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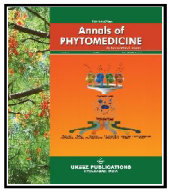

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Biosynthesis of silver nanoparticles (AgNPs) using fresh oyster mushroom extract and their antifungal activity against *Rhizoctonia solani*

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Abstract

The current work aims to develop a low-cost and eco-friendly method for generating silver nanoparticles using the edible oyster mushroom extract (*Pleurotus sajor-caju*) and to evaluate the efficiency of that approach against *Rhizoctonia solani*. The capacity to precisely synthesize stabilized silver nanoparticles was evaluated using the following parameters: 0.1 Mm silver nitrate ($AgNO_3$) substrate concentration, reducing agent (2.5 and 5.0 ml mushroom extract) and dark incubation (12, 18, 24, and 72 h). Dark incubation acts as a catalyst in the synthesis and elements of the mushroom extract act as reducing and stabilizing agent. By using dynamic light scattering (DLS), FTIR, and UV-visible spectroscopy, the size of the biosynthesized silver nanoparticles was determined.

The biosynthesized silver nanoparticles had polydispersity index of 0.253 and Z average size was 99.30 nm, indicating that the surface of the silver nitrate nanoparticles was reduced and coated with oyster mushroom extract. The poisoned food technique was used to assess the *in vitro* antifungal activity of biosynthesized silver nanoparticles on *R. solani* colony formation in order to see how these AgNPs affected the mycelium growth of *R. solani*. We employed seven different concentrations of mushroom nanoparticles (150, 250, 350, 450, 550, 650, and 750 µl.). It is clear from all of the concentrations that the amount of AgNPs increased along with the amount of pathogen growth inhibition. The 750 µl doses showed the largest growth inhibition (49.42 per cent), whereas the 650 µl concentrations only saw growth of 29.81 per cent.

The implementation of the oyster mushroom in the development of green synthesis of nanoparticles enhanced biological control of *R.solani* at higher concentration of 750 µl.

1. Introduction

In human devices, silver nanoparticles are used as a therapy as antibacterial, antifungal, antiviral and anti-inflammatory agents; however, their potential use in agricultural systems against plant viruses has not yet been investigated. Biogenic nanoparticles are those that can be created by the use of organic processes in nanoscale synthesis. It is the safest, most cost-effective and environmentally responsible method for producing AgNPs, Hence, the term “green synthesis” is also used. Because it does not use any chemical compounds, it is also the most reliable method for producing AgNPs. According to Lee *et al.* (2010); Torresdey *et al.* (2003), the use of plants, yeast, bacteria and fungus as well as other organic resources in the production of nanoparticles is a clean, safe and cost-effective method. A large variety of microorganisms are known to be poisoned by silver and silver nanoparticles have shown promise as effective antimicrobial substances (Jain *et al.*, 2009). Oyster mushrooms, or *P. sajor-caju*, are a popular type that can be eaten. Oyster, also known as the common oyster, is a member of the *Pleurotaceae* family and has a variety of documented therapeutic properties, including those for diabetes, HIV, cancer, immune modulation, antibacterial, antiviral, anti-inflammatory, antifungal, and decreasing LDL cholesterol (Shamtsyan *et al.*, 2004; Wang *et al.*, 2002; Ahmad *et al.*, 2002, 2003; Kim *et at.*, 2015).

The remarkable characteristics of nanoparticles, such as biocompatibility, excessive productivity, speed of production, cost effectiveness and protection, have an impact on biomedical food, and engineering programmes. Nanotechnology is a field that is unexpectedly rising. Because of full-size advancements produced with nanotechnology, such as improvements in healthcare programmes, humanity has benefited greatly from the development of this field of study (Liu *et al.*, 2010). Silver nanoparticles (AgNPs) are thought of as fantastic nano-guns that can be used as active antimicrobial sellers in the eradication of multidrug-resistant (MDR) microorganisms (Rai *et al.*, 2012). Fungi have been successfully employed in several studies to reduce retailers in the synthesis of AgNPs (Mukharjee *et al.*, 2001; Bhainsa and Souza, 2006). The medicinal properties of *Pleurotus* plants have a long history. Aqueous *Pleurotus florida* extract, an edible oyster mushroom, has been used as a lowering agent and picture-irradiated extracellular AgNP generation, has also been covered previously (Bhat *et al.*, 2011). In less than 30 min, according to Jagadheeswari *et al.* (2020), silver nanoparticles were created using *W. somnifera* plant leaf extracts, and their antibacterial efficacy against food pathogens

