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Myco-Synthesis, Characterization of Nanomaterials and its Agricultural Applications: Scope and Challenges

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Abstract

Myco-synthesis of nanomaterials is new and developing area of research in recent years due to its broad variety of applications in different fields. It is considered a cost-effective, less toxic, and eco-friendly way of synthesizing nanomaterials using fungi. The present article covers the synthesis of zinc oxide nanoparticles (ZnO NPs) using fungi cultures varieties namely Penicillium, Aspergillus, Mucor, Trichoderma and Rhizopus. Synthesized nanomaterials were characterized using different techniques. The impact of synthesized nanomaterials on the growth of cauliflower and rice seedlings was investigated in the current study. Based on the experimental results, it was observed that despite having the huge potential of myco-synthesized nanomaterial in agriculture, a lot of further research needs to be carried out to make it more variable for field applications.

Keywords

Myco-synthesis, Agricultural applications, Microbes

Introduction

Nanoscience have risen to prominence in technological breakthroughs due to their adjustable characteristics and performance. Synthesis of nanomaterials make the researchers to be worked more on it, as their applications are totally depended on the synthesis process. NPs synthesized through chemical, physical, and biological route have definite size and shape. Compared to biological method, chemically and physically synthesized techniques are very much costly, time-consuming, and involves toxic chemical substances [1]. The biological method for NPs synthesis involves extra benefits over other methods because of the use of bio-based entities plants (all parts) and micro-organisms (viruses, bacteria fungi, and algae) for extraction. Biological based approach is gaining popularity in recent years because of the production of high scale products in the safe, reliable, and simple manner [2]. Myco-synthesis of nanomaterials is a novel and emerging research topic in recent years due to its vast variety of applications in various sectors [3]. Biosynthesis of NPs via fungi and their subsequent applications are studied under "Myco-nanotechnology" [4]. Protein and enzymes present in fungi act as absorbing and reducing compounds that reduce metal ions to form metallic NPs. Growth of fungi is fast compared to bacteria in same conditions. Synthesis of NPs through fungi are more advantageous over other microbes, because their mycelia offer a large surface area for interaction. It is also found that microbes secretes inorganic materials by two modes: 1) Intra-cellularly and 2) Extra-cellularly. In intra-cellular mode, in the presence of enzymes/proteins, transportation of ions occurs into the microbial cell wall for the formation of NPs while, in extra-cellular method, enzymes/proteins reduced metal ions at the surface of microbial cell wall for synthesizing NPs [5].

The study on synthesis of nanomaterials using fungi has gained significant momentum in recent years [6]. Multiple types of NPs like silver [7], gold [8], palladium [9], iron [10], and ZnO [11] have been synthesized easily via biological method. In the list of metals after iron, Zn act as second most abundant metal [12]. ZnO Nps production and characterisation have attracted a lot of interest in recent years due to its numerous applications in diverse fields [13]. In the year 2012, Lakshmi et al., studied the anti-bacterial activity of ZnO NPs which synthesized from some bacteria and fungi like *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* [14].

The increasing need on agriculture/food (agri-food) has become challenge for the society as the world's population increases rapidly. It indeed brings laurels to nanotechnology, as it makes sure to modernize agriculture/food sector with advance techniques for crop management, smart distribution systems for controlled and targeted insertion of agro-chemicals, farm management and noticing early crop's diseases, nano-based antimicrobial in food pathogen observation [15, 16]. It was indicated that to make ZnO NPs as effective anti-microbial agent, various functional groups (C=O C-N, N-H, O-H, and N=O) are involved. ZnO NPs synthesized from fungus can be worked as good antifungal agent. It was validly approved, that different *Aspergillus* sp., can be mixed in the food to decrease food poisoning effect. Rice weevil (*Sitophilus oryzae*) and grasserie disease (Baculovirus; *Bombyx mori* nuclear polyhedrosis virus were efficiently controlled by the function of verity of nanoparticles like aluminium oxide, silver nanoparticles, titanium dioxide, and ZnO [17].

