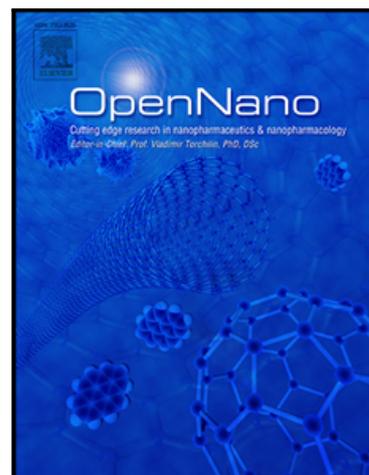


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Biosynthesis of Zinc oxide nanoparticles using culture filtrates of *Aspergillus niger*: Antimicrobial textiles and dye degradation studies

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Abstract

The main goal of undertaking the current investigation was to evaluate the extracellular synthesis of ZnO NPs using culture filtrates of *Aspergillus niger*. The obtained UV-Visible spectrum of the culture filtrate showed an absorption peak at 320 nm. The FTIR band showed the appearance of carboxylic acid and strong aromatic ring that could be responsible for the synthesis of ZnO NPs. Studies of the antimicrobial activity of ZnO NPs coated fabric showed a reduction of viable *E. coli* and *S. aureus*. 90% of decolorization of Bismarck brown dye was obtained by treating it with 100 μ l ZnO NPs. The Germination (%) of the seeds of plants was lower with the raw dye when compared to the dye treated with ZnO NPs. The present study concludes that the *A. niger* mediated synthesis of ZnO NPs proved well in degrading dye and can be incorporated onto cotton fabrics since it reveals antibacterial activity.

Keywords: *A. niger*; Zinc Oxide Nanoparticles; Bismarck Brown; *Pisum sativum*

1. Introduction

Due to the recent advancements in science and technology, research attempts for creating nanoparticles of size within 100 nm [1] due to its wide range of benefits [2]. It can be synthesized through the biological, chemical and physical methods. Out of these, biological methods seems effective and ecofriendly one because of other two methods has some poisonous compounds confines their application. Further, researchers tend to exploit biological methods by developing high-yielding, low-cost and non-toxic nanoparticles [3-7]. The biological method was applied with the help of using the biologically active [8] products of bacteria, fungi, plants, and yeasts represent the excellent sources for the production of nanoparticles [9-12]. Mostly fungi were chosen instead of bacteria because of their tolerance, better metal bioaccumulation ability, and high binding capacity. There are wide applications of fungi as they produce huge enzymes, ease in the scale-up process, economic viability, and ease in handling the biomass [13-14].

Generally, Zinc (Zn) acts as the second most abundant metal after iron and it is the only metal represented in the six classes of enzymes (lyases, transferases, oxidoreductases, hydrolases, isomerases, and ligases)[15]. Due to its applications, studies on the synthesis, properties, and characterization of ZnO NPs have received much attention recent years [16]. Specifically, ZnO NPs synthesis has gained much attention because of its extensive antimicrobial activity, eco-friendliness, and simplicity. This nanoparticle was applied in various fields such as Rubber industry, Pharma industry, Textile industry, Biosensor, Cosmetics, etc [17]. Moreover, this method for nanoparticle synthesis is more advantageous because it helps to synthesize nanoparticles at mild pH, pressure, and temperature [18-22]. Lakshmi et al., (2012) examined the ZnO NPs antibacterial activity through some bacteria and fungi like *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [23]

Thus, it is clear from the above information that the main aim of this research work is to develop an easy and effective method for ZnO NPs synthesis and its characterization, and as well as to devise a distinct technique for coating of ZnO NPs over cotton fabrics to provide antimicrobial capacity and eventually assess the degradation of dye (Bismarck Brown) and its phytotoxicity study.

2. Materials and Methods

2.1. Fungi used and growth conditions

The fungus *A. niger* was procured from the Microbiology Laboratory, VIT, Vellore. The process of sub-culturing is done for 96 h at 32°C by cultivating on Czepak Dox agar slants and refrigerated at 4°C [24].

