Research Article

Biosynthesis of Zinc Oxide Nanoparticles from Soil Fungi and their Characterization by Using Analytical Methods

Amitha Ganapathy Bottangada, Akarsh Subhakar, Sowmya Hirakannavar Veeranna, Thippeswamy Basaiah*

Department of Microbiology, Bioscience Complex, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shivamogga, Karnataka, INDIA.

Submission Date: 21-06-2023; Revision Date: 14-08-2023; Accepted Date: 12-09-2023.

ABSTRACT

Aims: The main aim of the study is to synthesize the zinc oxide nanoparticles from fungal isolates of soil and characterize the physicochemical properties of synthesized ZnO NPs for agricultural purposes. **Materials and Methods:** The soil samples have been collected, isolated, and examined for the solubilization of zinc. The promising isolate was molecular characterized and deposited in NCBI GenBank. This isolate was then utilized to synthesize and characterize ZnO nanoparticles. The synthesized ZnO NPs were characterized using UV-vis DRS, UV-vis spec, DLS, XRD, FT-IR, and SEM-EDAX for confirmation and quantification. **Results:** In the 14 fungal isolates, Aspergillus fumigatus (Db5) has shown the highest zinc solubilization index of 2.75mm. The fungal culture filtrate displayed an absorption peak at 315 nm in UV-visible spectrum analysis whereas, in UV-vis DRS, it showed 330nm with a band gap found to be ~3.1 eV, which indicated the presence of zinc oxide. The synthesized ZnO-NPs measure 27.35 nm in particle size and have a zeta potential of -2.35 mV. The average size of the ZnO NPs was found to be 23nm through XRD. The functional hydroxyl (O-H) group of the ZnO NP showed an intense band near 3447.16 cm^{-1} through FT-IR analysis. In SEM studies, the morphology of ZnO NPs was found to be irregular, spherical, and granule-like structures. **Conclusion:** All the studies indicate that the fungal isolate *Aspergillus fumigatus* (Db5) can synthesize ZnO NPs which can be further tested for crop protection and production as an eco-friendly approach.

Keywords: *Aspergillus fumigatus*, Analytical techniques, Biosynthesis, Eco-friendly, Zinc oxide nanoparticles.

Correspondence:
Dr. Thippeswamy Basaiah,
P.G. Studies and Research, Department of Microbiology, Bioscience Complex, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shivamogga-577451, Karnataka, INDIA.

Email: thippeswamyb272@yahoo.in; thippeswamyb205@gmail.com

INTRODUCTION

Nanotechnology has evolved as a technology that has changed every sector of applied science during the past decade. The field of Nanoparticles (NPs) is one of the paths to nanotechnology that involves nano-scale materials with extremely tiny particles of various sizes from 1 to 100 nm. Because of their tiny size and high surface area to volume ratio, Nanoparticles (NPs) have unique features compared to those of their bulk counterparts[1] Due to their physiochemical features, metal oxide NPs have been extensively studied for their potential biological applications. Due to their high chemical and thermal endurance, even under demanding processing circumstances, Zinc Oxide Nanoparticles (ZnO NPs) have attracted a lot of attention. ZnO NPs are used in several cutting-edge industries, including sensors, cosmetics, environmental protection, and biological and pharmaceutical ones.[2] ZnO NPs have attracted the attention of many researchers all around the world. Its biological and chemical behaviour allows us to reconstruct morphology. ZnO NPs have been used for a range of biological applications, such as UV

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2023.12.36

filtering, medication transport, tumour cell eradication,[3] antibacterial and antifungal activities, and the death of tumour cells.[4] There are numerous ways the produce ZnO NPs, including chemical vapour synthesis,[5] the sol-gel approach.[6] Sono-chemical and thermal breakdown.[7] Processing steps and physical factors such as pH, pressure, and temperature are difficult to manage in addition to releasing by-products that may be detrimental to the ecosystem; thus, green synthesis of ZnO NPs is favoured. Fungi are simple to manipulate and contain a diverse array of proteins and enzymes in their cells, making them great choices for ZnO NPs synthesis.

In the current study, we aimed to isolate and screen the fungi using two different soil samples. Further, the soil samples were isolated and screened for the identification of fungi. *Aspergillus fumigatus* (Db5) were utilized in the biosynthesis of ZnO NPs. for a broad range of application process. Analysis using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) provided comprehensive descriptions of the NPs. Despite this, only a few microorganisms are capable of producing ZnO NPs. So, other microorganisms with the capacity to synthesize ZnO NPs must be investigated. As a result, this research was conducted to identify a new fungal strain capable of synthesizing ZnO NPs and to analyze its characteristics.

