Research and Reviews Journal of Agriculture and Allied Sciences

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

Antagonistic activity by microbial blended CuO NPs of endophytic actinomycetes isolated from Triticum vulgare against plant pathogens

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Research Article

Received date: 29/09/2021

Accepted date: 01/10/2021

Published date: 13/12/2021

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Keywords: *Triticum vulgare, Actinomycetes, Streptomyces, Pathogen, XRD, FT IR and Cu SO4 : 5H2O*

ABSTRACT

For the creation of nanoparticles, two endophytic actinomycetes were used, both of which belonged to the Streptomyces species. The 16SrRNA gene sequence was used to identify these species. Streptomyces noursei (A-1) and Streptomyces fradiae were identified as the two species (A-2). Cu oxide nanoparticles were made from biomass extracts of these species using the Cu SO4 : 5H2O method. UV visible spectroscopy, FTIR spectroscopy, X-ray Diffraction, and transmission electron microscopy were used to characterise the nanoparticles (TEM) Surface plamon resonance (SPR) absorption band in the region of 410 to 450 nm was measured in the biosynthesis of Cu oxide nanoparticles.

The Cu oxide nanoparticles produced via biosynthesis were spherical in shape and crystalline in structure. The size of Cu oxide nanoparticles produced via biosynthesis ranges from 50 to 100 nm. The crystalline nature of both A-1 and A-2 biosynthesized actinomycetal CuOnanaoparticles was revealed by XRD examination. The presence of a number of distinct bonds, including large C-O and N-H bonds, was determined using FTIR spectra.

When it came to medicinal applications, the biosynthesized CuO-NPs were remarkably efficacious against pathogenic microorganisms. These NPs were

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

more efficient against plant pathogenic fungi. They worked well against fungus.

INTRODUCTION

Now is the moment to conduct multidisciplinary study with the combination of many facts in the issue, as well as a need to look beyond the subject in order to protect human life. Because SARS CoV -19 has become a pandemic, researchers must concentrate their efforts on bio produced oxide nanoparticles and their therapeutic implications in society.

Nonotechnology is concerned with biological processes; for example, the employment of microorganisms for biosynthesis of bio nanoparticles is one of the tools in which microbial cells have proven to be highly effective. Although nanoparticles are useful, there is a need to develop nonopaticles, which are necessary in the sense that they are clean, simple to prepare, cost effective, and have a wide range of uses.

It is necessary to produce nanoparticles that are environmentally benign and do not have any negative effects. Nanoparticles must also be developed because they are time efficient. The application of nanoparticles in various fields is based on physic chemical features such as surface shape, surface area, size, zeta potential, and current charges on it. Various nanoparticles with metal oxides, such as gold, copper, silver, zinc, magnesium, and others, are now being examined and evaluated for their vast and diverse applications.The high surface to volume ratio of nanoparticles with their metal oxide is one of their most important qualities; as a result of these characteristics, nanoparticles are noble. [1] Copper oxide metal nanoparticles have demonstrated antibacterial [2,3], antioxidant [4], anticancer [5], antifungal [6], and larvicidal [7] properties in a variety of fields. Microorganisms are used to biosynthesise nanoparticles, and they are more successful than other nanoparticles due to their stability, low toxicity, lack of hazardous residue, environmental friendliness, and lack of side effects. [8]Most CuO- Nanoparticles are chemically synthesised using several ways such as alkoxide supported approach [9], sol gel method [10], electrochemical method [11], microwave irradiation method [12], and thermal decomposition method [13].

Chemical base nanoparticles have a high impurity content, which is their major disadvantage [14]. This might induce unfavourable effects and side effects during their applications.The green synthesis of nanoparticles is beneficial and beneficial over pure chemical base nanoparticles. Microorganisms such as bacteria, actinomycetes, fungus, algae, and plant species, as well as unicellular and multicellular cells, are used in the green synthesis of nanoparticles [15]. Green nanoparticles that have been produced are generally ideal for usage. As they are biosafety [16], these green produced nanoparticles are effective in a variety of sectors.

Actinomycetes are gram-positive bacteria found primarily in soil, air, and water. Soil is a great source of these microorganisms.

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p-ISSN: 2319-9857

The majority of these actinomycetes are utilised to make nanoparticles. Its own biomass or metabolites, such as protein enzymes and other actinomycetes' products, are used to make green nanoparticles that are employed to solve problems in several domains of nature.

Triticum vulgare is an economically important plant species that is extensively dispersed around the world, particularly in India. It is used to treat decubitus ulcers, sores, burns, scarring delays, dystrophic illnesses, and tissue regeneration problems, among other things. Triticum vulgare's leaves, roots, stems, and seeds are valued for their medicinal and nutritional properties.Triticum vulgare seeds provide nutritional benefits. Endophytic actinomycetes were isolated from Triticum vulgare, which was taken in the middle of winter from an agriculture area in the Sangli district of Maharashtra (India).

The study focuses on isolating endophytic actinomycetes from Triticum vulgare and using their extract for the production of CuO nanoparticles. Metabolites such as protein and enzyme present in extract generated by endophytic actinomycetes act as a capping and stabilizing agent during the manufacture of CuO nanoparticles[17].

The CuO nanoparticles that were biosynthesized were studied. Biosynthesized CuO nanoparticles were characterized using XRD, UV-Vis spectroscopy, TEM, and SEM.