2.2. *Aspergillus niger* mediated Zinc oxide nanoparticle synthesis (ZnO NPs)

To prepare a biomass for the biosynthesis of ZnO NPs, the culture medium of fungus should be comprised of the liquid(g/l) K₂HPO₄, 2.0; (NH₄)₂SO₄, 1.0; Yeast extract, 0.6; MgSO₄·7H₂O, 0.1; KH₂PO₄, 7.0; and glucose, 10.0. The pH level was fixed as 6.2 ± 0.2. The fungus was placed inside the flask and orbital shaker is used to incubate the fungus at 200 rpm and at the temperature of 37°C. The culture of the fungi was then filtered through Whatman no.1 filter paper. 5mM Zinc nitrate was added and kept in an shaking incubator at 200 rpm at 32°C for 2 days. The White precipitate is produced at the bottom of the flask that indicates the reduction process. The white precipitate was centrifuged at 10,000 rpm for 10 min. Simultaneously, a positive control and negative control were maintained by incubating the fungus mycelium with de-ionized water and Zinc nitrate solution [25].

2.3. Characterization of Zinc oxide nanoparticles

A double beam UV-visible spectrophotometer (ELICO, India) was utilized for the UV absorbance of ZnO NPs. Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer, Germany) is used to identify the functional groups at 4 cm⁻¹ resolution. The instrument Phillips RY "3:52" * ?307628" α. "EwM " tcfkcvkqp+" qrgtcvkpi "cv"52" o C"cpf"62"mX" y cu" wugf"vq" cff"vjg" X-ray diffraction model of whole samples at 20-:2ÅE"*4 +0The morphology of the prepared ZnO NPs was observed by scanning electron microscopy (JEOL, Model JFC-1600).

2.4. Application onto fabrics

A 30×30 cotton fabric material was placed in the solution comprising of acrylic binder (1%) and ZnO NPs (2%) for 5 min. The fabric was then air-dried and then cured for 3 min at 140°C. The cotton fabric was then soaked in sodium lauryl sulfate for 5 min to remove the unbound nanoparticles. Then, the fabric was rinsed for at least 5 times to fully eliminate the soap solution. Rinsed fabric was then dried and utilized for antibacterial action [26-27].

2.5. Evaluation of antibacterial activity of nano finished fabric

The antibacterial activity was evaluated against *S. aureus* and *E. coli*. Fabric samples were placed in the center of the agar plate which was previously swabbed with the bacterial inoculum. After an incubation of 24 hours, the zone of inhibition around the cotton fabric was examined and measured in mm [26-27].

2.6. Catalytic reduction of Bismarck brown using ZnO NPs

A. niger mediated synthesized ZnO NPs were tested against Bismarck Brown in the presence of UV light exactly at 365 nm. About 1 ml of Bismarck Brown (1×10^{-4} M) was mixed with 25, 50, 75, 100 μ l of ZnO NPs. Every 24-hour interval, sample was collected and subjected to UV-Visible Spectroscopy [28-29]. The percentage of dye degradation was estimated by

$$\% \text{ Decolourization} = \frac{100 \times (C_0 - C)}{C_0}$$

Where C_0 is the original concentration of dye and C is the dye concentration after catalytic degradation

2.7. Phytotoxicity study

The study was carried out with degraded dye (Bismarck Brown) using the seeds of *P. sativum*. The seeds were sterilized to remove fungal growth by wiping them on the surface with 1.2% sodium hypochlorite solution. A shallow, circular, transparent dish with a flat lid is used to place all the five seeds separately and water it regularly with 5 ml of degraded dye per day. Positive Control was set up with normal water, and the untreated dye was used as negative control. The plates were regularly monitored for germination. Seeds with emerging radical are considered to be germinated. The length of the radical (root) and plumule (shoot) and the rate of germination were noticed after 1 week [30-31].