MATERIALS AND METHODS

Collection of soil samples and Isolation

Soil samples were collected at 10 - 15 cm depth, where most of the microbial activity is present. Soil samples of approximately 500g were collected in sterile polythene bags, labelled and stored at 4°C until used. By using the serial dilution approach, fungi were isolated from collected soil samples. Each soil sample was serially diluted after being dissolved in 9 mL of sterilized distilled water. 0.1 mL of inoculum was pipetted from dilution 10^{-3} to 10^{-5} and plated on Potato dextrose agar amended with 0.03% of streptomycin sulphate to control bacterial growth. The plates were incubated at 28°C for 5 to 7 days in the incubator. The incubated plates were observed for the growth of different fungi.[8]

Screening of isolated fungi for Zinc solubilizing capacity

The solubilization potential of isolates were checked on zinc solubilizing agar medium

(Dextrose 10.0 g, Dipotassium hydrogenphosphate 0.1g, Potassium chloride 0.2g, Ammonium sulphate 1.0g, Magnesium sulphate 0.20g, Agar 15.0 g, Zinc oxide 1.0 g and 1000 mL distilled water).[9] To observe a clear halo zone, isolates were inoculated on agar media and incubated at 30 ± 1°C for seven days. The solubilization index was calculated by measuring the colony diameter and the halo zone diameter using the following formula.[10]

$$SI = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

SI: Solubilization Index

Molecular characterization of isolated fungi

By using 18s rRNA sequencing, isolated fungi were molecularly characterized. The CTAB method was used to extract fungal DNA, and a UV-vis spectrophotometer was used to quantify the amount of DNA extracted. The extracted DNA was subjected to PCR using ITS primers (ITS-1 and ITS-4). The sequences gathered from the PCR samples were put through the BioEdit program and Blast analysis. After that, the sequence was sent to Gen Bank, NCBI, in order to get an accession number.

Biosynthesis and Purification of ZnO NPs

In a flask with 1000 mL of modified Malt extract Glucose Yeast extract Peptone (MGYP) medium containing Malt extract 3.0 g, Sucrose 10.0 g, Yeast extract 3.0 g, and Peptone 5.0 g-metal tolerance fungi were cultivated. The flasks were sterilized and administered with 1 mL of spore suspension at a concentration of 10-6 conidia produced from 7-day-old cultures, and the pH of the medium was adjusted to 6.8. The flasks underwent a 72-hour incubation in a rotary shaker at 30°C and 150 rpm. The biomass was removed from the culture broth after 72 hr by centrifuging it at 4000 rpm, 4°C for 10 min, and then washing it with sterile distilled water to get rid of any components. The recovered 15 g (wet weight) of fungal biomass was suspended in 100 mL of distilled H_2O and kept shaking once more for 62 hr at 28°C and 150 rpm. After the incubation period, Whatman no. 1 filter paper was used to separate the biomass of each fungus.[11] The cell-free filtrate that had been collected was used to biosynthesize ZnO NPs. The culture filtrate was given 1 mM zinc nitrate, and it was shaken for 48 hr at 28°C at 150 rpm. When the mixture was converted to a hazy, white precipitation, ZnO NPs were formed. For 5 minutes, the white mixture was centrifuged at 18,000 rpm.[12] After being washed with alcohol and three times with distilled H_2O, precipitates were dried at 70°C for 4 hr. The ZnO NPs were then crushed into a fine powder and calcined at 500°C for 1 hr.[13] For upcoming research, the fine powder was

gathered and kept in an airtight glass vial at room temperature.

Characterization of Biosynthesized ZnO NPs

Ultraviolet-visible spectrophotometer and UV-vis Diffuse Reflectance Spectra (DRS) analysis

The band gap energy spectra of synthesised ZnO NPs powders were obtained by UV-vis diffuse reflectance spectra (UV-vis, Shimadzu 2450) in the range of 200 to 900 nm by using the reference material Barium Sulphate ($BaSO_4$), at a scanning speed of 100 nm/min. The optical properties of Zinc Oxide nanoparticles were characterised by UV-vis Spectroscopy (Systronics).

Particle size and zeta potential measurements

Utilising a particle size analyzer (Zetasizer, Malvern, nano 383, England), zeta potential and dynamic light scattering were assessed to estimate the size and surface charge of the nanoparticles. The cuvette containing the nanoparticle solution was filled to the top ¾th of its volume before being set within the zeta sizer's dynamic light scattering chamber. The experiment was performed with a scattering angle of 90° and a temperature of 25°C. ZnO NPs dispersion and refractive index were set at 1.365 and 1.330 (viscosity 0.8872 Cp), respectively.[14] The average particle diameter (d. nm) was recorded for all ZnO Nps from the intensity graph by using Malvern software (Version 7.12).[15]

Phase identification of zinc oxide nanoparticles using an X-ray Diffractometer

X-ray Diffractometer (XRD) measurements were made on synthesised and standard ZnO NPs (M/s. Rigaku, Ultima 4, Tokyo, Japan). The collected data were compared to approved standard data made available by the Joint Committee on Powder Diffraction Standards (JCPDS Card No. 36-1451). The average particle size of ZnO NPs was calculated from the XRD pattern using Debye-Scherrer's formula.[16]

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

Where 'θ' is the Bragg angle from 2θ value of intensity peak from the XRD pattern, 'D' is the crystallite size of the ZnO NPs, 'k' shape factor (0.9), 'λ' wavelength of X-ray (1.54 Å) Cu Kα radiation, and 'β' is the full-width half maximum of the diffraction from XRD pattern.

Functional group analysis

The functional group in the synthesized ZnO NPs was analyzed by using an FT-IR (IRTracer 100 AH, Shimadzu, Japan). Using a hydraulic press to combine ZnO NPs and potassium bromide KBr, the sample was created. The moisture content was then removed before drying. The instrument was initialized with Lab Solution software (Version, 100) and infrared spectra were recorded in the range of 350-7800 cm^{-1} at a resolution of 4 cm^{-1}.