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was assessed. Extreme challenges have been presented to the ability of various synthetic fungicidal compounds to remain effective against various fungi in order to protect plant life. Because of their constant exposure to the environment, plants are particularly susceptible to multiple fungal attacks. *R. solani* is a plant pathogenic fungus that has a wide range of hosts and a widespread distribution. It was discovered in a location from more than a century ago. Known for producing a number of plant diseases, including wire stem, root rot, damping off and shadow rot; *R. solani* is regarded as a soil-borne pathogen. *R. solani* attacks hosts that are still developing, such as seeds and seedlings, which are often found in the soil. The plant is more susceptible to infection due to a variety of environmental conditions, including *Rhizoctonia*, which prefers warmer, moister temperatures for contamination and growth. Additionally, *R. solani's* attack is delayed by post emergent damping off. The early stages of the seedling are when it is most susceptible to illness (Cubeta and Vilgalys, 1997). The evaluation of the *R. solani* inhibitory action of synthesized AgNPs derived from mushroom extract; however, was not disclosed. In the current study, oyster fit for human consumption, mushroom extract was employed as a lowering agent for the green synthesis of AgNPs, along with their characterization by UV spectroscopy, DLS, FTIR and Xeta ability. AgNPs generated were further evaluated for their antifungal inhibitory capacity (Zhang *et al*., 2016).

2. Materials and Methods

A fresh, mature fruit body of the oyster mushroom was cultivated in Mushroom Research Laboratory at the Department of Plant Pathology, Anand Agricultural University, Gujarat, India and was used along with AR-grade silver nitrate ($AgNO_3$). For the creation of silver nanoparticles, milli-Q water (18.2MQ) was employed.

2.1 Cultivation of mushroom

From the mushroom research lab, the mother culture for spawn production was brought. According to the method described in Randive (2012), a species of *P. sajor kaju* was grown in the Mushroom Research laboratory.

2.2 Prepration of mushroom extract

A sterile knife was used to cut the basidiocarp into small pieces. After being cleansed under running water to remove any dust, rinsed with double-distilled water and allowed to air dry for 30 min in the sun, the pieces were then crushed in a mortar with liquid nitrogen. 10% crushed mushroom powder and 100 ml of milli Q water should be combined in a flask. For 40 min, boil the flask. The extract was centrifuged for 10 min at 8000 rpm after cooling to room temperature and filtering through muslin linen. In order to use it later, the supernatant was stored at 4°C and used as an extract in the environmentally friendly production of silver nanoparticles.

2.3 Effect of two factors, concentration of mushroom extract and incubation period on the production of silver nanoparticles

The final volume was increased to 100 ml by adding 2.5 and 5.0 ml of mushroom extract to 97.5 and 95 ml, respectively, of freshly produced 0.1 mM silver nitrate solutions. Different lengths of dark incubation were applied to the treatments (12, 18, 24 and 72 h). Ag^+ had been reduced to Ag^0, as evidenced by the solution's colour changing from yellowish to reddish brown.

2.4 Characterization of environmentally friendly silver nanoparticles

The UV-visible spectra of the samples were used to identify the bioreduction of the Ag^+ to Ag^0 ion spectrum. The finding of silver nanoparticles was made possible *via* UV-visible spectroscopy. Using the Beckman Coulter DU730 visual spectrometer, the absorbance spectra of the colloidal material were measured. Using a zetasizer Nano ZS90, measurements of size, PDI and zeta potential were taken (Malvern Instruments Ltd., U.K.). Using 1 mg of dried AgNPs and 100 mg of potassium bromide (KBr), a potassium bromide (KBr) pellet was produced. In the following step, the pellet was analyzed using FTIR Perkin Elmer Spectrum II spectroscopy. For each spectrum, scans with a spectral resolution of 4 cm^{-1} were gathered in the 400-4000 cm^{-1} range.