3. Results and discussion

The ZnO NPs synthesized using *A. niger* was characterized, and the results are discussed below

3.1. Visual observation of Zinc oxide nanoparticles synthesized by *A. niger*:

By mixing the *A. niger* culture filtrate with the aqueous solution of zinc nitrate, the culture filtrate colour was changed from light yellow to colorless liquid after 48 hours. White precipitation of ZnO NPs is formed as a result of zinc nitrate ions reduction by the proteins as shown in Fig. 1. This indicated the colour change in fungal culture.

3.2. Characteristics of the synthesized ZnO Nanoparticles

3.2.1. Analysis of UV spectrum

The UV-Vis Spectrophotometer technique was used to measure the structural characterization of nanoparticles by determine the absorbance of measurement and the UV-Vis spectrum findings revealed that the *A. niger* exhibits the ZnO NPs stable synthesis. At 320 nm absorption bands as shown in Fig. 2 confirms the existence of ZnO NPs in culture filtrate. Therefore, these ZnO NPs absorption spectra will have a strong blue shift signifying these particles should not be greater in size than the exciton Bohr radius [32]. This peak is the characteristic of the ZnO formation while blue shift proves confinement in nanoscale. The absorption peak was appearing as similar the absorption band for the results obtained by Talam et al., 2012 [33]. Similar to this, the study of Jamdagni et al., 2018 examined the UV spectrum range of ZnO is 320-390nm [34]. Santhosh kumar et al., 2017 also specified the UV spectrum range of ZnO was measured at 380nm [35]. Another study of Gao et al., showed the colour change of *A. paniculata* extract treated with Zinc nitrate might be because of vibrations in surface plasmon resonance [36].

3.2.2. Analysis of FT-IR spectrum

To recognize the diverse functional groups found in synthesized nanoparticles, these ZnO NPs were characterized under FT-IR. The functional group of ZnO NPs is indicated using the peaks. It is calculated from the fig. 3 that samples have 3199.91, 1587.42, 1315.46, 1074.35, 1041.56, and 823.60 absorption peak. The absorption peak at 3199.91 corresponds to O-H stretch and 1587.42 correspond to C=C stretch. In line with our finding, the study like Kavitha et al., 2017 examined the O-H axial stretching band at 3378.58 cm⁻¹ [37]. Another study has showed the peak at 1637.56 cm⁻¹ is due to C=C aromatic stretching [38]. Thus, the strong aromatic ring and carboxylic acid appearance in FTIR band is responsible for ZnO NPs synthesis.

3.2.3. XRD analysis of ZnO NPs synthesized by *A. niger*

X-ray diffraction is performed with the intention of substantiate various phases of ZnO nanoparticles. In the Fig. 4, the standard wurtzite and XRD patterns of ZnO NPs are depicted. The phase identification of ZnO NPs is done using the X-ray powder diffraction (XRD) with 2θ ranges from 20° to 80°, 0.02° s⁻¹ of scanning rate and $\lambda = 0.1540$ nm. The XRD peaks at 30.1°, 33.91°, 36.60°, 44.10°, 56.56°, 60.0°, 65.1°, 69.1° and 70.48° were identified as (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2), and (2 0 1) reflections, respectively. The product is found to have a fine crystalline structure as demonstrated by the sharper and stronger diffraction peaks. The average crystallite size (d) of

also stated the similar diffraction peaks at (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0) [39,40]

3.2.4. SEM analysis

Morphology and the size of the ZnO NPs were detected using the Scanning Electron Microscope (SEM). It is concluded from Fig.5 that the particles in the samples were compactly arranged and were almost spherical in shape. Study like Emad et al., 2017 also examined the practices and morphology of ZnO NPs and found that the size is spherical in shape [41]. However, the study shows the size range is 41-75 nm but our study findings examined the ZnO NPs diameter was found to be 53-69 nm with an average size of 61 ± 0.65 nm. According to Raut et al., 2015 found the nanoparticle size is 11-25 nm and shows hexagonal in shape [42].