Surface morphology and elemental detection of synthesized ZnO NPs

The surface morphology and elemental detection of synthesized ZnO NPs were studied using a Scanning Electron Microscope (SEM) with an Element Detection Sensor (EDS) Oxford instruments X-MaxN 80, United Kingdom (Carl Zeiss, EVO 10, Germany).

RESULTS AND DISCUSSION

Collection of soil samples for isolation of fungi

A total of 14 fungi were obtained from Doddaballapura agriculture field and forest soil samples from Shankaraghatta. After purification in fresh PDA media, each strain was given a unique code ranging from Sg1 to Sg9 and Db1 to Db5 to facilitate further study (as shown in Table 1 and Figure 1). These fungal strains were then identified based on their morphological characteristics and microscopic observation, using a standard manual.[8]

Screening of isolated Fungi for Zinc solubilizing capacity

Zinc solubilizing

Zinc solubilizing potential varied with different zinc solubilizing isolates (as shown in Table 2 and Figure 2). The 14 fungal isolates were examined for solubilization

Table 1: Isolation of fungi from the soil sample.

Sample site	Isolate Code	Name of Organism
Shankaraghatta	Sg1	*Fusarium* sp.
Shankaraghatta	Sg2	*Penicillium* sp.
Shankaraghatta	Sg3	*Penicillium* sp
Shankaraghatta	Sg4	*Penicillium* sp.
Shankaraghatta	Sg5	*Fusarium* sp.
Shankaraghatta	Sg6	*Fusarium* sp.
Shankaraghatta	Sg7	*Penicillium* sp.
Shankaraghatta	Sg8	*Penicillium* sp.
Shankaraghatta	Sg9	*Aspergillus* sp.
Doddaballapura	Db1	*Aspergillus* sp.
Doddaballapura	Db2	*Aspergillus* sp.
Doddaballapura	Db3	*Fusarium* sp.
Doddaballapura	Db4	*Fusarium* sp.
Doddaballapura	Db5	*Aspergillus* sp.

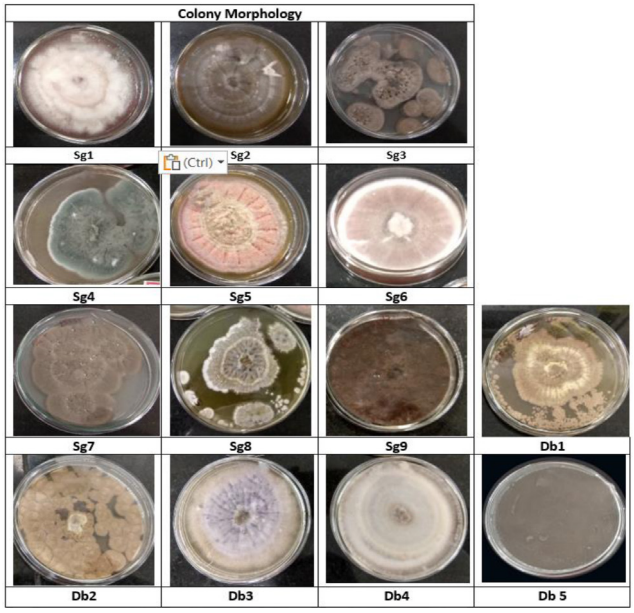

Figure 1: Different fungal species isolated from soil samples.

Table 2: Screening of isolated fungi for Zinc solubilization Index.

Code	Zone Diameter (mm)		
	Solubilization Zone	Colony Diameter	Zinc solubilization Index (ZSI)
Sg1	48	40	2.20
Sg2	30	26	2.15
Sg3	21	20	2.05
Sg4	25	23	2.09
Sg5	35	34	2.03
Sg6	23	20	2.15
Sg7	23	20	2.15
Sg8	36	22	2.64
Sg9	60	45	2.33
Db1	31	20	2.55
Db2	23	22	2.05
Db3	40	30	2.33
Db4	40	25	2.60
Db5	70	40	2.75

to determine their capacity to solubilize insoluble zinc oxide (ZnO), and the results ranged from 21 mm to 70 mm. Strain Db5 demonstrated the highest solubilization with a zone of 70 mm, while Sg3 showed the lowest with a zone of 21 mm.

Db5 fungal isolate showed a high zone of zinc solubilization index of 2.75mm and the rest of the isolates showed in the range of 2.03-2.64mm. This data suggests that all the fungal strains are capable of solubilizing ZnO, but strain Db5 had significantly

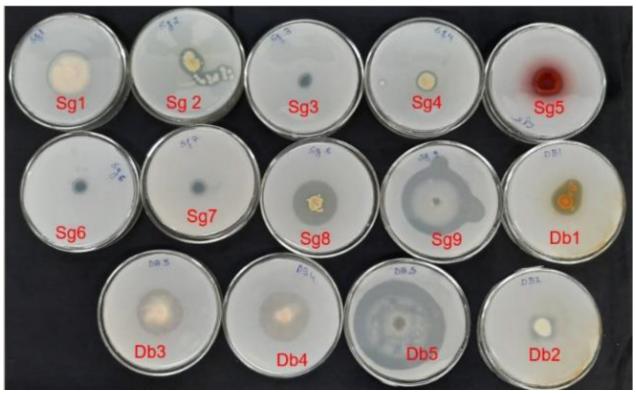

Figure 2: Zinc solubilization of fungal isolates on media plates after 7 days of incubation.

