

Textile Dye Degradation and Antibacterial Potential of Myco- Synthesized Silver Nano Particles (AgNPs)

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Abstract:- The present study is focused on rapid mycosynthesis, characterization, and potential applications of silver nano particles (AgNPs). A novel fungal strain *Beauveria sp. MTCC 5184* was incubated in 1% glucose media for 48 hours, and the cell-free broth was used for nanoparticle synthesis. The presence of possible biomolecules that are accountable for the formation and capping of AgNPs was detected in FTIR analysis. AgNPs showed an absorption peak at 439 nm, and TEM images illustrated the smaller size of the nanoparticles with variable shapes. The powder XRD pattern indicated five diffraction peaks at 2θ values 38.280, 44.480, 64.760, and 77.690, which correspond to the (111), (200), (220), (311), and (222) planes of face-centered cubic. DLS analysis illustrated an average particle size of 58nm and zeta potential of 11.57mV. Further in functional characterization, AgNPs and sodium bromide showed promising dye-reducing ability against two textile dyes, Reactive Blue HERD and Orange M2R cold brand. Individual dyes and a mixture of both dyes were shown to reduce by 99% at the concentration up to 80 ppm at neutral pH. AgNPs showed strong antibacterial activity towards various entero-pathogens by well diffusion method. The findings suggest that using 1% glucose media for the growth of new fungal species is enough to synthesize silver nanoparticles with potential applications.

Keywords:- *Beauveria sp. MTCC 5184*, AgNPs, Dye degradation, Antimicrobial activity.

I. INTRODUCTION

Manipulation of an object on a nano-scale level had an evolutionary effect on various fields of science, including surface science, molecular biology, organic chemistry, microbiology, semiconductor physics, and many more.[1–4]. Silver has been best known for its antimicrobial potential since ancient times in its different forms (metallic, salt, and colloidal). Due to their antimicrobial properties, AgNPs have been used in coating water filters and medical devices. In the textile industry, silver-surrounded fabrics are now used in sporting gear. These AgNPs have been used as antimicrobial agents in bandages, sprays, sanitizers, detergents, socks, wipers, toothpaste, washing machines, shampoos, soaps, and many other products[5]. Along with the antimicrobial activity, AgNPs exhibit some other properties such as degradation of organic dyes, cytotoxic activity against cancer cells, and antioxidant activity[6–10].

Microbiology, together with nanotechnology, has become an interesting field for many researchers as it has been providing effective solutions in order to protect the environment. The ability to reduce metals to metal ions by both prokaryotes & eukaryotes makes them interesting nano-factories and interesting candidates for the synthesis of nano particles[11]. In order to survive in the high metal concentration, microorganisms tend to produce reducing enzymes. Employments of these reducing enzymes in the biological reduction of metal ions provide an immense application of nano particle bio-synthesis[12]. Mycosynthesis of nano particles includes the use of fungal strains for nanoparticle synthesis[13]. Fungi, compared to bacteria, produce more proteins and extracellular enzymes, which reduce metal into nano particles[14]. Fungi synthesize nano particles by intracellular and extracellular methods (Pavani et al., 2011). The intracellular method involves the addition of metal precursors in the culture. Once added, it gets incorporated into the fungal biomass and gets reduced by reducing enzymes. This biomass must then be separated from culture by chemical treatments such as centrifugation and filtration. The reason why researchers prefer extracellular synthesis over intracellular synthesis is perhaps the direct conversion of the metal precursor to nanoparticles by biomolecules present in the cell-free broth rather than the internalization of the metal precursor in the biomass. Madhanraj et al. studied AgNPs synthesis assisted by mushroom fungi[9]. There are several reports on the mycosynthesis of AgNPs using fungal biomass, viz. *Aspergillus flavus* and *Fusarium oxysporum*[15, 16]. Growing concern about the environmental challenges faced due to textile industry dyes has gained scientific fraternities' attention[17]. The existence of dye molecules in the water affects the health of aquatic organisms by altering the regular metabolic activity, inhibiting sunlight penetration, and reducing photosynthetic activities[18]. Several methods, including filtration, adsorption, coagulation, and flocculation, have been incorporated to reduce the pollution of the dyes[19]. However, each method has drawbacks, including high costs and energy inputs. Recent reports suggest the applicability of AgNPs in curbing these textile dyes. However, more scientific data regarding the catalytic property of nano particles is required to deal with the heavy load of dye in the aquatic environment[20]. The main objective of this study is the mycosynthesis of AgNPs using a culture filtrate of a new strain of *Beauveria* and exploring their applications. Textile dye degradation and antibacterial activity against pathogenic bacteria are described in this paper.