The common plant pathogenic fungus are decreases the yield of crop plants. The plant pathogenic fungus are Didymella bryoniae causes Gummy stem blight disease affecting in watermelons, Septoria apiicola causes Late blight disease in Celery, also causes Bean Rust. Blast disease of rice disease caused by Pyricularia oryzae. The microbial blended CuO NPs of endophytic actinomycetes isolated from Triticum vulgare was used in this study for antimicrobial activity against these pathogenic plant fungi.

Material and Method

Fresh, newly growing stems of Triticum vulgare, a common wheat plant planted in a region of black soil near the edge of the Krishna river in Maharashtra, India, are available. Maxima's Hot Start PCR Master Mix (Thermo K1051, Sigma) was used to amplify 16 rRNA genes. Purification was done with the Gene Jet Kit (Thermo K0702), and the phylogenetic analysis was done with MEGA 6.0 software. CuSO4.5H2O (HiMedia) was used as a biosynthetic precursor for CuO nanoparticles.

Perkin Elmer Lambda 35 UV-VIS spectrophotometry was used, and a Varian 670 FTIR spectrometer was used for Fourier Transform Infrared [FTIR] spectroscopy.

For XRD, a Philips Analytic X-ray B.V. PW-3710 based model diffraction analysis utilising Cu-K radiation with a wavelength of 1.5418 was utilised, and for TEM, a JEOL JEM series JED-2300T transmission electron microscope was employed.

Plant sampling and study area.

Triticum vulgare was a widespread plant grown by farmers in India, especially in the Maharashtra region. The isolation of endophytic actinomycetes from such a Triticum vulgare was carried out.

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In the mid-winter season, Triticum vulgare was gathered from an agriculture field in the Sangli District (16.8530N. 74.5830E) in Maharashtra (India). For this investigation, the mature stem was chosen. The stem in Fig. 1 was sliced into small pieces ranging from 1 to 2 cm in length, collected in a sterile conical flask, and delivered to the Departmental Microbiology laboratory. The conical flask containing Triticum vulgare stems was then kept at 4 ° C to keep it cool.

The identification of the plants was accomplished using botanical nomenclature and classification. Species identification was done using the categorization process up to the genus and species level.

Isolation of endophytic actinomycetes

After that, the stem of Triticum vulgare was washed with sanitized and cooled tap water, which helped to remove the surface pollution on the stem. Surface sterilization was conducted out using a 1.5 to 3% hydrogen peroxide solution, which was effective in killing the remaining contaminants on the stem's surface. The stems were then immersed in a 70 percent ethanol solution for 15 seconds. It enables for surface sterilization of stems by washing them in sterilized distilled water for 5 minutes, then confirming surface sterilization by plating the washed distilled water on nutrient agar media. The absence of any growth of bacteria, fungus, or actinomycetes after four days at room temperature demonstrated that the plate had been properly sterilized.

The stems were crushed with a sterile needle and plated on glycerol aspargine agar media with griseofulvin 10 g/ml [antifungal pill] and incubated at room temperature for one to two weeks.

One of the signs of an actinomycetal colony is the colony's powdery appearance when embedded in media on a plate. This colony will be used for additional examination and will be retransferred on a slant for preservation. This is referred to as endophytic actinomycetes, and the endophytic actinomycetes used for nanoparticle production serve as a green supply during the preparation process.

Molecular identification of endophytic actinomycetes.

The isolated endophytic actinomycetes were separately merged in 0.08 % saline NaCl, during the treatment of saline they were purely washed by the normal saline water. The marmur extraction method was specifically used for the isolation of genetic material from endophytic actinomycetes. Isolated genetic material was then used for the identification of isolated endophytic actinomycetes.

16S-rRNA genes were used for the further analysis to identify the particular species of actinomycetal isolates. 16S-rRNA genes were amplified using Hot Start PCR Master

Mix of Maxima (Thermo K1051, Sigma) in PCR technique. Then amplification of isolated genetic material (16S-rRNA genes) was carried out with PCR technology in the amount of 50 µL. The universal primer should be used for the amplification and identification of isolates.

In PCR technique the reaction was carried out by using universal primers like 27f containing (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492r containing (5′-TAC GGC TAC CTT GTT ACG ACT-3′) [18]

The PCR product was then purified. using the Gene Jet Kit and then proper sequencing were carried out by DNA sequencers, in which forward and reverse primers were used for the sequencing.

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The specific sequence was observed by 16S-rRNAtechnology. Then were matched with the standard database by phylogenetic techniquewhich was depends on database and watched isolated sequence to closely related sequence of gene bank. MEGA 6 software was used for this analysis.

BiosynthesisofCuO nanoparticles

CuO nanoparticles were biosynthesised using Cu SO4 5H2O, which is a precursor and a good, readily available source for CuO nanoparticle biosynthesis. For the production of CuO nanoparticles, this reaction mixture was treated with separated actinomycetes' metabolites, specifically biomass filtrate of actinomycetes. During the procedure, actinomycetes biomass filtrate served as a reducing agent, and it played a key role in the biosynthesis of CuO nanoparticles.

To generate actinomycetal biomass, isolated actinomycetes were inoculated individually in 100 mL of starch nitrate broth and cultured at room temperature for 48 hours on a mechanical shaker rotating at 100 rpm. After the required incubation period had passed, the broth was filtered using filter paper to separate the filtrate from the biomass. For the next step, the actinomycetal biomass was employed. About 5 to 10 gm of actinomycetal biomass was washed with sterilised distilled water, which was helpful in freeing the surface media particle from the actinomycetal biomass.