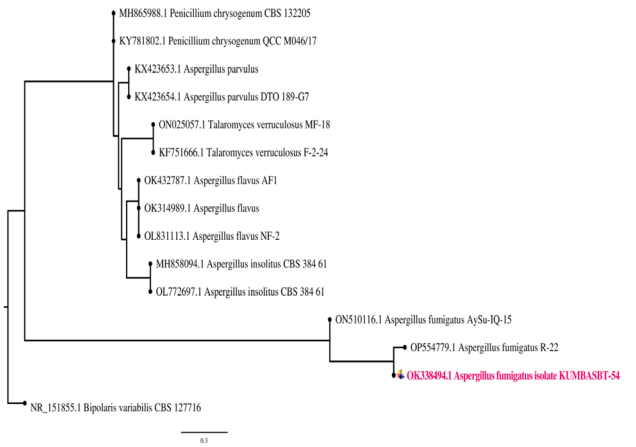

Figure 3: Phylogenetic tree of isolate Db5.

higher solubilization potential. Microorganisms that can solubilize zinc possess enzyme complexes that aid in zinc metabolism.[17] Consequently, the solubilization of zinc can be used as a primary selection mechanism for microorganisms with the potential for nanoparticle synthesis.

Molecular characterization of the selected fungi

The ITS sequence of Db5 was deposited in the NCBI GenBank under accession no. OK338494. The similarities between the ITS nucleotide sequence of Db5, with the *Aspergillus fumigatus* isolate MEBP0065 sequences obtained in the GenBank database were shown a similarity of 96.89% (Figure 3).

Characterization of Biosynthesized ZnO NPs

Ultraviolet-visible spectrophotometer and UV-vis diffuse reflectance spectra analysis

The production of ZnO NPs was verified by UV-visible spectroscopy. Zinc nitrate, the starting material, is transformed into ZnO NPs, as shown by the absorption spectra of the synthesised ZnO NPs in Figure 4 (a). The

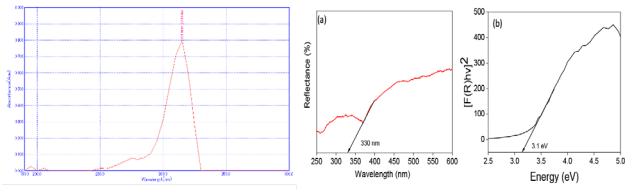

Figure 4 (a) **Figure 4 (b)** **Figure 4 (c)**

Figure 4: Characterization of synthesised zinc oxide nano particles (a) UV-visible spectra (b) UV-visible diffuse reflectance spectrum, (c) energy band gap of Db5 nanoparticles.

absorption peak at wavelengths of 315 nm, which is attributed to the intrinsic band-gap of Zn-O absorption, confirms the existence of ZnO NPs in culture filtrate. The maximum peaks of ZnO NPs at 300 and 359 nm.[18] The obtained ZnO NPs absorption peaks are in good accordance with those published in earlier studies, where the absorbance peak is recorded between 310 nm and 360 nm of wavelength.[19] According to the data, the absorption peak appeared to be identical to the absorption band.[20] Our result also agrees with the study[19] that examined the UV spectrum range of ZnO NPs as 310-360 nm which is a characteristic feature of pure ZnO NPs. Additionally, it has been noted that there is a correlation between nanoparticle particle size and the UV-visible spectrum's absorption peak intensity. The absorption peak changes towards a lower wavelength as particle size decreases.

UV-visible DRS is used for the determination of the optical bandgap of materials, which is depicted in Figure 4 (b) and whose maximum reflectance occurs at a wavelength of 330 nm. The absorption band of the produced Db5 sample from DRS was determined using the Kubelka-Munk (K-M) theory. The $[F(R)E]^{1/2}$ intercept plot against photon energy delivery bandgap. Kubelka-Munk equation is found in equation (2).

$$F(R_\infty) = 1 - R_\infty / 2R_\infty$$

Where R_∞ is the reflection coefficient. The band gap of ZnO NP's is found to be ~3.1 eV (Figure 4 (c). This is comparable with the energy bandgap values for ZnO NPs that have previously been published.

Particle size and zeta potential measurements.

A common approach for determining particle size in colloidal solution is dynamic light scattering and the stability of colloidal solutions is assessed based on the surface charge of the produced nanoparticles by zeta potential. The results represented in Figure 5 (a) and Figure 5 (b) make it evident that the mean Z-average diameter (nm) and zeta potential value of the synthesized ZnO-NPs using *Aspergillus fumigatus* is 27.35 nm and

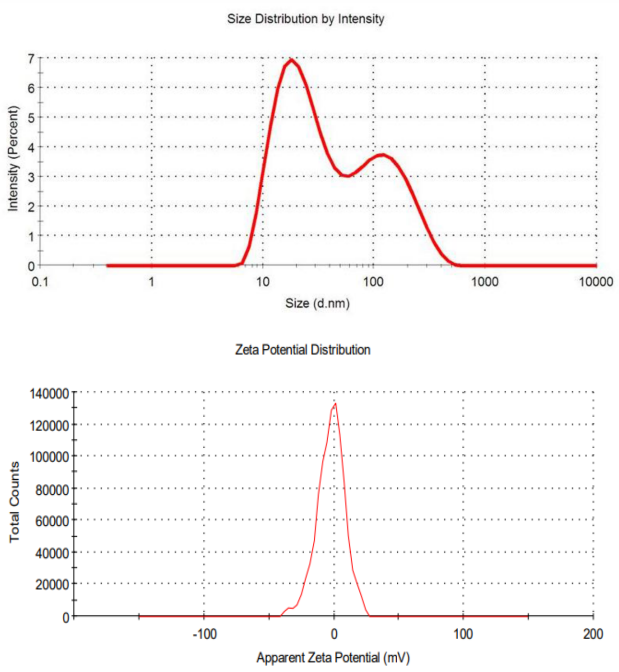

Figure 5 (a): The size distribution of Db5 nanoparticles.
Figure 5 (b): Zeta potential of Db5 nanoparticles.