3.3. Application studies

3.1. Evaluation of antibacterial activity of nano finished fabric

The bacteriostatic activity of the Zinc Oxide nanoparticles (ZnO NPs) impregnated fabrics against *E. coli* and *S. aureus* were studied and this activity was indicated by a zone of inhibition (Table 1). However, the cotton fabric with ZnO nanoparticles showed maximum antibacterial activity. This result demonstrated that ZnO nanoparticles can be used to prepare sterile fabrics. ZnO NPs generate active oxygen species but there is no clear evidence regarding this study. Yamamoto et al [43] detected the presence of active oxygen species. It is already revealed that the bacterial growth is inhibited by micron-sized and nano-sized ZnO suspensions; The performance of nanoscale ZnO suspension is more when compared to micron-sized ZnO suspension [44]. Ferris, 2010 reported that ZnO NPs exhibit high level of antifungal and antibacterial activity even at lesser concentrations [45]. Likewise, study of Gunalan et al., 2012 exhibited the high level of antibacterial activity of ZnO NPs [46].

3.2. Catalytic reduction of Bismarck brown using ZnO NPs

Dyes with different concentration of ZnO NPs were observed (Fig. 6) for decolorization. Bismarck Brown showed maximum degradation only at 72 hours (100 μ l ZnO NPs showed 89% decolorization, 65 μ l ZnO NPs showed 72% decolorization, 50 μ l ZnO NPs showed 52% decolorization and 25 μ l ZnO NPs showed only 38% decolorization). In line to our finding, the study of kalpana et al., 2016 examined the maximum degradation of dye at 72 hours [47].

3.3. Phytotoxicity study

Evaluation of *P. sativum* towards the dye and its degradation products was carried using the assays of plant growth and seed germination (Fig.7). There was 100% Germination in control (Water). The germination(%) of *P. sativum* was also found to be 100% when treated with degraded dye compounds and the untreated dye showed only 40% germination. The length of shoot and root were also significantly affected by the untreated dyes rather than the treated one. The plumule and radicle length of *P. sativum* was represented in Table2. The result of germination was in line with the study of kalpana et al., exhibits 40% germination but the evaluation was done with *Cicer arietinum* [47] .

4. Conclusions

Globally, the application of nanotechnology in the field of nanomedicine and agriculture is very much important. This investigation guarantees that ZnO NPs play a dual role as an effective nanomedicine and as an agrochemical sector. This study reveals that the *A. niger* can be used for the large-scale synthesis of ZnO NPs. The study also demonstrated that the Zinc nitrate may be reduced extracellularly using *A. niger* to generate stable ZnO NPs. The biosynthesized nanoparticles have been characterized by XRD, UV-Vis spectroscopy, FTIR and SEM. The functional groups present in the ZnO NPs were affirmed by using FT-IR analysis and the range of peaks is 823.60 - 3199.91. Sharper and stronger diffraction peaks was observed in XRD analysis and confirmed the synthesized ZnO NPs. Synthesized ZnO NPs were in the size range of 84 - 91nm and found to be spherical in shape as confirmed by the SEM analysis. The cotton fabric coated using Nano ZnO NPs possesses the antimicrobial property when compared with the untreated one. The synthesized ZnO NPs were also used to degrade dye (i.e. Bismarck brown). The reduction in absorbance suggests that the dye molecules were completely mineralized along with the colour removal. It is concluded that the *A. niger* mediated synthesis of ZnO NPs proved very well in degrading bismarck brown and it can be used in waste water treatment as well as it can be incorporated onto cotton fabrics since it exhibits antimicrobial activity. Overall, the biosynthesized ZnO NPs showed excellent antimicrobial activity and also showed effective catalytic activity towards degradation of Bismarck brown hence having the possible for industrial application.