II. MATERIALS & METHODS

A. Microorganisms and chemicals

Fungal strain *Beauveria sp.* (MTCC-5184) was isolated and maintained as described earlier (More S.V. 2017). The bacterial strains *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* were procured from the National Collection of Industrial Micro organisms, Pune, Maharashtra. All the media chemicals were obtained from Hi-media chemicals, India. A precursor for the synthesis of the nanoparticles was obtained from Merck. Sample powders of Reactive Blue HERD (Blue) and Orange M2R cold brand (Orange) were collected from DKTE's Textile & Engineering Institute, Ichalkaranji, Maharashtra, India.

B. Extracellular synthesis and characterization:

Extracellular synthesis of AgNPs was carried out as described below. Vegetative inoculums were developed by using the spores of *Beauveria sp.* MTCC 5184 were inoculated in 5 ml of MGYB broth. This was incubated at 28°C in a rotary shaker at 140 rpm for 24 hours. After the incubation, it was transferred to 45 ml of 1% sterile glucose media and further incubated at 28°C in a rotary shaker at 140 rpm for 48 hours. Cell-free supernatant was collected by centrifugation of broth for 15 minutes at 9000 rpm and collected the supernatant. To the supernatant, 1 ml of 10 mM AgNO₃ was added and inoculated in the water bath at 70°C for 2 hours. Confirmation of AgNPs production was marked by a color change from white to brown after 2 hours. Further, the biophysical properties of AgNPs were evaluated by using various techniques. UV-visible spectroscopy of a liquid sample of the nanoparticles was done in the range of 300 nm to 900 nm by Systronics (India) limited double beam UV-Visible spectrophotometer Au- 2704-X. FTIR of the lyophilized AgNPs sample was recorded from 5000 to 500 by Jasco FTIR 4100. The XRD characterization was performed on an X-ray diffractometer Equinox 100 Thermo scientific operated at 40kV, over the 2θ range of 20°–80°. For XRD, the samples were freeze-dried and obtained in powdered form. For TEM, EDX, and SAED patterns, a drop of AgNPs solution was placed on a copper grid (300μm mesh) and allowed to air-dry. After drying, the particles were examined under JEOL Model 1200 EX. The Zeta potential and size of AgNPs were measured by Brookhaven Zeta potential analyser.

C. Dye degradation activity:

In order to get the absorption maxima of both the dyes, UV-Vis spectral analysis (300 nm to 900 nm) was performed before the dye degradation study. Dye degradation activity was performed by mixing 1ml of dye with 1% (v/v) of 0.1M sodium borohydride and 1% (v/v) AgNPs suspension at room temperature and neutral pH. Dye degradation activity was estimated as described by [20].

D. Effect of dye concentration on degradation activity

Different concentrations of individual dyes, namely Orange M2R cold brand and Reactive Blue HERD from 60 to 100 ppm were studied for effective dye removal. A reaction mixture containing 1 ml of dye solution at neutral pH, 1% (v/v) of 0.1M sodium borohydride, and 1% (v/v) AgNPs solution, was incubated at room temperature.

E. Effect of a mixture of dyes on degradation activity

To study the effect of a mixture of two dyes, orange and blue dyes were mixed in a 1:1 ratio with an equal concentration of 80 ppm. This mixture was then allowed to react with 1% (v/v) AgNPs solution & 0.1 M Sodium borohydride at room temperature. All the experiments were carried out in triplicates. In all the experiments, deionized water was taken as a control instead of AgNPs. All the experiments were monitored by performing the spectral analysis of the test and control samples at a specific interval of 5 min during incubation. The degradation of dyes was calculated using the following equation.

$$\% \text{ reduction} = \left(\frac{A_o - A}{A_o} \right) \times 100$$

where

A_o = Absorbance of control,

A = Absorbance of the test.

F. Antimicrobial activity

Antimicrobial Activity of AgNPs was estimated against various opportunistic pathogens entero-bacteria, namely *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* by well diffusion method as described earlier by [21]. Serial dilutions of each culture up to 10⁻³ were made, and plating was done by spread plate technique on LB agar (Yeast extract, 0.5%; Tryptone, 1%; NaCl, 1%; Agar, 1.5%). After spreading, wells were prepared on a plate measuring 6 mm, and nanoparticle test samples were added to the well. As a control, the nano particle precursor, i.e., AgNO₃, distilled water, and supernatant of *Beauveria sp.*, was also taken in a separate well. The plates were observed for azone of inhibition after 24 hours of incubation at 37°C.