-2.35 mV respectively. A similar result was observed in earlier findings.[21] Reported that size distribution of 84.79 nm and a zeta potential value of -14.4 mV from ZnO NPs prepared from *Aspergillus niger* extracellular extracts.[22] The capping effect of the biomolecules in the culture filtrate may be the cause of the nanoparticle size and the negative potential value.[21] Negative values indicate nanoparticles have high stability due to the electrostatic repulsive force and preventing nanoparticle clumping makes them extremely stable.[23-25]

XRD Studies

The XRD pattern of *Aspergillus fumigatus* (Db5)-ZnO NPs is shown in Figure 6. The diffraction peaks of ZnO NPs appeared at 2θ = 31.82°, 34.51°, 36.27°, 47.71°, 56.76°, 62.95°,66.53°, 68.12°, 69.13°, 72.45°, and 77.09° which corresponded to the (100), (002), (101), (102), (110), (103), (200), (201), (004) and (202) was confirmed. All of the ZnO NPs' diffraction peaks match JCPDS file No. 3-888 with hexagonal phase and wurtzite manner. Further, no impurity peaks were observed in the XRD patterns of ZnO NPs which indicates purity and good crystallinity. Because of this, it has been discovered that *Aspergillus fumigatus*-produced ZnO nanoparticles have a fine crystalline structure with an average crystal size of 23 nm. This is demonstrated by the finer and stronger diffraction peaks. The XRD results are the consistent study of Sharma and others, which showed several peaks at 2θ between 31.77° and

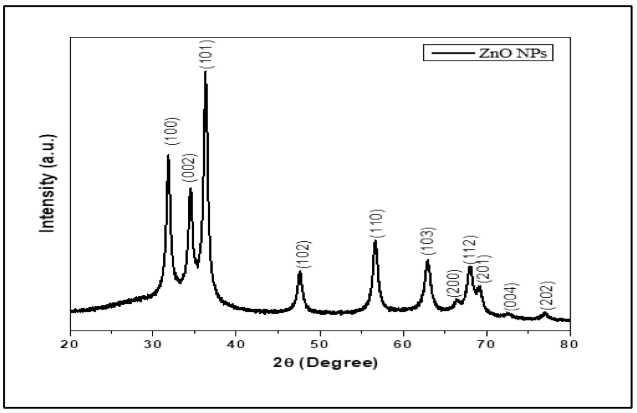

Figure 6: XRD pattern of Db5 nanoparticles.

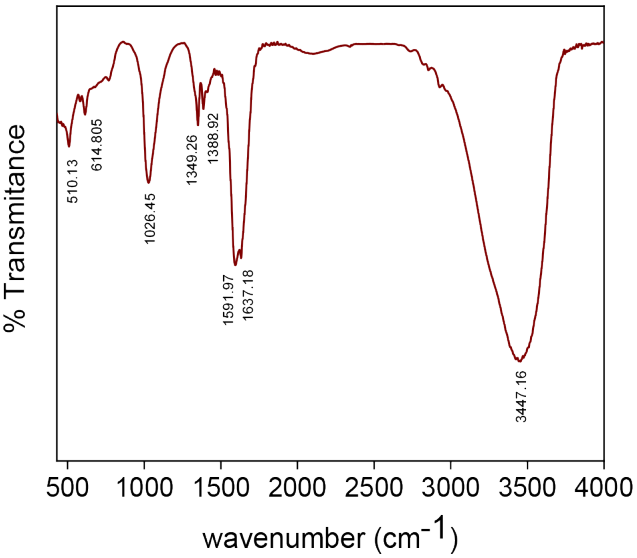

Figure 7: FT-IR Spectrum of Db5 nanoparticles.

98.67°. Several studies have reported consistent findings concerning the XRD analysis of ZnO nanoparticles synthesized using *biological* materials.[26-28]

FT-IR Spectrum

The FTIR study was performed to analyse the functional groups present in the synthesized zinc oxide nanoparticles. Measurements were carried out in the spectral range of 350-7800 cm^{-1} which showed various absorption peaks in the range of 3447.16 cm^{-1}, 1637.18. cm^{-1}, 1591.97 cm^{-1}, 1026.45 cm^{-1}, and 510.13 cm^{-1} (Figure 7). The intense band near 3447.16 cm^{-1} in samples indicates the presence of strong stretching vibration of the hydroxyl (O-H) group of water stretching. A similar result with O-H stretching vibration at the peak of 3334.71 cm^{-1} and -O axial stretching band appears at 1656.36 cm^{-1}.[29] The absorption peak at 510.13 to 614.80 cm^{-1} corresponds to metal-oxygen (ZnO stretching vibrations) vibration mode is still visible.

