparticles have shown the good antimicrobial activity against gram negative bacterial strains under study and can be consider for further analysis.

CONCLUSION

Highly pure and stable copper nanoparticles were synthesised by using two different stabilisers. The different bio stabilisers stabilise the copper nanoparticles from getting readily oxidise, and this makes these particles more efficient. Antimicrobial activity of copper nanoparticles get enhanced because of the capping of *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*. The copper content and the elemental analysis was done by EDX analysis. TEM and SEM analysis confirms the spherical and granular structure of the CuNPs. Lastly the both the type of CuNPs have shown good antimicrobial activity against the selected bacterial strand with MIC value of 125 µg/ml which is very significant value. Thus, the two types of copper nanoparticles can be successfully employed as an antimicrobial agent against plant pathogens and can be considered as the good and effective alternative to other chemical-based antimicrobials. Utilizing bacterial strains with nanoparticles is not the common practice and quite a new thing. The current study has shown positive results and more research is needed to confirm the enhanced ability of nanoparticles after coating of biosurfactant.

REFERENCES

- [1]Ahamed, M., Alhadlaq, H. A., Khan, M. M., Karuppiah, P., & Al-Dhabi, N. A. (2014). Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. *Journal of Nanomaterials*, *2014*, 17-17.
- [2] Crisan, M. C., Teodora, M., & Lucian, M. (2021). copper nanoparticles: Synthesis and characterization, physiology, toxicity and antimicrobial applications. *Applied Sciences*, *12*(1), 141.
- [3]Fan, Q., Liao, Y. Y., Kunwar, S., Da Silva, S., Young, M., Santra, S., ... & Paret, M. L. (2021). Antibacterial effect of copper composites against Xanthomonas euvesicatoria. *Crop Protection*, *139*, 105366.
- [4] Giri, A. K., Jena, B., Biswal, B., Pradhan, A. K., Arakha, M., Acharya, S., & Acharya, L. (2022). Green synthesis and characterization of copper nanoparticles using Eugenia roxburghii DC. extract and activity against biofilm-producing bacteria. *Scientific Reports*, *12*(1), 8383.
- [5] Kumar, Y., Singh, V., Pandey, A., Genwa, M., & Meena, P. L. (2020, November). Synthesis, characterization and antibacterial activity of ZnO nanoparticles. In *AIP Conference Proceedings* (Vol. 2265, No. 1). AIP Publishing.
- [6] La Torre, A., Iovino, V., & Caradonia, F. (2018). Copper in plant protection: Current situation and prospects. *Phytopathologia Mediterranea*, *57*(2), 201-236.
- [7] Mali, S. C., Dhaka, A., Githala, C. K., & Trivedi, R. (2020). Green synthesis of copper nanoparticles using Celastrus paniculatus Willd. leaf extract and their photocatalytic and antifungal properties. *Biotechnology Reports*, *27*, e00518.
- [8] Murthy, H. C., Desalegn, T., Kassa, M., Abebe, B., & Assefa, T. (2020). Synthesis of green copper nanoparticles using medicinal plant hagenia abyssinica (Brace) JF. Gmel. leaf extract: Antimicrobial properties. *Journal of nanomaterials*, *2020*.
- [9] Strayer-Scherer, A., Liao, Y. Y., Young, M., Ritchie, L., Vallad, G. E., Santra, S., ... & Paret, M. L. (2018). Advanced copper composites against copper-tolerant Xanthomonas perforans and tomato bacterial spot. *Phytopathology*, *108*(2), 196-205.
- [10] Varympopi, A., Dimopoulou, A., Papafotis, D., Avramidis, P., Sarris, I., Karamanidou, T., ... & Skandalis, N. (2022). Antibacterial activity of copper nanoparticles against Xanthomonas campestris pv. vesicatoria in tomato plants. *International Journal of Molecular Sciences*, *23*(8), 4080.
- [11] Parikh, P., Zala, D., & Makwana, B. (2014). Biosynthesis of copper nanoparticles and their antimicrobial activity. *Inst Post Studies Res KSV Uni. India*, 1-15.
- [12] Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., & Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia*, *4*(3), 707-716.