This study covers the synthesis of ZnO NPs through 8 selected fungal cultures extracellularly. Eight fungal cultures of Penicillium (*Penicillium chrysogenum*- Pc1, *Penicillium cammberti* II Thom- Pc2), *Aspergillus aculoatus*- As1, Trichoderma (*Trichoderma hamatum*- Tc1, *Trichoderma longibrachiatum* Rifai- Tc2), Mucor (*Mucor minutes*- Mu1, *Mucor bainieri*- Mu2) and *Rhizopus nigricans*- Rz1 were used in the present species. ZnO NPs were synthesized using these fungal natural factories. Different approaches including characterization of mycosynthesized ZnO NPs, mycosynthesized mechanism, and the application of ZnO NPs on two agricultural crops (rice and cauliflower) are also covered in this study. Obtained results were thoroughly investigated. It has been observed that despite the huge promise of myco-synthesized nanomaterials in agriculture, the experimental results indicate that much more research is required to make it more adaptable for field applications.

Green synthesis of nanoparticles through microbes

Green synthesis of NPs mainly involves: a) solvent medium used for the synthesis, b) environment friendly reducing agent, and c) non-toxic materials for stabilization of nanoparticles. Synthesis of ZnO NPs through 8 fungal cultures (Pc1, Pc2, As1, Tc1, Tc2, Mu1, Mu2, and Rz1; (Figure 1 and Table 1) were collected from ITCC (Indian Type Culture Collection), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi.

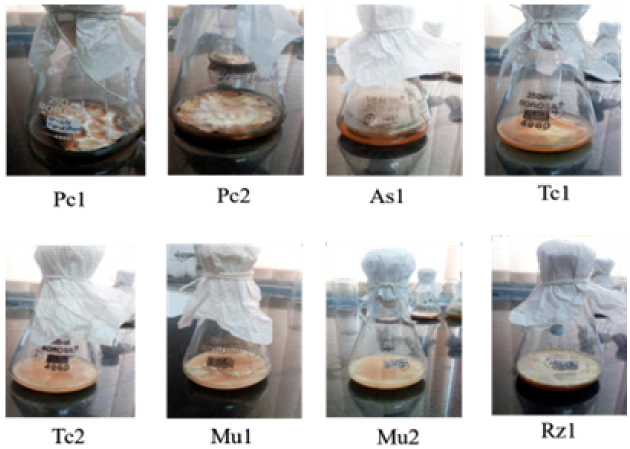

Figure 1: Fungal cultures.

Table 1: List of fungal filtrates used for the synthesis of ZnO NPs

S. No.	Name of fungus	ITCC No	Abbreviation used in this study
1	*Penicillum chrysogenum*	419	Pc1
2	*Penicillium camemberti II thom*	1502	Pc2
3	*Aspergillus aculoatus*	2589	As1
4	*Trichoderma hamatum*	3380	Tc1
5	*Trichoderma longibrachiatum rifai*	7002	Tc2
6	*Mucor minutes*	4485	Mu1
7	*Mucor bainieri*	3951	Mu2
8	*Rhizopus nigricans*	1584	Rz1

Extraction method

For obtaining beneficial economic viability, recovery and the purification of biosynthetic products, and high scale production, NPs synthesized from fungus through extracellular method is very useful [18].

All these isolates of the fungi mentioned above were cultured in PDA slants (for storage and further use) and PDB (for the extracellular synthesis of NPs) and incubated at 25 °C in the BOD incubator for 10 days.

PDB – For 1000 ml – 200 ml Potato extract + 800 ml distilled water + 20 gm Dextrose or Artificial Potato dextrose Agar can also be used – 36 gm PDA and rest is distilled water

PDA – For 1000 ml – 200 ml Potato extract + 20 gm agar + 20 gm dextrose + 760 ml distilled water.

Synthesis of zinc-oxide nanoparticles by fungi

Microbes mediated synthesis of NPs is more advantageous because it does not need very stringent condition like a pure starting material compared to chemically synthesized NPs. The fungal cell components such as cell walls, cell membranes, proteins, enzymes, and other intracellular components perform an significant role in the synthesis of NPs. Previous literature shows *Aspergillus terreus* a fungus, was developed aerobically and was stirred in an orbital shaker at 160 rpm for 4 days at 32 °C. In vacuum condition, culture of fungus was filtered. Concentrated 1 mM of $ZnSO_4$ salt was mixed into filtrate and left for 2 days at 32 °C in a shaking incubator at 150 rpm. Transformation process started as deposition of white precipitate [19]. In this study, 8 fungal strains were used to synthesize ZnO NPs extracellularly after extraction, in which 25 ml of fungal culture mixed with 1 mM of zinc nitrate and place the solution in BOD shaker incubator at 200 rpm at 270 C for 5 days under dark conditions.

Synthesis mechanism

How does extracellular synthesis of ZnO NPs by fungi occur?