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Compliance with ethical standards

Conflict of interest: The authors have declared no conflict of interest

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Table 1: Evaluation of antimicrobial activity

Culture	Zone of inhibition
<i>Staphylococcus aureus</i>	12±0.23 mm
<i>Escherichia coli</i>	10±0.78 mm

Table 2 Plumule and Radicle length of *Pisum sativum*

Treatments	<i>Pisum sativum</i>	
	Plumule (cm)	Radicle (cm)
Control (Water)	20±0.64	6±0.68
Untreated Dye (Bismarck Brown)	6.5±0.59	3±0.02
Treated Dye (Bismarck Brown+ ZnO NPs)	14±0.79	5.2±0.14



Fig 1. The color change of *A. niger* cell free supernatant from light yellow to colorless after addition of ZnNO_3 solution (5 mM) and ZnO NPs formation

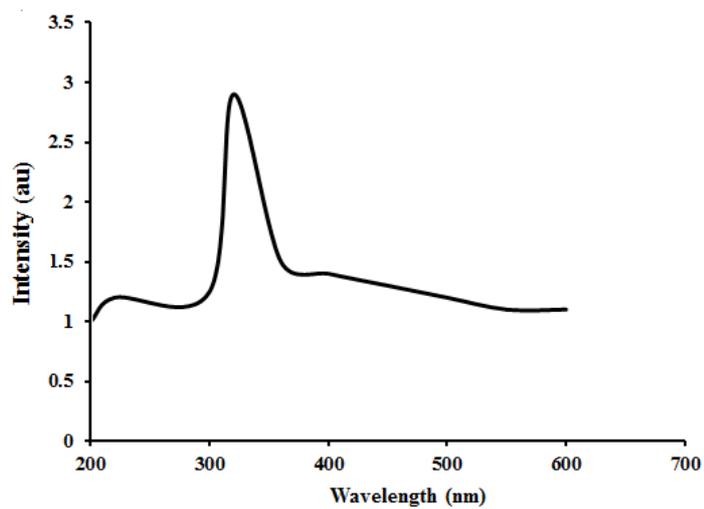


Fig 2. UV-Visible spectrum of ZnO nanoparticles.