III. RESULTS

A. Synthesis and Characterization

AgNPs were synthesized using *Beauveria sp.* MTCC 5184 culture filtrate is described in the materials and methodology section. After the synthesis of AgNPs in 2 hours, the supernatant became brown (Figure 1). The absorption spectrum of the colored supernatant was recorded in the 200-800 nm range on a UV-visible spectrophotometer. This absorption spectrum has shown a peak centered at 439 nm (Figure 2). TEM images of synthesized AgNPs, as shown in Figure 3(a) to 3(f), clearly shows nanoparticles with a spherical appearance. EDX image shown in Figure 3(g) indicates the presence of elemental silver. The presence of Cu maybe due to the copper grid used. The powder XRD pattern (Figure 4) was

recorded for the phase recognition exhibited by the AgNPs. The Ag NPs indicated five diffraction peaks at 2θ values of 38.28° , 44.48° , 64.76° , and 77.69° , which correspond to the (111), (200), (220), (311), and (222) planes of face-centered cubic. FTIR spectrum shows absorption bands at 3335, 1748, 1659, 1290, and 1236 cm^{-1} (Figure 5). The Zeta potential and DLS analysis are presented in Figures 6(a) & 6(b). DLS analysis indicated the size of the particles ranging from 1 nm to 200 nm, and the zeta potential was found as an average of -11.51 mV.

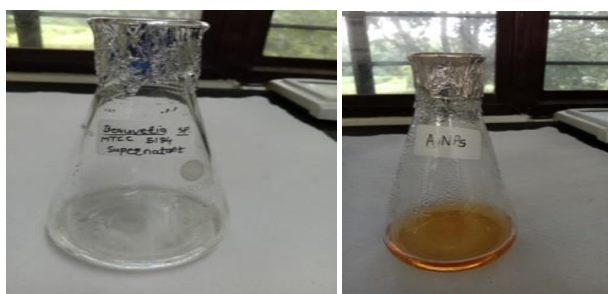


Fig. 1(a): Control

Fig. 1(b): Test

B. Degradation of dyes

a) Effect of dye concentration

Dye degradation activity was checked using the dye concentration from 60 to 100 ppm. Maximum absorbance for Reactive Blue HERD was obtained at 605 nm, and for Orange M2R cold brand, it was at 497 nm (Figure 7). Hence, the absorbance of control and test (Reaction mixture) for Reactive Blue HERD was studied at 605 nm, and for Orange M2R cold brand, it was studied at 497 nm. Spectral analysis of the test sample during the incubation period revealed that the absorption spectrum of the sample reduces with time, indicating a reduction of the dye, which could be observed visually as well [Figure 8 (a), 8 (b), S1 & S2]. The dye degradation activity of AgNPs was approximately 96 to 99% up to 80 ppm. With increasing concentration of dyes, i.e., at 90 and 100 ppm, the dye degradation activity decreased [Figure 9(a) & 9(b)]. Hence all the further experiments were carried out at 80 ppm fixed concentration.

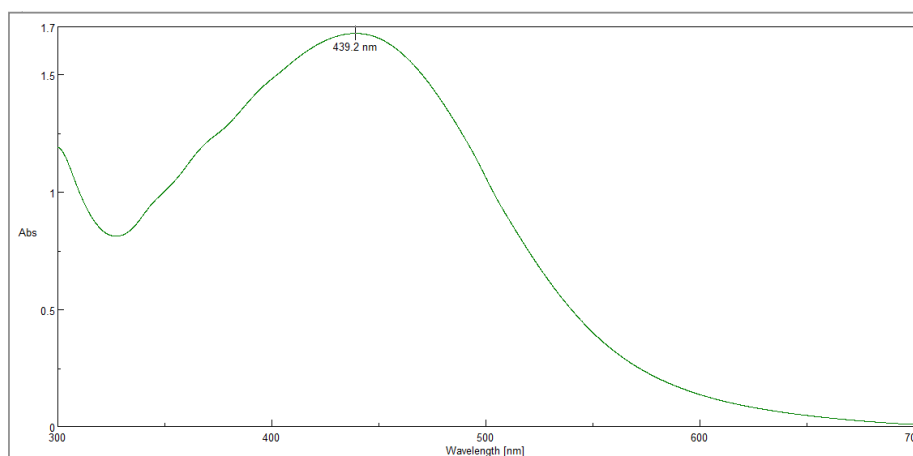


Fig. 2: UV-visible spectra of synthesized AgNPs

b) Effect of a mixture of dyes

When the degradation activity was checked with the mixture of two dyes (80 ppm concentration), both dyes were reduced effectively within 35 minutes. Figure 9 indicates the degradation of the mixture. Degradation of the mixture took slightly more time than the individual dye. However, it was also illustrated from a spectral analysis that blue dye degraded at a faster rate than orange.

c) Antimicrobial potential of AgNPs

Antimicrobial activity was carried out according to the protocol mentioned in the materials and methodology. To confirm the activity was exclusively by AgNPs, three control samples, viz., a precursor for the synthesis of the AgNPs, i.e., AgNO_3 in two different concentrations, distilled water and the supernatant of glucose media, were used. It can be concluded from Figure 11 that AgNPs show very effective antimicrobial properties against various bacteria, viz., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Table 1 represents the diameter of the inhibition zones due to antimicrobial activity.