Although limited studies are reported on the synthesis of ZnO NPs using fungi, that is why the specific mechanism involved have not yet been fully elucidated. In studies, it was reported that extracellular synthesis of NPs is a nitrate reductase mediated process, by which metal ions reduced into metal NPs [20-25]. In extracellular pathway, cell membrane contains enzymes which acts as a growth medium. In the synthesis of ZnO NPs, the bio-reduction of Zn^{2+} was carried out [25]. After obtaining electron, Zn^{2+} reduced in Zn^{0} and form ZnO NPs. The graphic of the extracellular synthesis mechanisms is mentioned in Figure 2.

So, basically nitrate reductase enzyme involved in the mechanism of synthesis of NPs through fungus, by this enzyme reduction process occur and it help in the growth of NPs. After reduction, extracellular protein secreted by microbes help in capping, higher stabilization and dispersion of the NPs [20]. Resulted white precipitation in the medium shows the production of NPs.

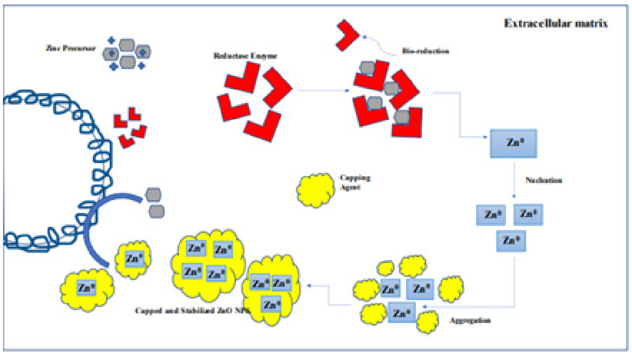

Figure 2: Graphic representation of extracellular synthesis mechanisms of ZnO NPs.

Spectroscopic characterization of synthesized ZnO NPs

Ultraviolet-Visible spectrophotometer (UV-Vis)

The synthesized ZnO NPs from 8 fungal strains were characterized by using UV-Vis was performed on UV-Vis spectrometer Perkin Elmer. Analysis was recorded in the range of 200 - 800 nm. Figure 3 analysis indicates that all the synthesized ZnO NPs show maximum absorbance between 200 nm to 400 nm due to surface plasmon resonance which occurs due to resonance of collective conduction electrons with incident electromagnetic radiations. Bulk Zn shows absorption at 385 nm, the high blue shift absorption for ZnO NPs is due to high decrease in particle size [26].

FTIR (Fourier tranform infrared spectroscopy)

FTIR measurement was performed to identify the possible capping and stabilizing agents for the synthesis of ZnO NPs by fungal strains. Images in Figure 4 confirms the reduction of metal ions by the fungus as peaks are observed around 3000 - 3400 cm^{-1} leads to N-H stretching for primary, secondary amines, and amides [27, 28]. Here, the observed

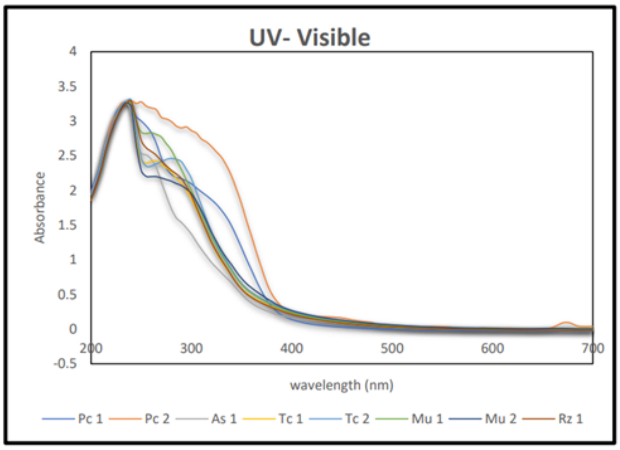

Figure 3: UV-Vis spectra of synthesized ZnO NPs from fungal strains.