After that, the washed biomass was suspended in sterilised distilled water and left at room temperature for another 48 hours, while actinomyctal metabolites percolated in the distilled water. Whatman No. 1 filter paper was used to separate the filtrate, which included actinomyctal metabolites, from the rest of the mixture. Because this filtrate acts as a reducing agent, it was collected and employed in the reduction process to produce CuO Nanoparticles biologically. In the procedure, 100 ml of separated actinomycetal filtrate was mixed with 25 mM Cu SO4 5H2O.This reaction mixture was kept at room temperature in the dark until a colour change occurred, at which point it turned dark.

Characterization of biosynthesized CuO nanoparticles.

The presence of biologically synthesisedCuO Nanoparticles was confirmed by a change in colour in dark appearance, which is the primary indicator of biologically synthesisedCuO Nanoparticles. UV-Vis spectrophotometry was used to confirm the biologically synthesisedCuO Nanoparticles, and the UV- Vis spectrophotometer revealed the firm biologically synthesisedCuO Nanoparticles. The wavelength range should be between 100 and 600 nm. Fourier Transmission (FT) infrared (IR) spectroscopy was used to investigate the presence of various functional groups in biologically produced CuO nanoparticles [17].

Transmission electron microscopy was used to investigate the form and size of biomass filtrate-mediated biosynthesized CuO nanoparticles (TEM). The true morphological size was displayed.

XRD Philips Analytic X-ray B.V. (PW-3710 Based Model diffraction study utilising Cu-Kg radiation) with a wavelength of 1.5418 nm, All of the methods were useful in determining the differences in their characterisation of various nanoparticles. [20]

Antimicrobial activity of biologically synthesized CuO-NPsandminimal inhibitory concentration (MIC)

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p-ISSN: 2319-9857

It is a sort of therapeutic analysis of NPs-prepared materials. CuO-NPs were biologically produced and tested against human pathogens, some of which were gram-positive and some gram-negative. The antimicrobial activity was evaluated against plant pathogen fungi such as Septoria apiicola (ATCC, 34705)Didymellabryoniae (ATCC, 200532) andPyriculariaoryzae (ATCC 66063).andThe zone of inhibition assessment was used to evaluate antibacterial activity. During the antimicrobial activity, CuSO4.5H2O was tested as a control.

The CuO-NPs' antibacterial properties were studied using the well diffusion method, which involved pouring pure cultures of fungal spore into molted sterilized potato dextrose agar media. All plates were given time to solidify. Wells of 0.7 mm diameter were made in aseptic conditions with a cork borer in seeded potato dextrose agar plate for eukaryotic cells.

The antimicrobial activity was measured using a concentration of 25 mM of biologically produced CuO-NPs. In each well, 100 µl of a 25 mM solution of biologically produced CuO-NPs was added. For diffusion purposes, all plates were incubated at a low temperature for 15 to 25 minutes. Plates were kept at 37˚C±2˚C for 24 h to 96 h in an incubator, and the diameter of the zone of inhibition for each culture of fungus around the well was measured. The measurements were taken in mm. The experiments were carried out in triplicate [17].

Result and Discussion

Isolation and Molecular identification of endophytic actinomycetes

The two strains of endophytic actinomycetes were isolated and identified from stem of *Triticum vulgare* of located in the region mentioned in study area were name as A-1 and A-2.

Fig . 1 The stem of *Triticum vulgare*

The identification of these stains A-1 and A-2 was done using 16S rRNA sequencing. The A-1 and A-2 strains were found to be grouped within the Streptomyces genus. In terms of A-1 and A-2 species, the clustered actinomycetal Streptomyces resembled Streptomyces noursei and Streptomyces fradiae by 92 percent and 97 percent, respectively.

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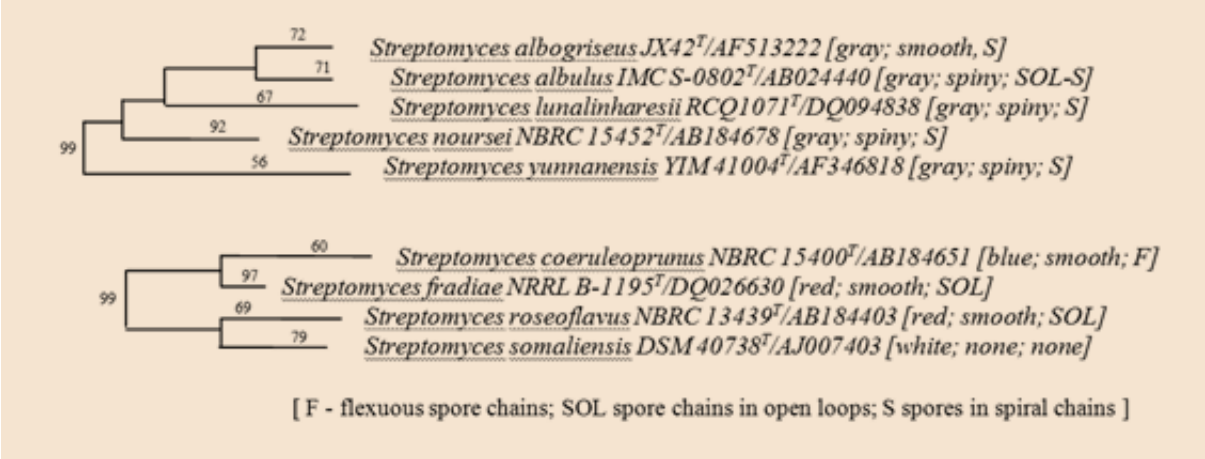

Fig. 2 Phylogenetic analysis of 16S rRNA sequences of isolated endophytic actinomycetes and matched sequence with the sequences from gene bank.