IV. DISCUSSION

As described in the results, the supernatant with precursor undergoes a color change from colorless to brown, indicating the synthesis of AgNPs. The color change is in agreement with the results obtained by [8]. According to the literature available, AgNPs show a characteristic peak in the region between 350-450 nm. This typical peak appears due to the Surface Plasmon Resonance [22]. A characteristic peak range of yeast-derived AgNPs was obtained between 350 nm-450 nm [23]. The highest absorbance peak of AgNPs produced using plant extracts was 372 nm [24]. Jyoti et al. reported a peak of AgNPs at 419 nm [25]. Chauhan et al. reported an absorption peak at 420 nm of biosynthesized AgNPs by using *Pichia fermentans* [26].

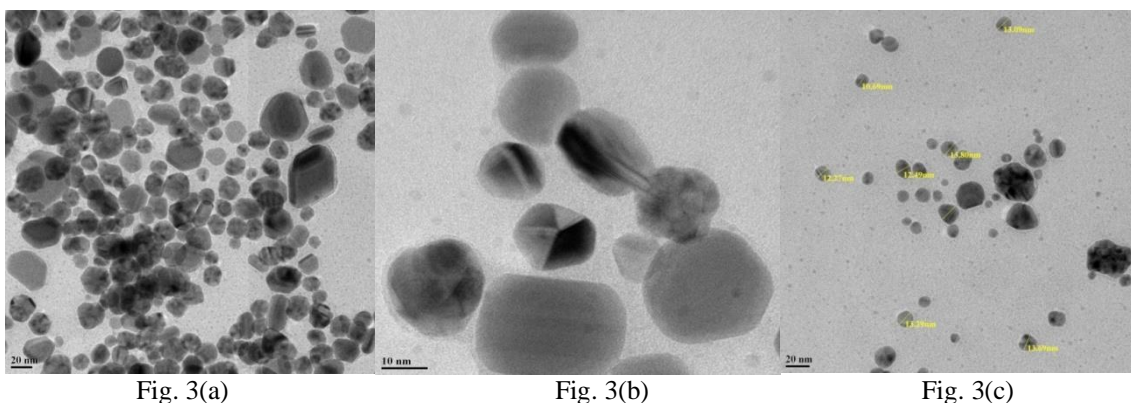


Fig. 3: Transmission electron micrographs and EDX image of AgNPs at various scale bar sizes of the insets. Figure 3 (c) presents diameter of the particles

Perhaps due to the presence of encapsulated proteins, there was a peak before 300nm[27, 28]. There was no evidence of flocculation even after two months of nanoparticle synthesis, indicating the myco-synthesized nanoparticles' long-lasting stability.

The FTIR is useful for analyzing the functional groups required to maintain nano particles stable and capped[22]. The basic principle behind FTIR analysis is that groups and bonds tend to vibrate at specific frequencies. The vibrations can be in the form of stretching or bending, depending on the nature of the bond or group.