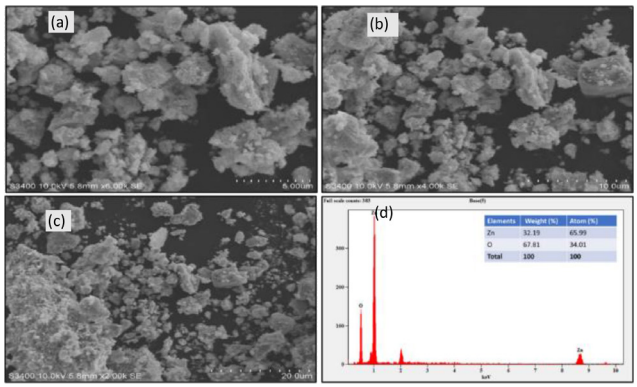

Figure 8: (a-c) SEM micrograph of ZnO NPs as shown in different magnification, (d) EDAX of Db5 nanoparticles.

The presence of the signal at 1591.97 cm^{-1} is caused by asymmetric carboxyl groups. There is a surge at 1388.92 and 1349.26 due to C-C aromatic stretching. The peak at 1637.56 cm^{-1} is due to -C=C- aromatic stretching.[30] There are further peaks at 1026.45 cm^{-1} of C-O group stretching.[31,32] Therefore, the existence of a potent aromatic ring and a carboxylic acid in the FTIR band can be used to explain how ZnO NPs were synthesized.

SEM-EDAX analysis

The Scanning Electron Microscope (SEM) was used to examine the morphology of the synthesised ZnO NPs. SEM micrograph of ZnO NPs as shown in different magnifications in Figure 8 (a-c). The results of the current investigation showed that ZnO NPs exhibit an irregularly spherical, granule-like shape with different-sized particles. It demonstrates a huge surface. A similar result for SEM analysis was reported, ZnO nanoparticles were spherical and agglomerated with nanocrystallites.[33,34]

In addition to confirming that the synthesised nanoparticles are mostly made of zinc and oxygen, the EDX spectrum offers unique peaks that correspond to zinc and oxygen. The EDAX of *Aspergillus fumigatus* (Db5) - ZnO NPs Figure 8(d) showed that Zn and O formed the NPs that were created. It validates the purity of ZnO NPs and shows that there are no other impurities in the sample spectrum. Zn and O have precise atomic percentages of 65.99% and 34.01%, respectively, but their weight percentages are 32.19% and 67.81%. The obtained values are near to those in existing reports.[35,7]

CONCLUSION

This study concludes that the fungal isolate *Aspergillus fumigatus* (Db5) can synthesize zinc oxide nanoparticles in the laboratory. The characterization

and quantification studies indicate the confirmation, potentiality and stability of the zinc oxide nanoparticles. *Aspergillus fumigatus* is a fast-growing fungus and easy to mass produce which can be used for the production of zinc oxide nanoparticles with a cost-effective process. Further ZnO NPs have to be evaluated on plant growth parameters as well as plant disease management for commercial purposes. This can be used as an ecofriendly approach for crop protection and production in INM and IPM practice for sustainable agriculture.

ACKNOWLEDGEMENT

The authors express their gratitude to the Department of Microbiology, Kuvempu University, Shivamogga, Karnataka, India, for providing the necessary laboratory facility. The authors are grateful to Chloros Energy Private Ltd., Tumkur for providing UV-vis DRS, XRD, FT-IR, SEM and EDAX analysis facility and also wish to thank Centre for Nanotechnology UAS Raichur for DLS analysis. The authors also would like to acknowledge Criyagen Agri Biotech Pvt. Ltd., Bangalore for spectrometer observation and lab facility for completing the research project.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

ZnO NPs: Zinc oxide nano particle; **Zn (NO3)2 .6H2O:** Zinc nitrate hexahydrate; **SEM:** Scanning Electron Microscopy; **EDX:** Energy Dispersive Spectrometry; **UV-vis spec:** UV-visible Spectroscopy; **UV-vis DRS:** UV-vis Diffuse Reflectance Spectra; **DLS:** Dynamic light scattering; **FT-IR:** Fourier Transform Infrared spectroscope; **XRD:** X-ray Diffractometer; **mm:** Millimeter; **cm:** Centimeter; **SI:** Solubilization Index; **CTAB:** Cetyltrimethyl ammonium bromide; **DNA:** Deoxyribonucleic acid; **RNA:** Ribonucleic acid; **PCR:** Polymerase chain reaction; **ITS:** Internal transcribed spacer; **PDA:** Potato Dextrose Agar; **BaSO4:** Barium sulfate; **MGYP:** Malt extract Glucose Yeast extract Peptone; **JCPDS:** Joint Committee on Powder Diffraction Standards; **nm:** Nanometer; **mV:** millivolts; **NCBI GenBank:** National Center for Biotechnology Information; **Sg:** Shankaraghatta; **Db:** Doddaballapura; **ZnO:** zinc oxide; **ZSI:** Zinc solubilization Index; **K-M theory:** Kubelka-Munk (K-M) theory.