Isolated A-1 and A-2 were subsequently recognised to the genus and species level, with A-1 being named Streptomyces noursei and A-2 being named Streptomyces fradiae. Streptomyces noursei and Streptomyces fradiae ribosomal RNA sequences were deposited in Gene Bank [20, 21], with their association numbers listed in this paper.

The identification of actinomycetes was carried out by V.V. Chougule and A.M. Deshmukh [22] with the help of amino acid, sugar pattern and different biochemical tests matched with bergey's manual.

Biosynthesis of CuO nanoparticles

Biosynthesis of CuOnanoparticleswere carried out by using $CuSO_45H_2O$, this is one of precursor and suitable, easily available source for the biosynthesis of CuO nanoparticles. Examine the biomass filtrate protein and enzyme of the isolated and identified strains A-1 and A-2, which act as the catalyst for the green synthesis of CuO Nanoparticles. In which Cu^{2+}ions under goes reduction process to form CuO Nanoparticles [1] the yielding capacity of biosynthesis of CuO Nanoparticles was tested by the change in color of biomass filtrate from faint blue to dark greenish brown shade.

Also the biosynthesized CuO Nanoparticles was tested by UV- vis spectrophotometer analysis for its confirmation. The bio synthesis of Cu oxide nanoparticles showed maximum absorption band at 415 nm and 430 nm for A-1 and A-2 respectively. Shown in figure No. 3 band at 415 nm and 430 nm for A-1 and A-2 respectively. Shown in figure No. 3

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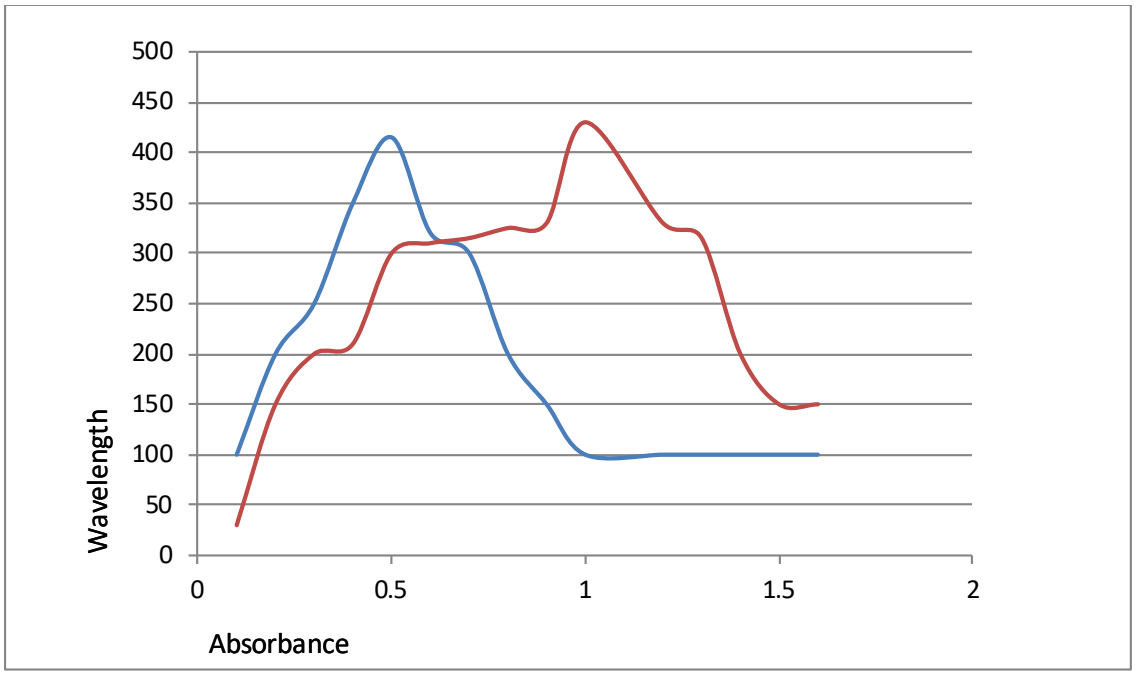

Fig. 3 Absorbance peak of A-1, A-2 in UV- vis spectroscopy

It was previously reported that the change in colour indicated the reduction of metal ions in the reaction mixture during the reaction, indicating the formation of CuO nanoparticles [23].

According to Krithiga and co-authors, the change in colour in the reaction mixture during the biosynthesis of CuO nanoparticles was caused by the Surface Plasma Resonance (SPR) phenomenon achieved by biomass filtrate containing metabolites [24]. Naila and Kannabiran investigated the maximal abosorption peak of biosynthesized CuO nanoparticles by actinomycetes, which was found to be between 380 and 450 nm [25].

Sathiyavimal and his co-authors used UV-vis spectroscopy to validate the biogenesis of CuO nanoparticles at a wavelength of 337 nm in one investigation [26]. The emergence of a single peak in UV–vis spectroscopy showed the morphology of the biosynthesized CuO nanoparticles; the presence of spherical shaped nanoparticles was indicated by the appearance of a single peak [27].