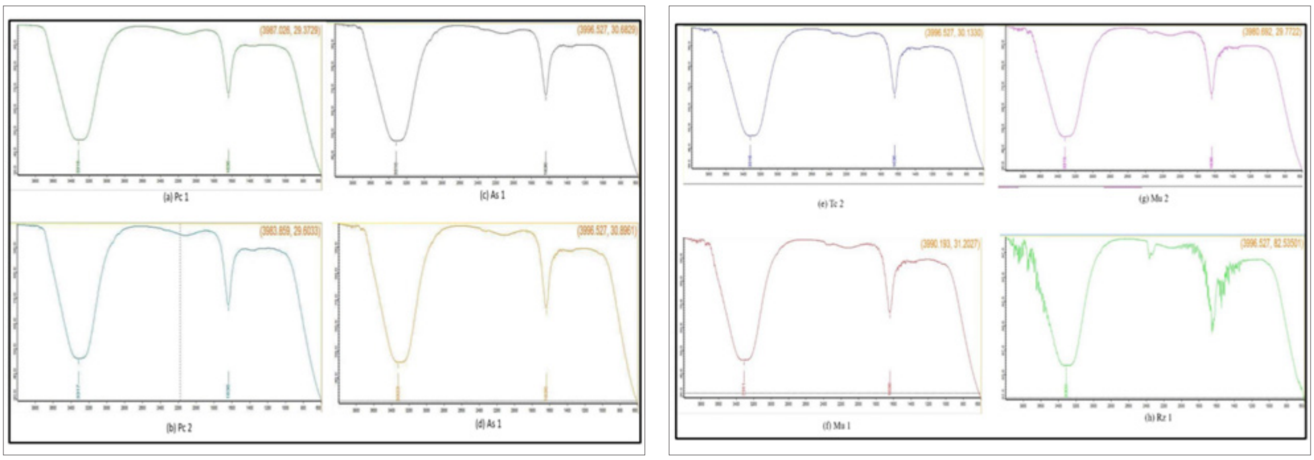

Figure 4: FTIR spectrum of synthesized ZnO NPs.

peak around 1600 - 1640 cm^{-1} leads to N-H bend primary amines [29]. There is a sharp peak shown in Rz1 spectra at 2200 cm^{-1}, which shows C=C stretching bond of alkynes molecules. The peak of ZnO NPs obtained at big decrease at 600 cm^{-1}. Therefore, synthesized ZnO NPs are encircling by proteins and metabolites with the attachment of functional groups [30].

Zeta potential

Zeta potential determines the charge generated on the surface of colloidal particles and also defines how much the particles of synthesized NPs are stable. Generally, suspension that are ≥ 15 mV featured as stable colloids [31]. Figure 5 displays that the zeta potential of fungal strains synthesized ZnO NPs was recorded as Pc1= -1.46 mV, Pc 2= -1.84 mV, As 1= -6.94 mV, Tc 1= -0.532 mV, Tc 2= -0.242 mV, Mu 1= -3.23 mV, Mu 2= -13.3 mV, and Rz 1= -21.4 mV and thus considered as stable solution. Negative charge on particles wasbecause of the high binding affinity of the extract (fungal) on metallic ions capping the particles, it disperses the stability and avoid aggregation [32].

Application in agriculture

Group of pathogens are active in agriculture field and destroyed vast variety of crops. Chemical fertilizers are used for the prevention of crop pathogens that are harmful for indigestion. So, there is need of organic and non- toxic methods that are easily available. Crop protection can be assisted through advance and effective technology, that is known as 'Myco-nanotechnology'. In this technology, metal NPs have demonstrated efficacy against a variety of crop pests, allowing them to be used in novel pesticide formulations [33, 34]. Rice weevil (*Sitophilus oryzae*) and grasserie disease (Baculovirus; *Bombyx mori* nuclear polyhedrosis virus) were efficiently treated with variety of nanoparticles such as TiO, AlO_2, Ag NPs and ZnO NPs [35]. In this research paper, two crop pathogens *Xanthomonas oryzae* (rice pathogen) and *Xanthomonas campestris* (cauliflower pathogen) were examined for antipathogenic activity. Synthesized ZnO NPs from 8 fungal strains were tested on these two crop pathogens.

Tables 2 and 3 represent the effectiveness of ZnO NPs against crop pathogens compared to fungal extracts, respectively. Results shows that there are possible chances that ZnO NPs synthesized through fungal strains can be used in crop management.