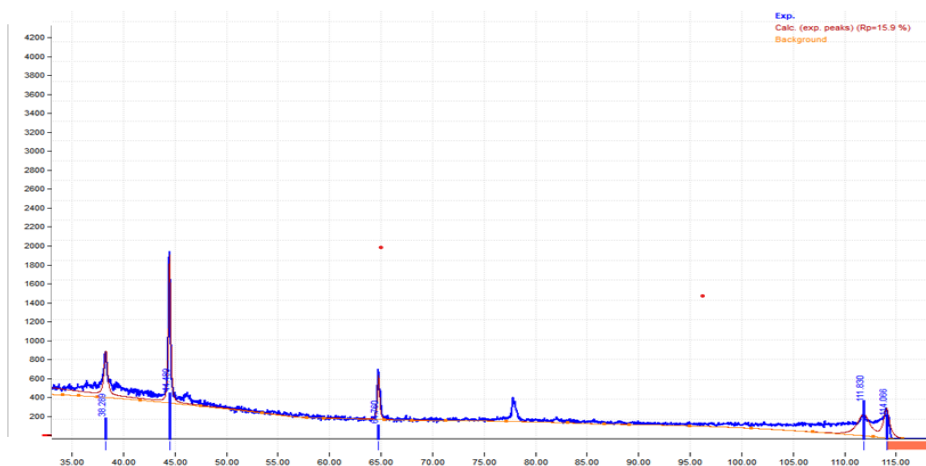


Fig. 4: X-ray diffraction pattern

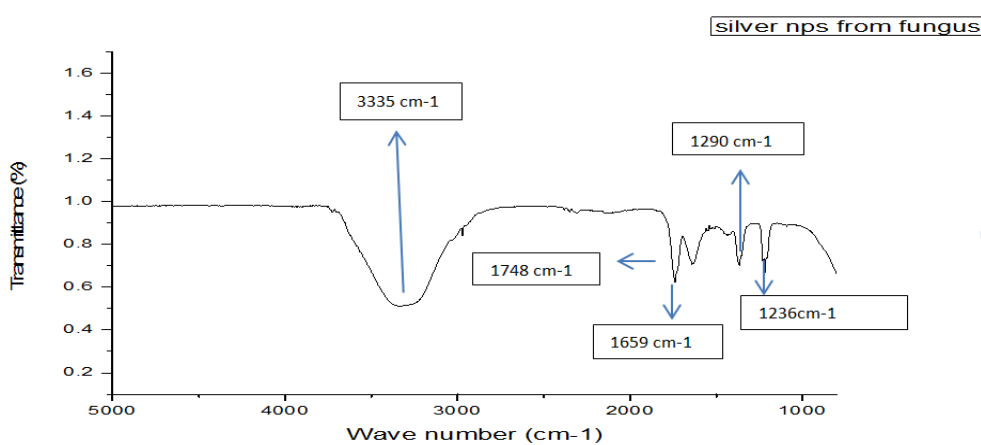


Fig. 5: FTIR of the nano particles

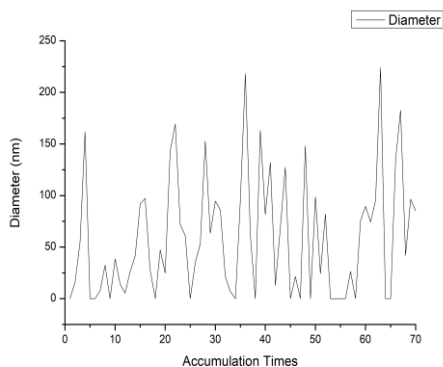


Fig. 6(a): DLS of the AgNPs

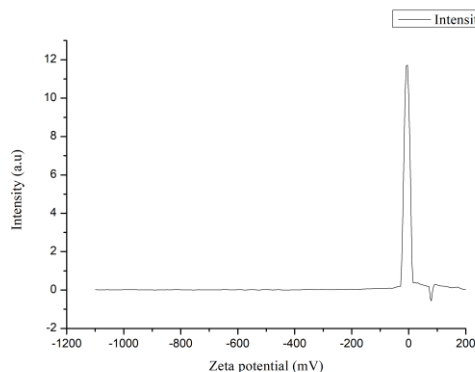


Fig. 6(b): Zeta potential of AgNPs

In the infrared spectrum, the single band in the region of 3350-3310 cm^{-1} indicates vibrations of N-H stretching. In the current spectrum, the band that appeared at 3335 cm^{-1} indicates the same. Overtone bands show the characteristic peak in the range between 2000 and 1650 cm^{-1} . The band that occurred at 1748 cm^{-1} indicates the overtone band of C-H bending vibration (aromatic compound). Stretching of the C=N bond occurs between 1690-1640 cm^{-1} . Hence, the band at 1659 cm^{-1} attributes to the stretching of the C=N bond. The band is at 1290 cm^{-1} , perhaps because of aromatic amine stretching. Amines display C-N stretching in the range of 1020-1250 cm^{-1} . So in the spectrum, the band at 1236 cm^{-1} indicates the stretching of amine [8]. From the UV-Visible and FTIR studies finding, it can be predicted that proteins from fungal broth reduce the precursor and behave as a capping material on nano particles. The capping of proteins gives nano particle stability, which has been thoroughly reported. Extraction, purification, and identification of the proteins involved in nano particle formation and stability are now being carried out in our laboratory. The TEM is an important technique for determining the morphology of nano particles. AgNPs with a size between 10-20 nm has been documented [28-30]. Nano particles that are spherical and smaller in size have more antibacterial action than those that are larger [25]. The display of C & O indicates the capping of proteins on the AgNPs [28]. The shiny circular bands in the SAED image [Fig. 3(h)] indicate various planes of AgNPs [30]. The negative zeta potential value of the AgNPs indicates repulsion among the particles [10, 30]. Shahzad et al. studied size-controlled mycosynthesis nanoparticles where the zeta potential of the nanoparticles is -9.91 mV [31]. Hence, the particles in the medium are well separated, ensuring the particles' stability. Degradation of the dye refers to the reduction of dye molecules by Sodium borohydride, where AgNPs act as a catalyst by providing a larger surface area for the electron exchange [32]. Various reducing agents have been used to reduce the dye. Malakootian et al. reported 98% removal of the reactive blue 19 by combining AgNPs and hydrogen peroxide [33]. AgNPs loaded on activated carbon could efficiently remove methyl orange. One can reduce the dye in the presence of UV light as well [34]. However, NaBH_4 is used preferably because of its high availability & low cost. The reaction between strong nucleophilic NaBH_4 & electrophilic dye molecules results in the degradation of dye molecules to form CO_2 , H_2O , etc. which are non-hazardous [35]. Among the various

concentrations of both the dyes (60 ppm to 100 ppm), effective results (90% to 100%) were obtained at up to 80 ppm. Hence, the study of dye removal for the mixture was carried out by keeping the dye concentration at 80 ppm.