Characterization study ofbiosynthesizedCuOnanaoparticles

XRD

Figure 4 shows the XRD patterns of calcined biosynthesized actinomycetal CuOnanaoparticles. In the standard data, all of the principal peaks are indexed as spinel biogenesis of CuO nanoparticles (JCPD No: 88-1935).The average crystallite sizes of biosynthesized actinomycetalCuOnanaoparticles samples were calculated from X-ray line broadening of the reflections of (222), (313), (401), (509), and (437) using Scherrer's equation (i.e., $D = 0.89k/(\beta \cos\theta)$, where k is the wavelength of the X-ray radiation, K is a constant taken as 0.89, h the diffraction angle, and b is the full width at half-maximum [28-31] , and were found to be 16 ± 4, 18 ± 1, 25 ± 2, and 26 ± 3 nm for the samples of biosynthesized actinomycetalCuOnanaoparticles calcined at 500, 600, and 7000C, respectively.XRD

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results of both A-1 and A-2 biosynthesized actinomycetalCuOnanaoparticles were same. XRD analysis shown that both A-1 and A-2 biosynthesized actinomycetalCuOnanaoparticles were crystalline nature.

Same type of rsults found for endophytic actinomycetal mediated biosynthesized copperoxide nanoparticles, where XRD analysis shown and found the crystalline nature of NPs [17]

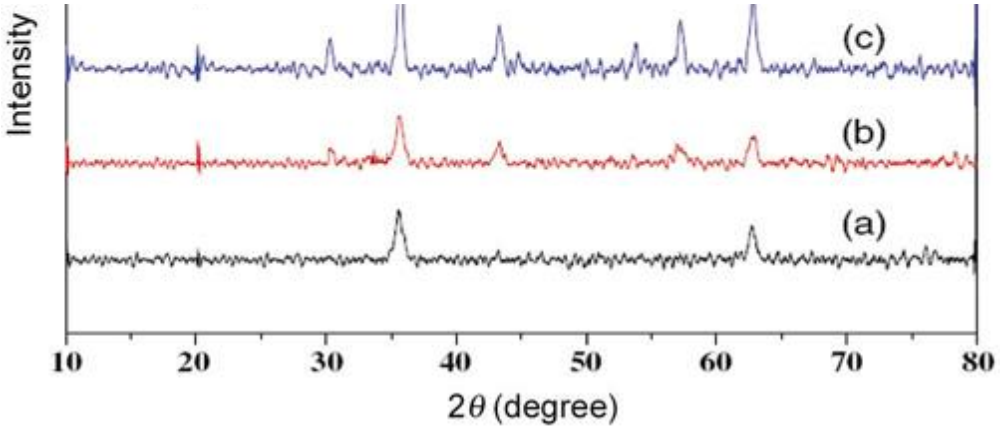

Fig. 4 XRD pattern of calcined actinomycetal CuO nanoparticles in air to: (a) 500 °C, (b) 600 °C, and (c) 700 °C.

TEM study

The morphology and size of biosynthesizedactinomycetal CuOnanoparticlesweredeteremined by TEM measurement. The comprehensive morphology and crystalline structure of two biosynthesized actinomycetal CuO nanoparticles samples, calcined at 600° C for 4 h were further studied using TEM. The TEM bright-field images with matching selected-area electron diffraction (SAED) patterns of the samples are shown in Figure 3. From the TEM bright field images, it was clearly seen that these two samples consist of packed biosynthesized actinomycetal CuO nanoparticles were crystallite particles of ~ 75 and 80 nmdiameter for the sample A-1 and A-2 respectively and particle size of these two samples of biosynthesized actinomycetal CuO nanoparticles was seen to be uniform and spherical shapedwere arranged in groups .

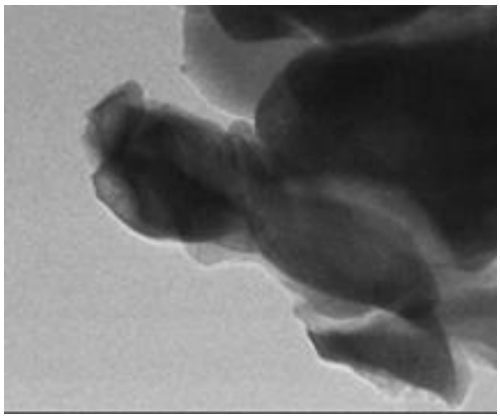

Fig.5 TEM images with corresponding SAED patterns of the biosynthesized actinomycetal CuO nanoparticles calcined in air for 4 h.

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Other study revealed and matched, where biologically synthesized CuO nanoparticles shown near about 61.7 nm, these CuO nanoparticles were synthesized using soil acinomycetes [32]

The study shown average size ofsynthesized biological CuO nanoparticles was near to 78nm and 80 nm for the two biological CuO nanoparticles synthesized from different species of Streptomyces, those they were endophytic actinomycetes [17].

FT- IR

FT – IR analysis was carried out for detection of functional groups which were involved in the biosynthesized actinomycetal CuO nanoparticles, where these involved functional groups were responsible for mechanisms of reduction, capping with the stabilization of synthesized biological CuO nanoparticles. [17] FT – IR analysis were achieved by FT - IR spectrum which was shown that the actinomycetal metabolite used in the preparation of biological CuO nanoparticles containing the various functional groups in the form of chemical bonds.