2.5 Antifungal activity of green synthesized mushroom silver nanoparticles

R. solani's pure culture was acquired from the Plant Pathology Department of the B.A. College of Agriculture at Anand Agriculture University, Anand. Utilizing the poisoned food technique, the relative effectiveness of biosynthesized mushroom silver nanoparticles was assessed *in vitro* at various concentrations of 150, 250, 350, 450, 550, 650, and 750 µl (Grover and Moore, 1962). After preparing the potato dextrose agar medium, it was sterilized for 20 min at 15 psi. PDA was combined with AgNPs before being poured into petri plates and cultured for 72 h at room temperature before antifungal action. A culture bit of 5 mm size of the test pathogen (*R. solani*) cut with a sterilized cork borer, pick up with the help of sterilized inoculation needle and place in the centre of each petri plate under aseptic conditions in the laminar airflow system. Petri plates poured with PDA and inoculated with the pathogen were served as control. Under a completely random design, each silver nanoparticle concentration was replicated three times in petri plates that were incubated at a temperature of 28 ± 1°C. From 24 h of incubation at 28°C until the test pathogen had fully grown on control plates, the radial growth was observed and the growth inhibition over control was computed using the method provided by Vincent (1947).

3. Results

3.1 Silver nanoparticles characterization

3.1.1 Effect of two substrate concentration and incubation time

By combining aqueous 0.1 mM $AgNO_3$ and two different volumes of mushroom extract, *i.e*., 2.5 ml and 5 ml, respectively, under dark incubation for 12, 18, 24 and 72 h, silver nanoparticles were produced in a green manner. The colour altered from yellowish to reddish brown at 72 h as a result of an increase in dark incubation time. With prolonged dark incubation periods, the colour changed from yellowish to reddish brown, as shown in Figure 1.

3.1.2 UV-Visible spectrophotometery

The generated green silver nanoparticles were identified by UV-visible spectroscopy. From the UV spectrometry analysis and absorbance peaks, it can be inferred that the sample 2.5 ml mushroom extract has an absorbance peak in the visible range. The absorbance peak was found to be at 480 nm. The sample, which included 97.5 ml of 0.1 mM $AgNO_3$ and 2.5 ml of mushroom extract, underwent additional characterization tests. Accordingly, the evidence suggests that mushroom extract aids in and promotes the transformation of silver nitrate into silver nanoparticles.

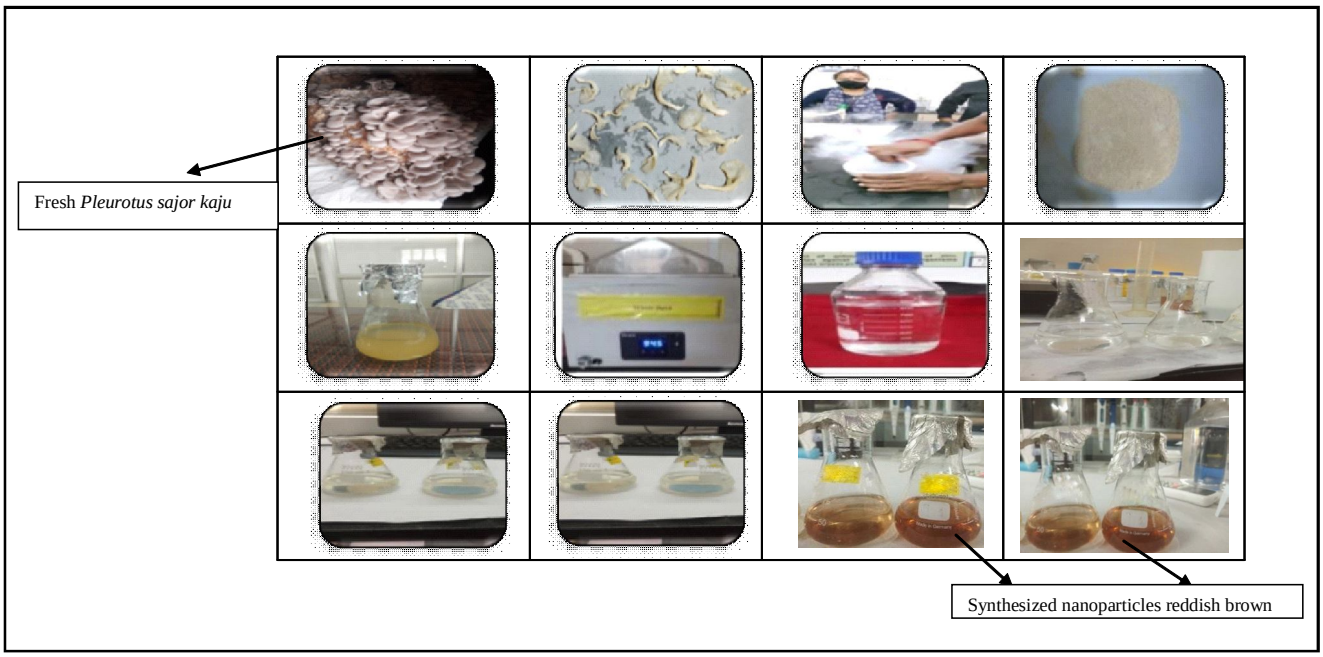

Figure 1: Synthesis of silver nanoparticles: Visual observation of the change in the reaction mixture from yellowish to reddish brown colour upon addition of silver nitrate solution.