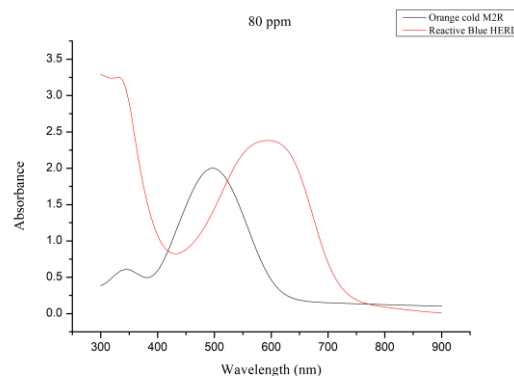


Fig. 7: Spectrum of Reactive Blue HERD & Orange M2R cold brand.

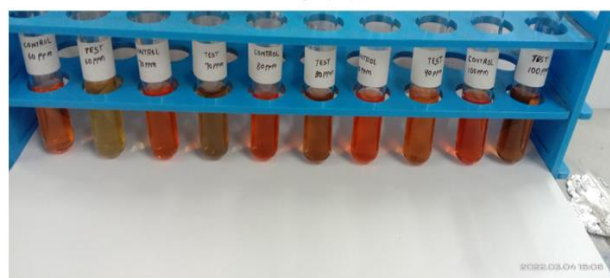
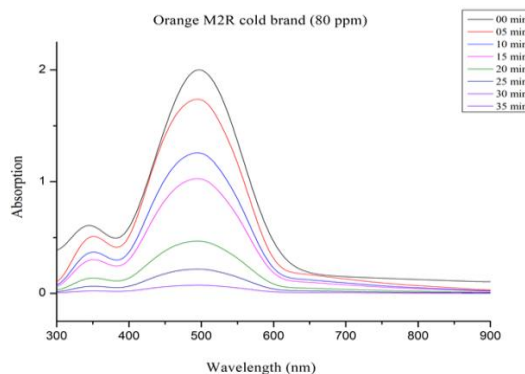


Fig. 8(a): Reduction of Orange M2R cold brand

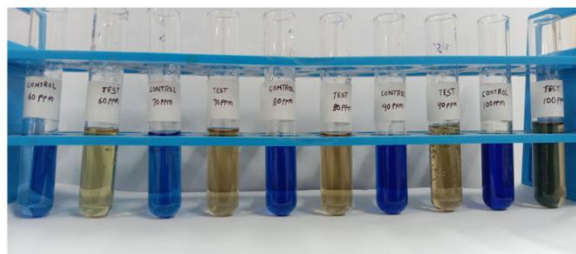
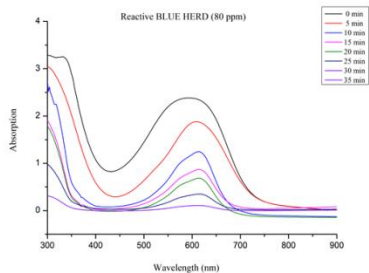


Fig 8 (b) Reduction of Orange M2R cold brand

The availability of enough catalytic surfaces is necessary to carry out the reduction process effectively[36]. At high concentrations of the dye molecules, the availability of AgNPs surface decreases for both the compounds, i.e., NaBH₄ & dye molecule[37]. This might be the reason why dye with high concentration could not get reduced properly. A low concentration of the dye molecule also decreases the interaction rate between AgNPs and dye molecules. Removal of a mixture of dyes is a necessary factor as textile industry effluent contains a mixture rather than just a single dye. Gola et al. reported the removal of a mixture of blue and orange dyes by AgNPs and NaBH₄ [20].

AgNPs generally show antibacterial activity and are commonly used in medical industries. The inhibition zone is shown by nanoparticles synthesized by *Beauveria sp.* MTCC 5184 proves that they are antibacterial. AgNPs have been studied for antibacterial potential towards various bacteria, including *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Yersinia pseudotuberculosis*, *Serratiamarcescens*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* has been reported[38].