FT – IR analysis was carried out for the actinomycetal filtrate and actinomycetal CuO nanoparticles. FT – IR analysis spectrum of biomass filtrate ofStreptomycesnoursei (A-1) and Streptomyces fradiae(A-2) were analyzed and the maximum peak was at 1710 cm-1 for Streptomycesnoursei (A-1) attributed that presence of C-O bond and maximum peak was at 3430 cm-1 for Streptomyces fradiae(A-2) attributes appearance of N-H bond shown in figure No. 6

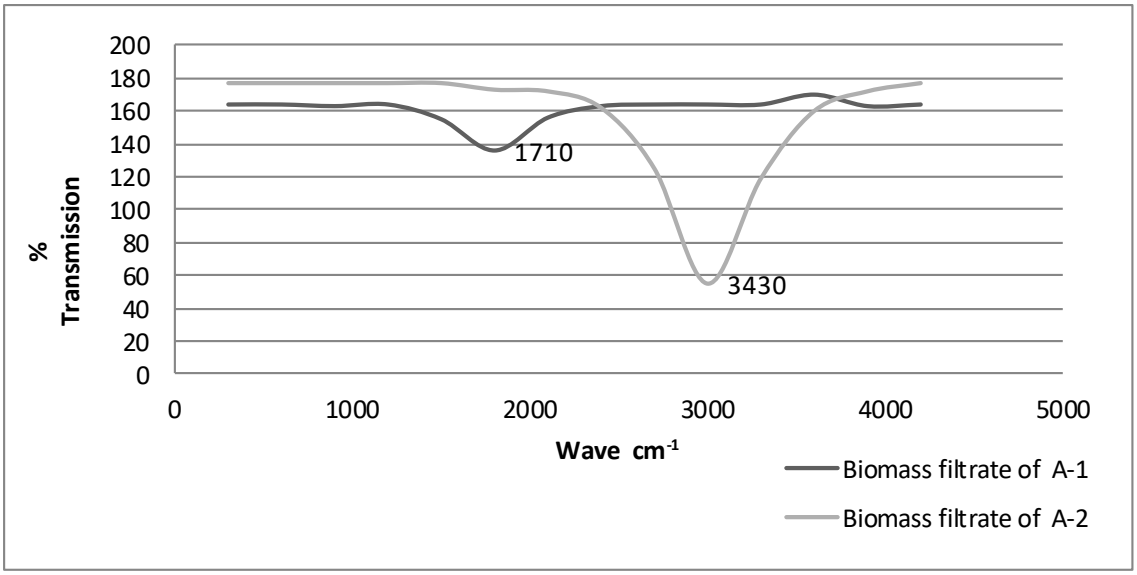

Fig 6 FT – IR spectrum of biomass filtrate of Streptomycesnoursei (A-1) and Streptomyces fradiae(A-2)

FT – IR analysis was carried out for the prepared actinomycetal CuO nanoparticles using Streptomycesnoursei (A-1) and Streptomyces fradiae(A-2). FT – IR analysis spectrum of these actinomycetalCuO nanoparticles was carried out in the range of 300 to 5000 cm-1 and shown that,

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The appeared peaks at 785.90 cm-1 by CuO nanoparticles of A-1 indicated that the related with (alkene) C=C. While the strong and wide peak at 3510. 67 cm-1 for the sample CuO nanoparticles of A-2 corresponds to alcoholic O-H bond. [33,34,35,36] The weak peak was appear at 2343.69 cm-1 for sample CuO nanoparticles of A-1 might be revels to the vibrations of atmospheric CO2 [37]. Different dominant peaks at 478.28 cm-1 for sample CuO nanoparticles of A-1 and 560.36 cm-1 for the sample CuO nanoparticles of A-2 might be recognized for the formation and presence of metal oxides as Cu-O vibrations., this was confirmed that the formation of highly pure CuO nanoparticles in the study [37]., shown in Figure No. 7

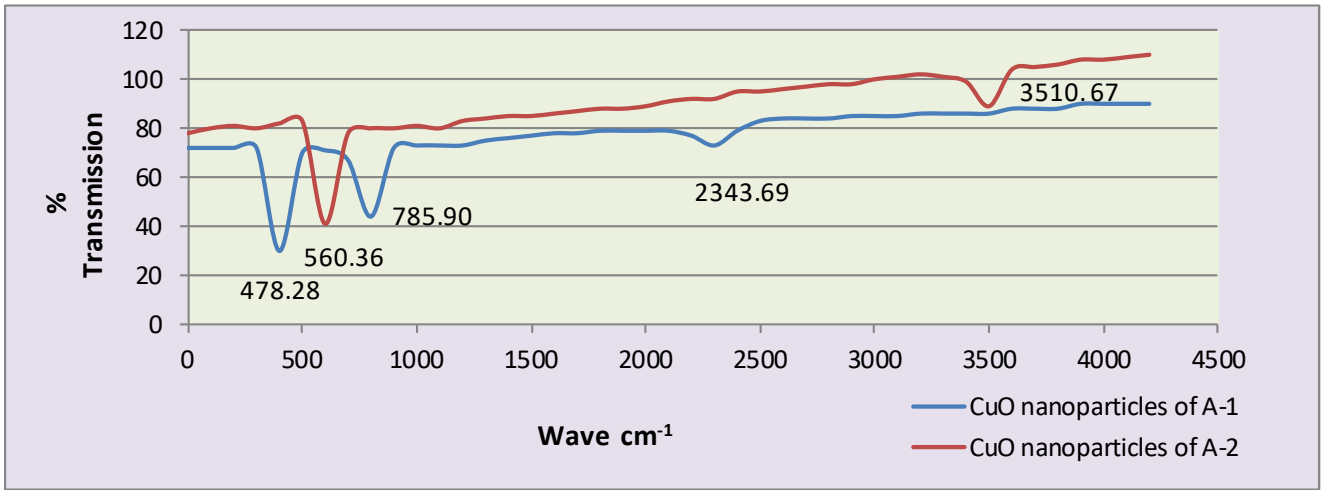

Fig. 7 FT – IR spectrum of actinomycetal CuO nanoparticles using Streptomyces noursei (A-1) and Streptomyces fradiae (A-2)