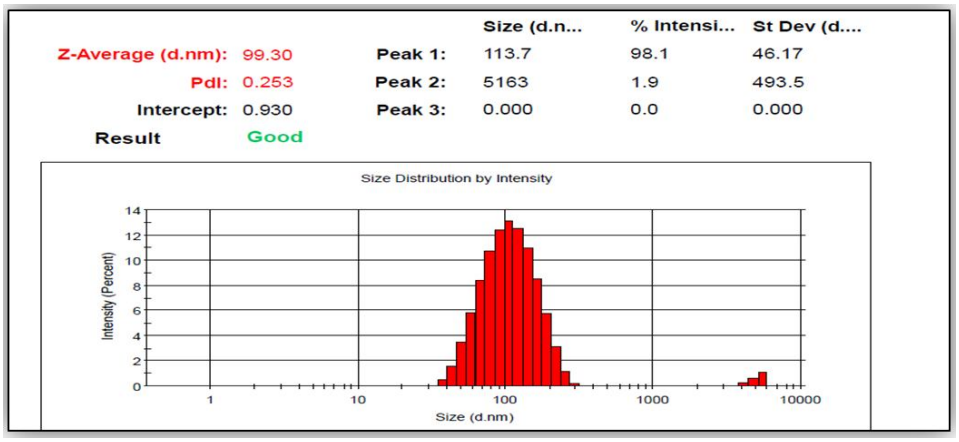

Figure 2: Polydispersity index of mushroom nanoparticles.

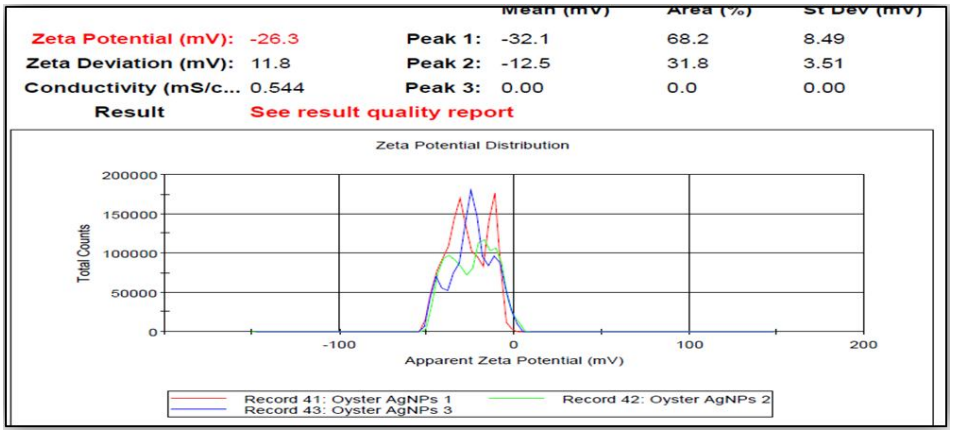

Figure 3: Zeta potential of mushroom nanoparticles.

3.1.3 Dynamic light scattering (DLS)

Mycosynthesized AgNPs exhibited a Z average diameter of about 99.30 nm and a polydispersity index of 0.253, which indicated that they were highly dispersed in aqueous medium, according to the DLS patterns utilized in the current work (Figure 2). These largely concur with the hypothesis that exposure time significantly affects particle size. The quantity of mushroom extract utilized is also an important element in the conversion of Ag^{+} to Ag^{0}.

3.1.4 Zeta potential (mV)

Synthesized mushroom nanoparticles were characterized by using the zeta potential of mycosynthesized AgNPs was –26.3, indicating that AgNPs are very stable in aqueous media (Figure.3).