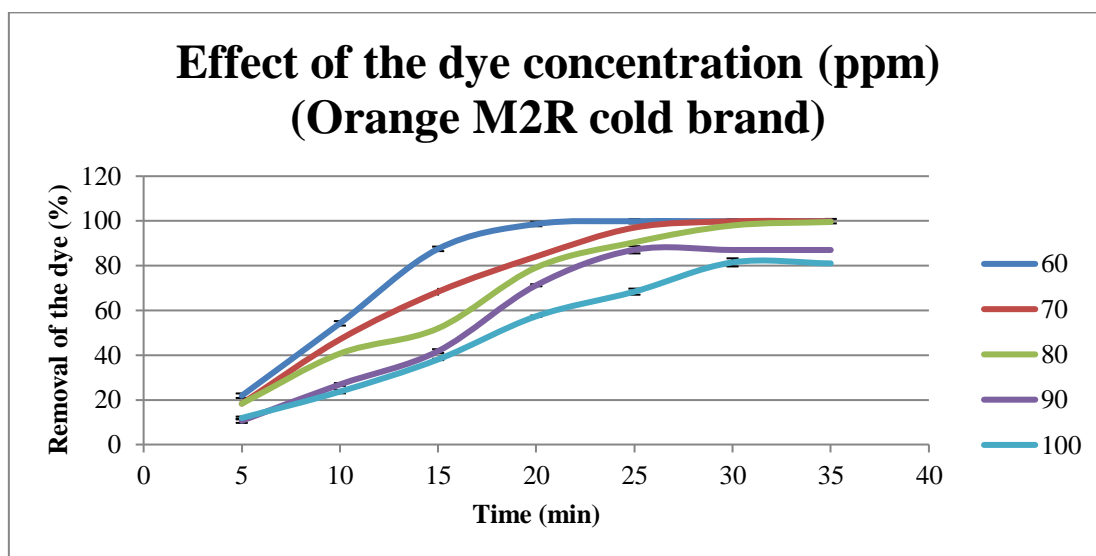


Fig. 9(a): Effect of dye concentration: Orange M2R cold brand

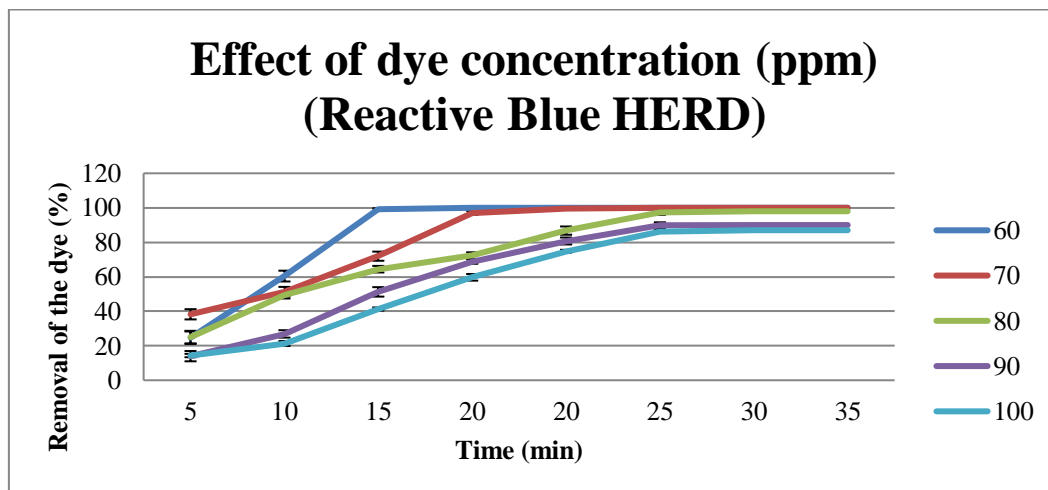


Fig. 9(b): Effect of dye concentration: Reactive Blue HERD

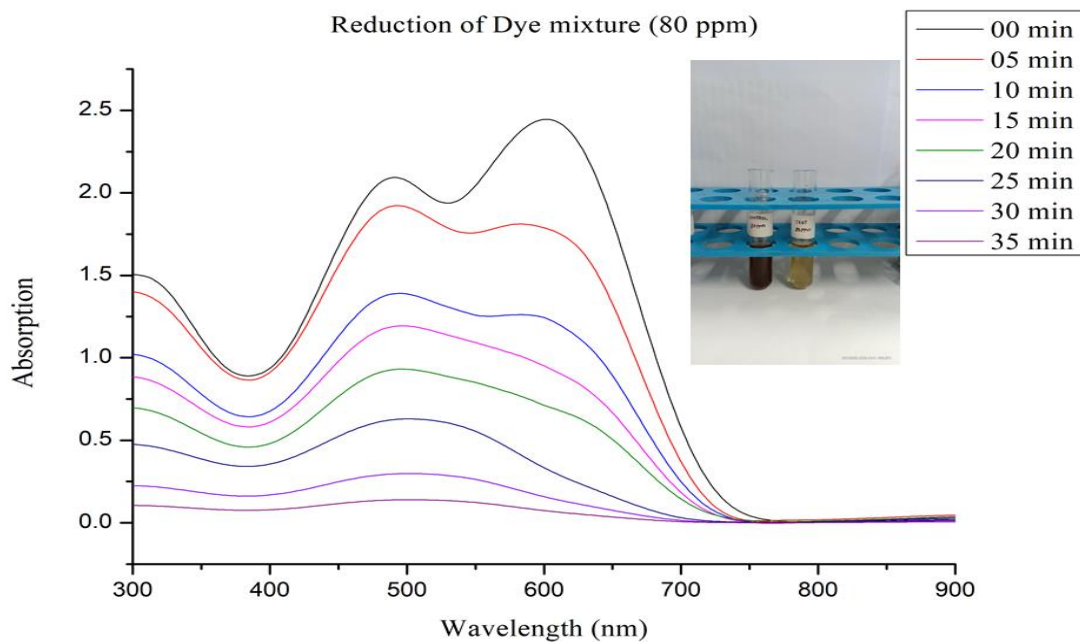


Fig. 10: Reduction of Dye mixture

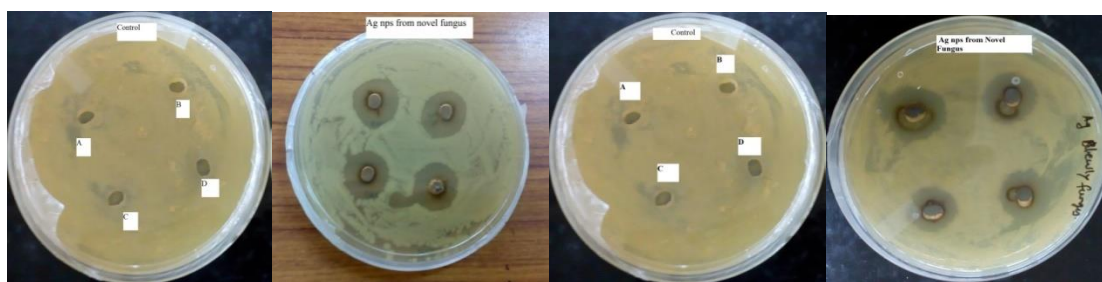


Fig. 11: (a) (b) (c) (d)

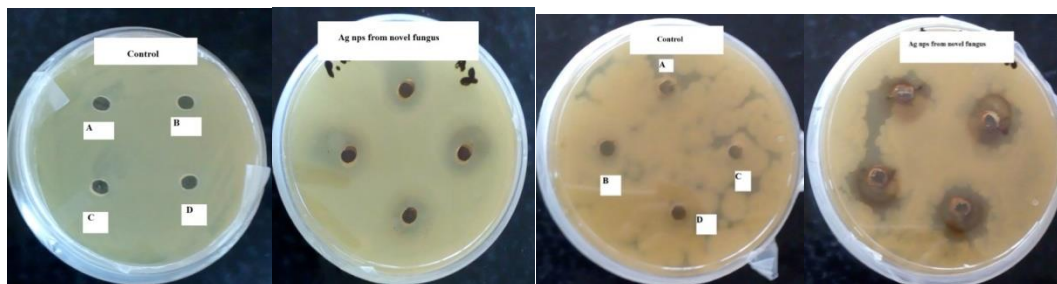


Fig. 11: (e) (f) (g) (h)

Fig: 11 (a), 11 (c), 11 (e) and 11 (g) are control in this (A) Distilled water as control, (B) Fungal supernatant of *Beauveria sp.* MTCC 5184 as control, (C) Precursor solution 10mM (D) precursor solution 100mM. Figure 11 (b), 11 (d), 11 (f) and 11 (h) shows the inhibition zone that formed by AgNPs against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* respectively

Organism	Average diameter of Inhibition zone of AgNPs
<i>Escherichia coli</i>	23 mm
<i>Staphylococcus aureus</i>	13 mm
<i>Pseudomonas aeruginosa</i>	12 mm
<i>Bacillus subtilis</i>	09 mm

Table 1: Average inhibition zone of AgNPs

AgNPs synthesized by using various fungal strains have been demonstrated to have antibacterial properties. The antimicrobial efficacy of AgNPs against *E. coli* and *S. aureus* was investigated by Li et al. using nanoparticles synthesized by cellular extracts of *Aspergillus japonicus*[39]. AgNPs synthesized using *Colletotrichum sp.* ALF2-6 also showed antibacterial activity[40]. The present study showed that the fungal strain *Beauveria sp.* MTCC 5184 is capable of AgNPs biosynthesis, having a size range between 10-200 nm, and the synthesized nanoparticles exhibit antibacterial activity. It has been confirmed that nanoparticles having smaller size shows more effective antimicrobial potential than larger-sized nanoparticles[41, 42]. It is hypothesized that these nanoparticles enter the cell by adhering to its cell wall membrane, causing damage to the cell organs, the generation of reactive oxygen species, and a change in the cell signalling pathway[42, 43].

V. CONCLUSION

A novel strain, *Beauveria sp.* MTCC 5184 was used for the biosynthesis of AgNPs. Characterization of AgNPs was carried out using UV-visible spectroscopy, TEM, FTIR, XRD, EDX, zeta potential, and DLS. Synthesized nanoparticles showed high catalytic activity for the reduction of two Azo dyes used in the textile industry: Reactive Blue HERD and Orange M2R cold brand. The AgNPs showed effective dye degradation activity even with the mixture of these two dyes. The antibacterial activity of nanoparticles was also tested against four entero-pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. These results suggest that the AgNPs can be effectively used against these pathogens, and the catalytic potential of AgNPs can be explored to degrade the harmful textile dyes used in the textile industry waste.

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