Conclusion

Biosynthesized ZnO NPs from 8 fungal strains is in its infancy stage but have numerous potentials. Much research is needed to improve the synthesis efficiency and controlling the size and morphology of particles. Spectroscopic characterization

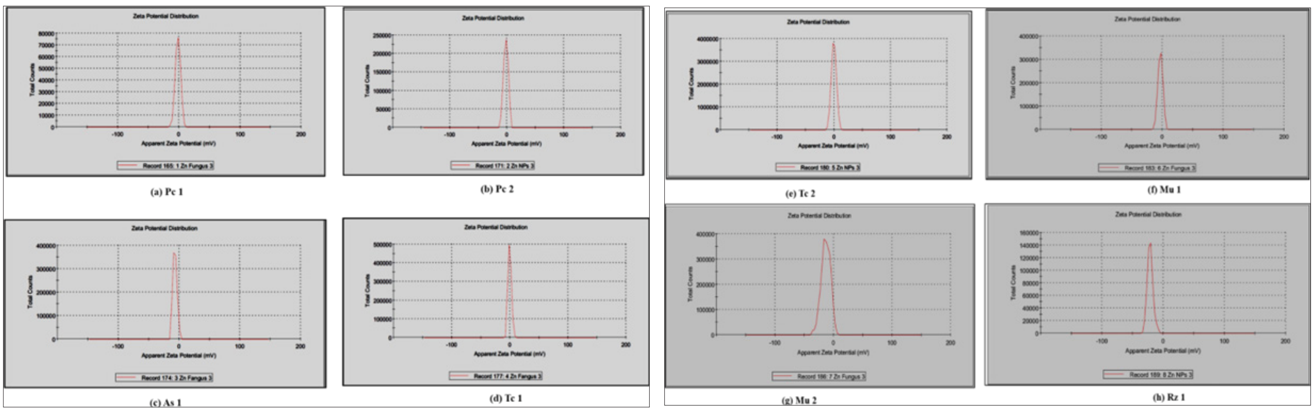

Figure 5: Zeta potential of ZnO NPs synthesized through fungal strains.

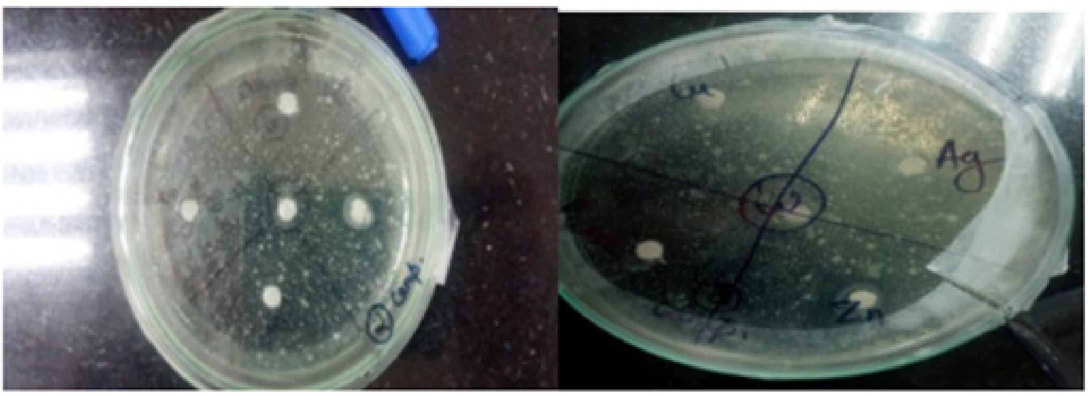

Figure 6: Antipathogenic images of inhibition zone.

Table 2: Against *Xanthomonas campestris.*

S. NO.	Abbreviation fungus name	Pure fungus	ZnO NPs inhibition zone
1	Pc1	0 mm	0 mm
2	Pc 2	2 mm	4 mm
3	As 1	0 mm	2 mm
4	Tc 1	0 mm	0 mm
5	Tc 2	0 mm	1 mm
6	Mu 1	0 mm	3 mm
7	Mu 2	3 mm	0 mm
8	Rz 1	0 mm	5 mm

Table 3: Against *Xanthomonas oryzae.*

S. NO.	Abbreviation fungus name	Pure Fungus	ZnO NPs inhibition zone
1	Pc 1	0 mm	7 mm
2	Pc 2	0 mm	10 mm
3	As 1	0 mm	12 mm
4	Tc 1	0 mm	8 mm
5	Tc 2	0 mm	12 mm
6	Mu 1	0 mm	6 mm
7	Mu 2	2 mm	6 mm
8	Rz 1	0 mm	14 mm

confirms the crystal nature and stability of synthesized ZnO NPs by UV- Visible, FTIR, and Zeta potential. Synthesized ZnO NPs have shown effective function of anti-pathogenic activity. The current study endorses the potential of biogenic ZnO NPs in the agricultural field like crop management and pesticide application, etc. However, more research needs to be carried in this direction both at *in vivo* and *in vitro* levels to determine its applications in the agriculture field.

Conflict of Interest

The authors declare no conflict of interest.

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