SUMMARY

The current study has created a market for the manufacture of ZnO NPs from the fungus *Aspergillus fumigatus*, which can be utilised to produce and protect plants. Current findings have shown the fungal zinc solubilization, synthesis, and characterization of ZnO NPs. This study addressed the confirmation and quantification studies of ZnO NPs and also helped to understand the easy synthesis of ZnO NPs. Further studies have to be carried out on the importance of ZnO NPs for plant growth promotion and its effect on controlling plant pathogens.

REFERENCES

1. Singh RP, Shukla VK, Yadav RS, Sharma PK, Singh PK, Pandey AC. Biological approach of zinc oxide nanoparticles formation and its characterization. Adv Mater Lett. 2011;2(4):313-7. doi: 10.5185/amlett.indias.204.
2. Dagdeviren C, Hwang SW, Su Y, Kim S, Cheng H, Gur O, *et al*. Transient, biocompatible electronics and energy harvesters based on ZnO. Small. 2013;9(20):3398-404. doi: 10.1002/smll.201300146, PMID 23606533.
3. Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumour cells and potential for drug delivery applications. Expert Opin Drug Deliv. 2010;7(9):1063-77. doi: 10.1517/17425247.2010.502560, PMID 20716019.
4. Shaheen TI, El-Naggar ME, Abdelgawad AM, Hebeish A. Durable antibacterial and UV protections of *in situ* synthesized zinc oxide nanoparticles onto cotton fabrics. Int J Biol Macromol. 2016;83:426-32. doi: 10.1016/j.ijbiomac.2015.11.003, PMID 26546870.
5. Lobiak EV, Shlyakhova EV, Bulusheva LG, Plyusnin PE, Shubin YV, Okotrub AV. Ni–Mo and Co–Mo alloy nanoparticles for catalytic chemical vapor deposition synthesis of carbon nanotubes. J Alloys Compd. 2015;621:351-6. doi: 10.1016/j.jallcom.2014.09.220.
6. Hasnidawani JN, Azlina HN, Norita H, Bonnia NN, Ratim S, Ali ES. Synthesis of ZnO nanostructures using sol-gel method. Procedia Chem. 2016;19:211-6. doi: 10.1016/j.proche.2016.03.095.
7. Moghaddam AB, Nazari T, Badraghi J, Kazemzad M. Synthesis of ZnO nanoparticles and electrodeposition of polypyrrole/ZnO nanocomposite film. Int J Electro Chem Sci. 2009;4:247-57.
8. Rohilla SK, Salar RK. Isolation and characterization of various fungal strains from agricultural soil contaminated with pesticides. Res J Recent Sci. 2011;1:297–303. doi: 10.1016/j.scienta.2009.07.019
9. Devi D, Gupta SB, Mishra BK, Verma NP. Isolation and identification of zinc solubilizing fungal isolates from cumin of semi-arid region of Rajasthan. J Pharm Innov. 2022;11(2):1036-40.
10. Birhanu G, Zerihun T, Genene T, Endegena A, Misganaw W, Endeshaw A. Phosphate solubilizing fungi isolated and characterized from teff rhizosphere soil collected from North Showa and Go jam, Ethiopia. Asian Journal of Microbiology Research. 2017: 11(17):687-696. doi: https://doi.org/10.5897/AJMR2017.8525.
11. Raliya R, Tarafdar JC. Biosynthesis and characterization of zinc, magnesium and titanium Nanoparticles: an eco-friendly approach. Int Nano Lett. 2014;4(1):93. doi: 10.1007/s40089-014-0093-8.
12. Hidayat C, Wina E, Jayanegara A, Jayanegara A. Characteristics of Nano Zn-Fitogenik (NZF) made by green synthesis process using guava leaves (*Psidium guajava*) for feed additives. In IOP Conference Series. IOP Conf Ser Earth Environ Sci. 2021;19(1). doi: 10.1088/1755-1315/888/1/012056.
13. Hsueh YH, Ke WJ, Hsieh CT, Lin KS, Tzou DY, Chiang CL. ZnO nanoparticles affect *Bacillus subtilis* cell growth and biofilm formation. PLOS ONE. 2015;10(6):e0128457. doi: 10.1371/journal.pone.0128457, PMID 26039692.
14. Bande F, Arshad SS, Hair Bejo M, Abdullahi Kamba S, Omar AR. Synthesis and characterization of chitosan saponin nanoparticle for application in plasmid DNA delivery. J Nanomater. 2015;2015:1-8. doi: 10.1155/2015/371529.