Antimicrobial activity of biologically synthesized CuO-NPs

Antimicrobial activity was measured using 5 to 25 mM concentrations of biologically produced CuO-NPs, employing the well diffusion method as seen in methods. The results were recorded with respect to zone of inhibition and triplicate. Plant fungal pathogens were identified to be more sensitive to CuO-NPs of both samples, i.e., A-1 and A-2,. The fungus was found to be highly sensitive to both samples of CuO-NPs, i.e., A-1 and A-2. The antimicrobial activity of biologically produced CuO-NPs of A-1 and A-2 was significantly different from the control (CuSO4.5H2O) at a concentration of 25 mM.

According to figure 8, the antimicrobial activity of biologically produced CuO-NPs of A-1 against Didymellabryoniae (ATCC, 200532) was higher than that of A-2. The diameters of the inhibition zones for CuO-NPs of A-1 and A-2 at 10 mM were 11.1 mm and 9.3 mm, respectively.

In comparison to A-2, the antimicrobial activity of biologically produced CuO-NPs of A-1 was higher against Septoria apiicola (ATCC, 34705), as shown in figure 9. The diameters of the zone of inhibitions for CuO NPs of A-1 and A-2 at 10mM were 10.4 mm and 9.8 mm, respectively.

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Figure 10 reveals that the antimicrobial potential of biologically produced CuO-NPs of A-2 was higher than that of A-1 against *Pyriculariaoryzae* (ATCC 66063) at 15 mM, with the diameters of the inhibition zones for CuO NPs of A-1 and A-2 being 6.7 mm and 9.4 mm, respectively.

Biologically produced CuO-NPs (A-1 and A-2) were of pathogens and could be used as a step forward in therapeutic applications. Copper, as a metal, may have an oligo-dynamic effect on bacteria [38]. Copper's antibacterial properties have been recognized and certified by the US Environmental Protection Agency (EPA) [39]. Furthermore, this study anticipated that endophyticactinomycetes' biologically produced CuO-NPs (A-1 and A-2) are more effective than Cu ions. CuO-NPs suppress microbial cells through a method in which the nanoparticles' tiny Size and high volume surface area enable them to interact with the cell membrane and resulting in inhibition [40]. According to some research, CuO-NPs produce hydroxyl molecules/radicals that bind to DNA and destroy the helical structure of DNA; moreover, these radicals attach to the sulfhydryl amino group and the carboxyl group of amino acids found in essential proteins and enzymes, resulting in inactivated essential enzymes [41]. These CuO-NPs are also implicated in the microbial cell penetration system. CuO-NPs inactivate the surface protein on the cell responsible for critical transport across the cell membrane, effectively stopping microbial cell permeability [42].Alternariaspp., Aspergillusniger, and Pythiumspp. are all inhibited by green-produced Cu-NPs. Green synthesized Cu-NPs inhibit Alternaria spp. by 57.1 %, Aspergillusniger by 63.8 %, and Pythium spp. by 58.1 % [6].tested for antibacterial efficacy against chosen pathogens. It was shown that biologically generated CuO-NPs (A-1 and A-2) by endophyticactinomycetes are effective against a variety

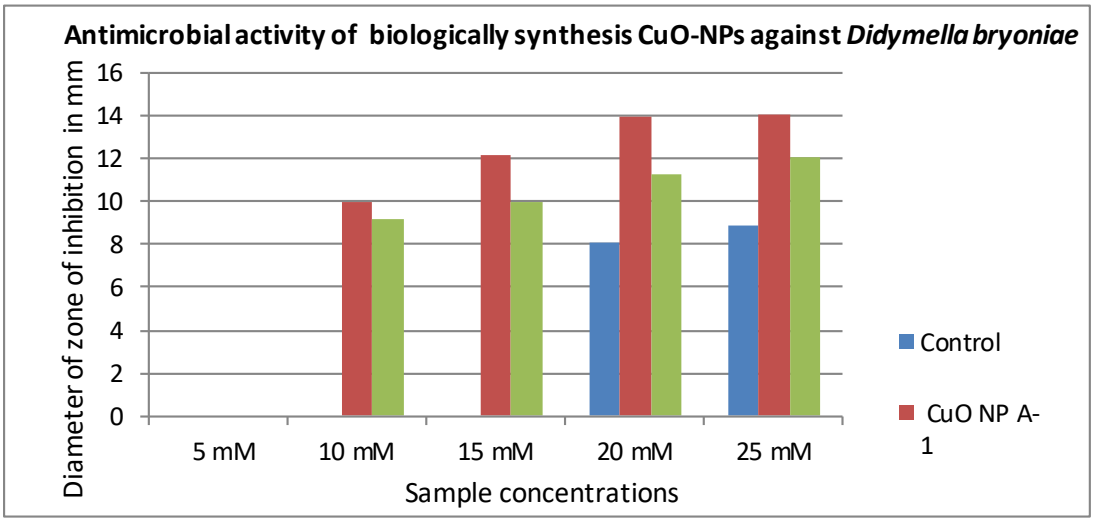

Fig.8 Antimicrobial potential of biologically synthesized CuO-NPs against *Didymellabryoniae* using a control