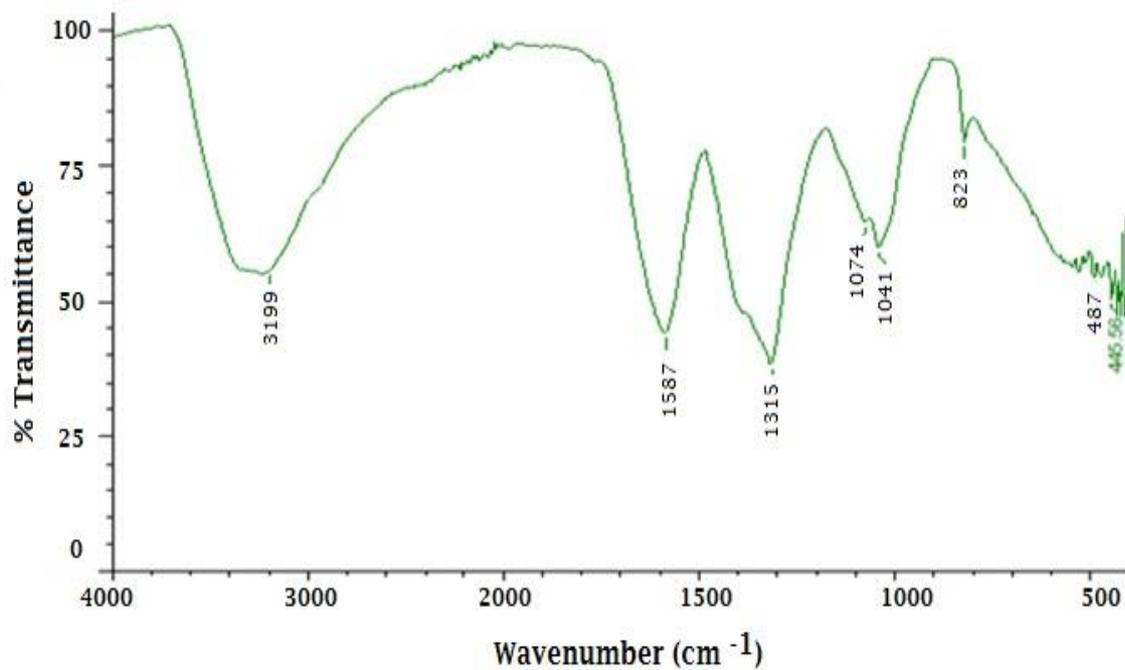


Fig 3. FT-IR analysis of ZnO nanoparticles

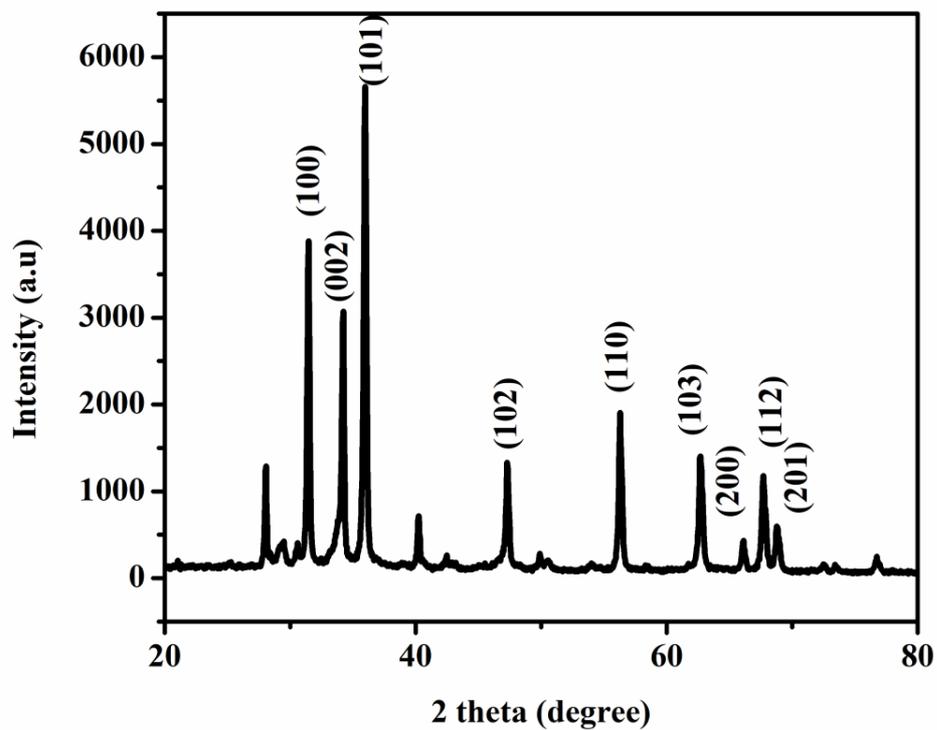


Fig 4. XRD pattern of ZnO nanoparticles

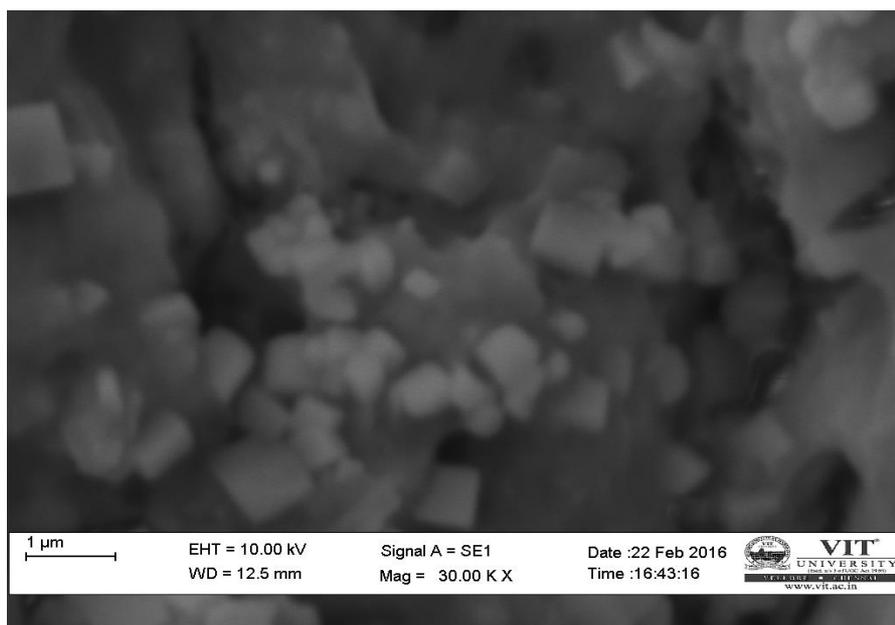


Fig 5. SEM images of biosynthesized ZnO NPs under different magnifications