- [13] Ramyadevi, J., Jeyasubramanian, K., Marikani, A., Rajakumar, G., & Rahuman, A. A. (2012). Synthesis and antimicrobial activity of copper nanoparticles. *Materials letters*, *71*, 114-116.
- [14] Usman, M. S., Zowalaty, M. E. E., Shameli, K., Zainuddin, N., Salama, M., & Ibrahim, N. A. (2013). Synthesis, characterization, and antimicrobial properties of copper nanoparticles. *International journal of nanomedicine*, 4467-4479.
- [15] Bogdanović, U., Lazić, V., Vodnik, V., Budimir, M., Marković, Z., & Dimitrijević, S. (2014). copper nanoparticles with high antimicrobial activity. *Materials Letters*, *128*, 75-78.
- [16] Chowdhury, M. N. K., Beg, M. D. H., Khan, M. R., & Mina, M. F. (2013). Synthesis of copper nanoparticles and their antimicrobial performances in natural fibres. *Materials Letters*, *98*, 26-29.
- [17] Ingle, A. P., Saxena, S., Moharil, M., Rai, M., & Da Silva, S. S. (2023). Biosurfactants in Nanotechnology: Recent Advances and Applications. *Biosurfactants and Sustainability: From Biorefineries Production to Versatile Applications*, 173-194.
- [18] Bilai, M., Khan, M. R., & Sayyed, R. Z. (2022). Biosurfactant mediated synthesis and stabilization of nanoparticles. In *Microbial Surfactants* (pp. 158-168). CRC Press.
- [19] Eid, A. M., Fouda, A., Hassan, S. E. D., Hamza, M. F., Alharbi, N. K., Elkelish, A., ... & Salem, W. M. (2023). Plant-based copper oxide nanoparticles; biosynthesis, characterization, antibacterial activity, tanning wastewater treatment, and heavy metals sorption. *Catalysts*, *13*(2), 348.
- [20] Shehabeldine, A. M., Amin, B. H., Hagras, F. A., Ramadan, A. A., Kamel, M. R., Ahmed, M. A., ... & Salem, S. S. (2023). Potential antimicrobial and antibiofilm properties of copper oxide nanoparticles: time-kill kinetic essay and ultrastructure of pathogenic bacterial cells. *Applied Biochemistry and Biotechnology*, *195*(1), 467-485.
- [21] Chaerun, S. K., Prabowo, B. A., & Winarko, R. (2022). Bionanotechnology: the formation of copper nanoparticles assisted by biological agents and their applications as antimicrobial and antiviral agents. *Environmental Nanotechnology, Monitoring & Management*, *18*, 100703.
- [22] Athira, K., Gurrala, L., & Kumar, D. V. R. (2021). Biosurfactant-mediated biosynthesis of CuO nanoparticles and their antimicrobial activity. *Applied Nanoscience*, *11*(4), 1447-1457.
- [23] Ouyang, L., Noël, V., Courty, A., Campagne, J. M., Ouali, A., & Vrancken, E. (2022). copper nanoparticles with a tunable size: implications for plasmonic catalysis. *ACS Applied Nano Materials*, *5*(2), 2839-2847.
- [24] Aderolu, H. A., Aboaba, O. O., Aderolu, A. Z., Abdulwahab, K. O., Suliman, A. A., & Emmanuel, U. C. (2021). Biological synthesis of copper nanoparticles and its antimicrobial potential on selected bacteria food-borne pathogens. *Ife Journal of Science*, *23*(1), 11-21.
- [25] Harishchandra, B. D., Pappuswamy, M., Antony, P. U., Shama, G., Pragatheesh, A., Arumugam, V. A., ... & Sundaram, R. (2020). copper nanoparticles: a review on synthesis, characterization and applications. *Asian Pacific journal of cancer biology*, *5*(4), 201-210.
- [26] Begletsova, N. N., Shinkarenko, O. A., Selifonova, E. I., Tsvetkova, O. Y., Al-Alwani, A. J. K., Zakharevich, A. M., ... & Glukhovskoy, E. G. (2019). Synthesis of copper nanoparticles using hydrazine in micellar solutions of sodium dodecyl sulfate. *Moscow University Chemistry Bulletin*, *74*, 14-19.
- [27] Nomoev, A. V., Khartaeva, E. C., Yumozhapova, N. V., Darmaev, T. G., Bardakhanov, S. P., Syzranthev, V. V., & Gafner, Y. Y. (2019). Receiving copper nanoparticles: Experiment and modelling. *Solid State Phenomena*, *288*, 140-147.
- [28] Wei, C., & Liu, Q. (2017). Shape-, size-, and density-tunable synthesis and optical properties of copper nanoparticles. *CrystEngComm*, *19*(24), 3254-3262.
- [29] Athira, K., Gurrala, L., & Kumar, D. V. R. (2021). Biosurfactant-mediated biosynthesis of CuO nanoparticles and their antimicrobial activity. *Applied Nanoscience*, *11*(4), 1447-1457.