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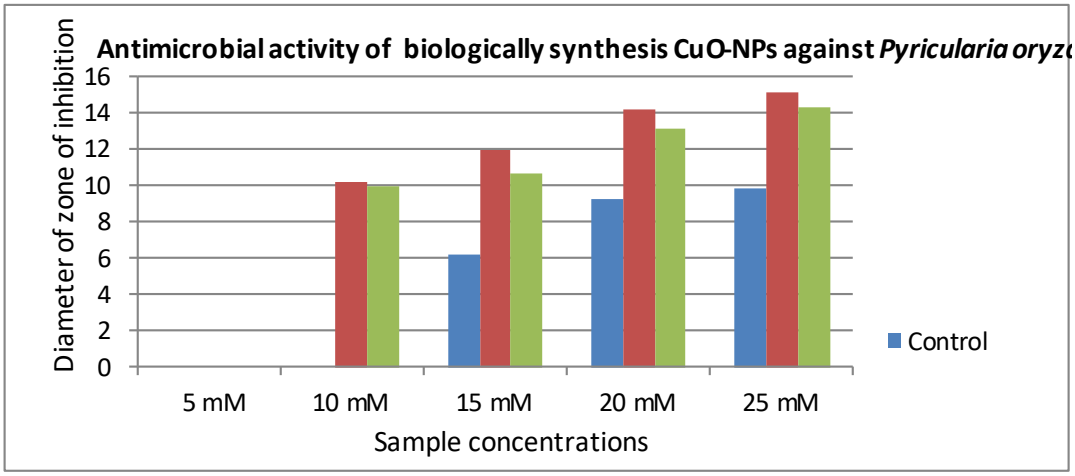

Fig. 9 Antimicrobialpotentialof biologically synthesis CuO-NPs against *Pyriculariaoryzae* using a control

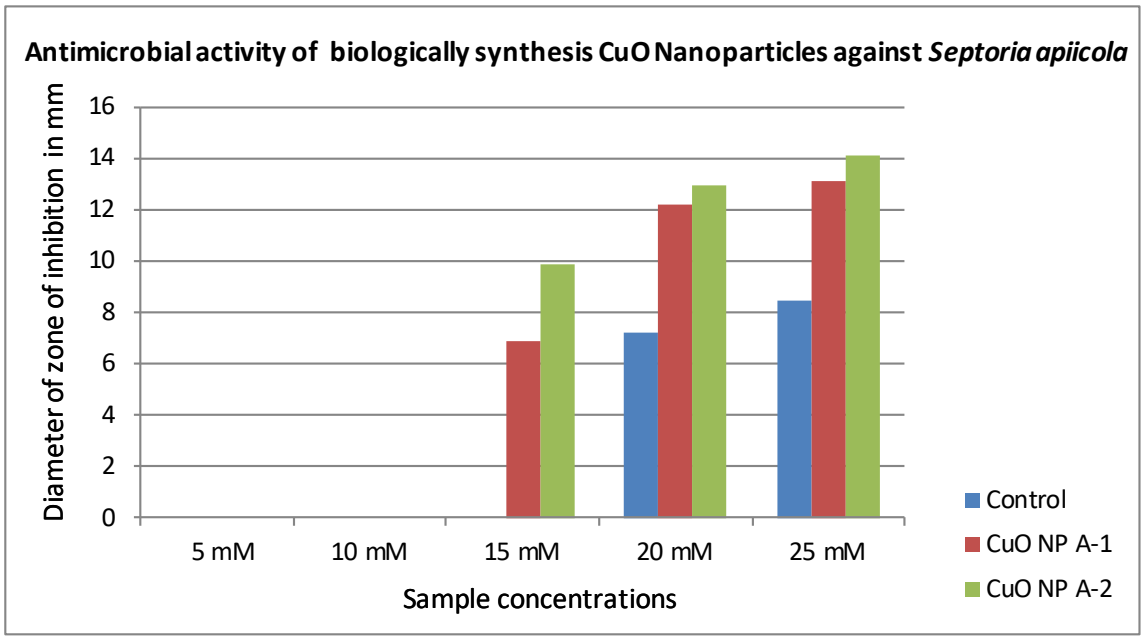

Fig. 10 Antimicrobial potential of biologically synthesized CuO-NPs against *Septoria apiicola* using a control

Conclusion

The work presented first was the endophytic actinomycetal driven synthesis of CuO nanoparticles utilising Streptomyces noursei and Streptomyces fradiae.

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Streptomyces noursei (A-1) and Streptomyces fradiae (A-2) biosynthesized CuO nanoparticles were validated by changing the hue from faint blue to dark greenish brown and obtaining an absorption band at 415 nm and 430 nm for A-1 and A-2, respectively.

XRD confirmed the crystal structure. Particle size was between 75 and 80 nm in diameter, and they were homogenous and spherical, according to TEM investigations. Both Biosynthesized CuO (A-1) and (A-2), which were evaluated by FT-IR, had a number of function groups.

Endophyticactinomycetes generated biogenic CuO-NPs (A-1 and A-2) are effective against a variety of fungal pathogens. All fungus are more sensitive to both types of CuO-NPs (A-1) and (A-2).The present study hence offers a novel and safe CuO-NPs multifunction as a possible means for diverse therapeutic applications.

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