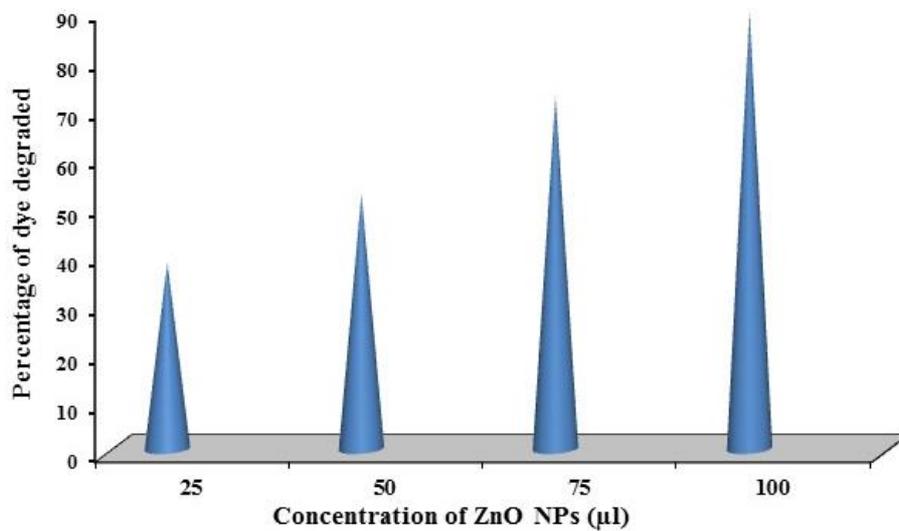


Fig 6. Catalytic reduction of Bismarck brown using ZnO NPs

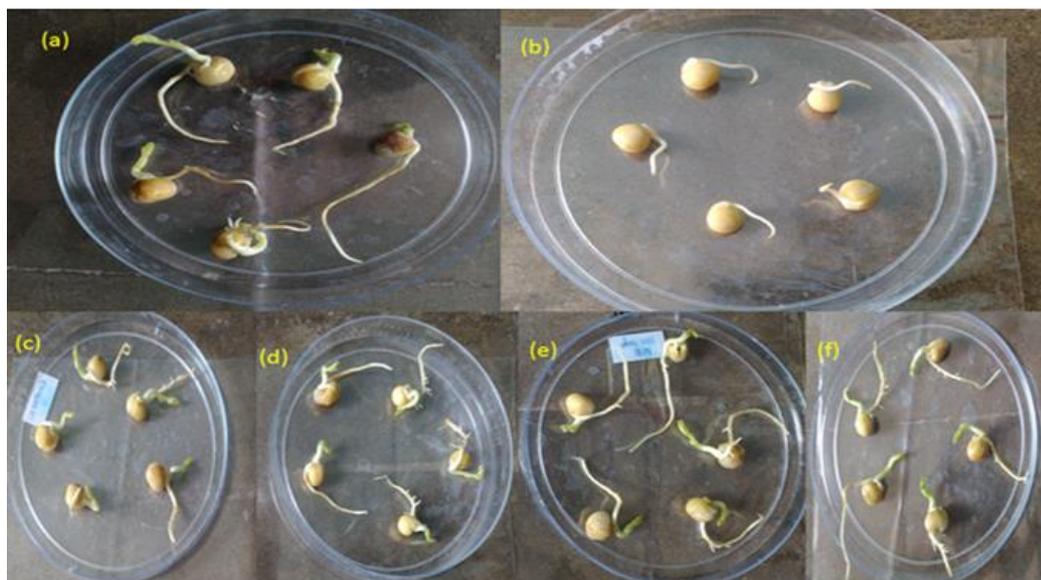
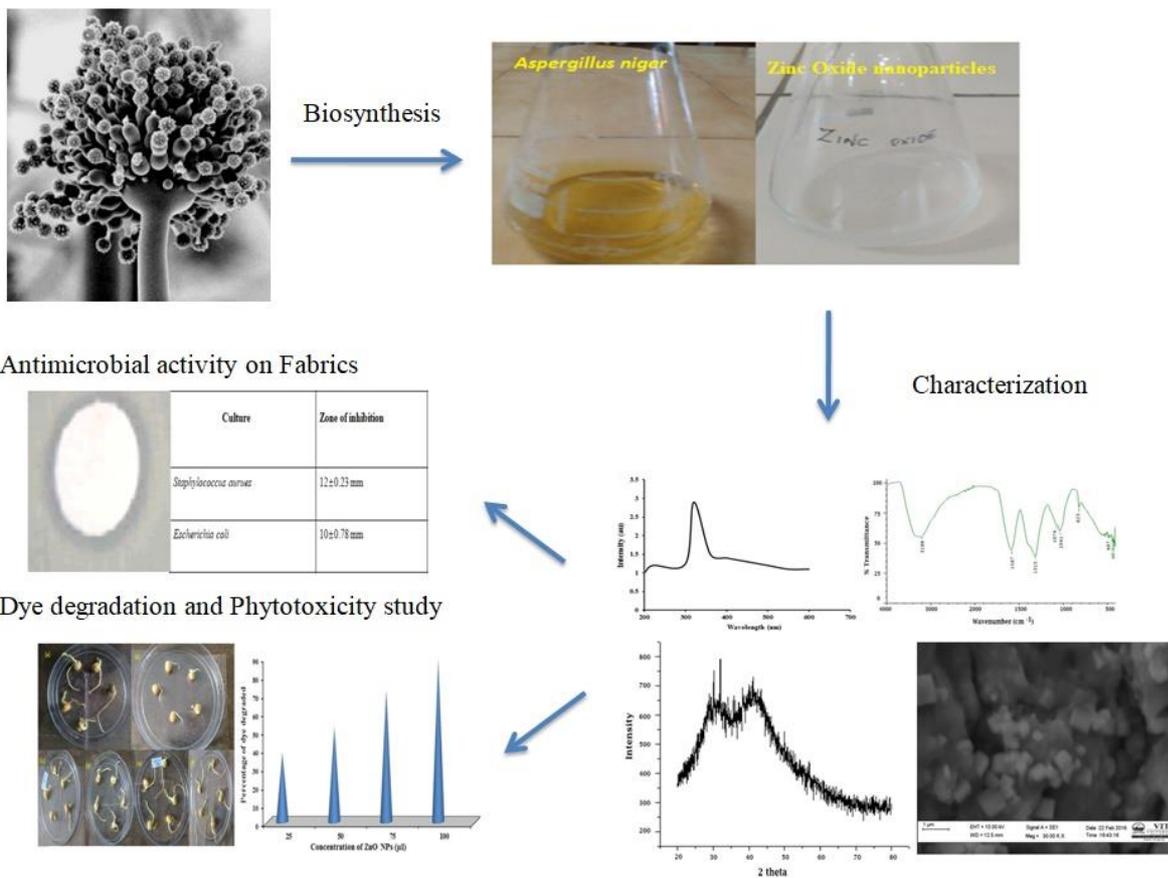


Fig 7. Catalytic Phytotoxicity study of synthesized ZnO NPs (a) Control (b) Untreated dye (c) Treated dye (25 μ l of ZnO NPs) (d) Treated dye (50 μ l of ZnO NPs) (e) Treated dye (75 μ l of ZnO NPs) (f) Treated dye (100 μ l of ZnO NPs)

Graphical abstract



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