15. Das S, Santra SC. Simultaneous biomass production and mixed origin wastewater treatment by five environmental isolates of Cyanobacteria. Biologija. 2010;56(1):9-13. doi: 10.2478/v10054-010-0010-7.
16. Vinila. Investigation of nanocrystalline ceramic superconductor GdBaCaCuO: structural and morphological aspects. J Phys Photon. 2016:220-8. doi: 10.4236/jcpt.2014.42016.
17. Harish Kumar K, Savalgi VP. Microbial synthesis of zinc nanoparticles using fungus isolated from rhizosphere soil. Int J Curr Microbiol Appl Sci. 2018;6(12):2359-64. doi: 10.20546/ijcmas.2017.612.272.
18. Aldalbahi A, Alterary S, Ali Abdullrahman Almoghim R, Awad MA, Aldosari NS, Fahad Alghannam S, *et al*. Greener Synthesis of Zinc Oxide Nanoparticles: Characterization and Multifaceted Applications. Molecules.2020:25(18), 4198. doi: https://doi.org/10.3390/molecules25184198.
19. Sasani Ghamsari M, Alamdari S, Han W, Park HH. Impact of nanostructured thin ZnO film in ultraviolet protection. Int J Nanomedicine. 2017;12:207-16. doi: 10.2147/IJN.S118637, PMID 28096668.
20. Kalpana VN, Kataru BAS, Sravani N, Vigneshwari T, Panneerselvam A, Devi Rajeswari V. Biosynthesis of zinc oxide nanoparticles using culture filtrates of *Aspergillus niger*: antimicrobial textiles and dye degradation studies. OpenNano. 2018;3:48-55. doi: 10.1016/j.onano.2018.06.001.
21. Mekky AE, Farrag AA, Hmed AA, Sofy AR. Preparation of zinc oxide nanoparticles using *Aspergillus niger* as antimicrobial and anticancer agents. J Pure Appl Microbiol. 2021;15(3):1547-66. doi: 10.22207/JPAM.15.3.49.
22. Fouda A, El-Din Hassan S, Salem SS, Shaheen TI. *In vitro* cytotoxicity, antibacterial, and UV protection properties of the biosynthesized zinc oxide nanoparticles for medical textile applications. Microb Pathog. 2018;125:252-61. doi: 10.1016/j.micpath.2018.09.030, PMID 30240818.
23. Kim KM, Choi MH, Lee JK, Jeong J, Kim YR, Kim MK, *et al*. Physicochemical properties of surface charge-modified ZnO nanoparticles with different particle sizes. Int J Nanomedicine. 2014;9;Suppl 2:41-56. doi: 10.2147/IJN.S57923, PMID 25565825.
24. Meléndrez MF, Cárdenas G, Arbiol J. Synthesis and characterization of gallium colloidal nanoparticles. J Colloid Interface Sci. 2010;346(2):279-87. doi: 10.1016/j.jcis.2009.11.069, PMID 20378122.
25. Chaudhuri SK, Malodia L. Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: characterization and its evaluation on tree seedling growth in nursery stage. Appl Nanosci. 2017;7(8):501-12. doi: 10.1007/s13204-017-0586-7.
26. Jain D, Shivani BAA, Bhojiya AA, Singh HK, Daima HK, Singh M, *et al*. Microbial fabrication of zinc oxide nanoparticles and evaluation of their antimicrobial and photocatalytic properties. Front Chem. 2020;8:778. doi: 10.3389/fchem.2020.00778, PMID 33195020.
27. Azizi S, Ahmad MB, Namvar F, Mohamad R. biosynthesis and characterization of zinc oxide nanoparticles using brown marine macro algae *Sargassum muticum* aqueous extract. Mater Lett. 2014;116:275-7. doi: 10.1016/j.matlet.2013.11.038.
28. Nazir S, Zaka M, Adil M, Abbasi BH, Hano C. Synthesis, characterisation and bactericidal effect of ZnO nanoparticles via chemical and bio-assisted (*Silybum marianum in vitro* plantlets and callus extract) methods: a comparative study. IET Nanobiotechnology. 2018;12(5):604-8. doi: 10.1049/iet-nbt.2017.0067, PMID 30095420.
29. Kavitha SM, Dhamodaran, Rajendra Prasad M, Ganesan. Int Nano Lett:7.2017:141-7. doi: 10.13005/ojc/360409.
30. Raghunandan D, Bedre MD, Basavaraja S, *et al*. Rapid biosynthesis of irregular shaped gold nanoparticles from macerated aqueous extracellular dried clove buds (*Syzygium aromaticum*) solution. Colloids surf. B Bio Interfaces. 2011;79:235-40.
31. Mousa MA, Khairy M. Synthesis of nano-zinc oxide with different morphologies and its application on fabrics for UV protection and microbe-resistant defence clothing. Text Res J. 2020;90(21-22):2492-503. doi: 10.1177/0040517520920952.
32. Getie S, Belay A, Chandra Reddy AR, Belay Z. Synthesis and characterizations of zinc oxide nanoparticles for antibacterial applications. J Nanomed Nanotechnol S. 2018;8(004). doi: 10.4172/2157-7439.S8-004.
33. Al-Kordy HMH, Sabry SA, Mabrouk MEM. Statistical optimization of experimental parameters for extracellular synthesis of zinc oxide nanoparticles by a novel haloalaliphilic *Alkali bacillus* sp. Sci Rep. 2021;W7;11(1):10924. doi: 10.1038/s41598-021-90408-y, PMID 34035407.
34. Vidya C, Hiremath S, Chandraprabha MN, Antonyraj MAL, Gopal IV, Jain A, *et al*. Green synthesis of ZnO nanoparticles by *Calotropis gigantea*. Int J Curr Eng Technol. 2013;1:118-20.
35. Brintha SRM, Ajitha M. Synthesis and characterization of ZnO nanoparticles via aqueous solution, sol-gel and hydrothermal methods. J Appl Chem. 2015:66-72. doi: 10.1088/2632-959X/abc813.

Cite this article: Bottangada AG, Subhakar A, Veeranna SH, Basaiah T. Biosynthesis of Zinc Oxide Nanoparticles from Soil Fungi and their Characterization by Using Analytical Methods. Asian J Biol Life Sci. 2023;12(2):263-70.