3.1.5 FTIR analysis

For the current experiment, the FTIR spectrum of mushroom AgNPs was collected in order to identify the functional groups present in the mushroom extract that were responsible for the reduction and capping of AgNPs. The functional groups of the mushroom extract were strongly imprinted on the AgNPs, as shown by the FTIR spectrum of the generated AgNPs, which is shown in Figure 4. The vibration stretches at 3334.02 cm-1 (0-H stretch of alcohol), 2110 cm-1, 1635.43 cm-1 (C=C band of cyclic alkene) and 621.07 cm^{-1} were discernible in the FTIR spectra of the mushroom AgNPs (C-Br stretch of halo compound). The mushroom extract's hydroxyl, amide and carboxyl functional groups were found to be essential for the reduction, capping and subsequent stability of the mycosynthesized AgNPs as a result of this vibrational stretching.

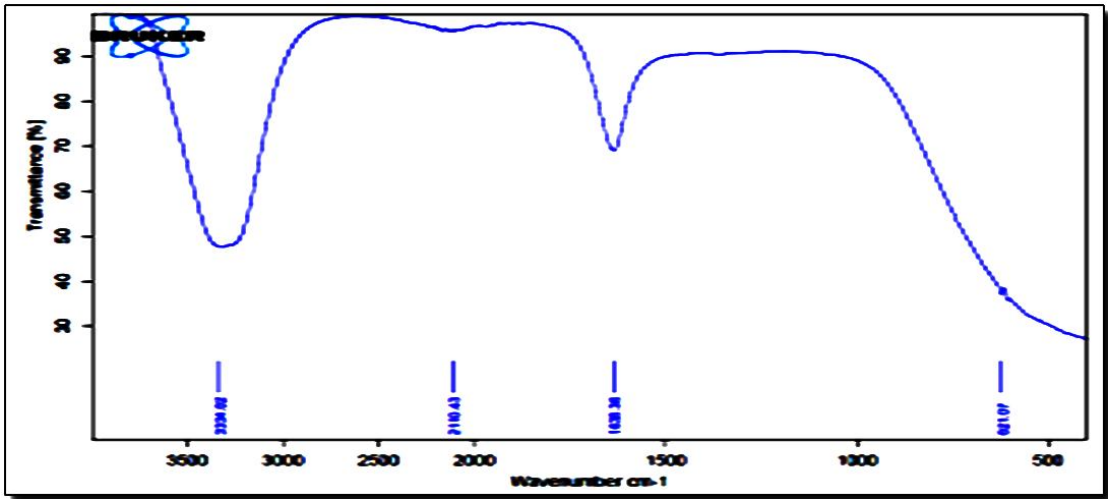

Figure 4: FTIR analysis of nanoparticles.

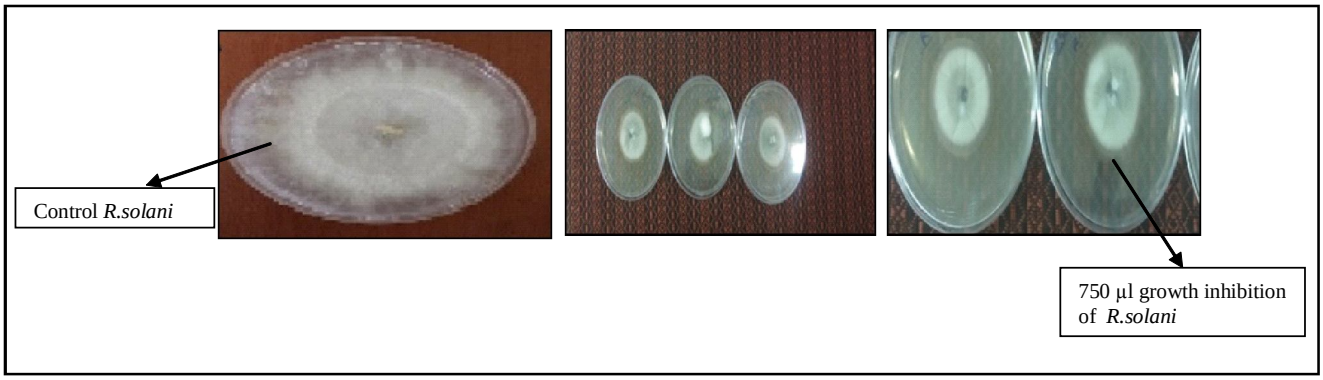

Figure 5: Growth inhibition of *R.solani* by different concentration of synthesized mushroom nanoparticles compared to control.

3.2 Antifungal activity of green synthesized mushroom sliver nanoparticles

The poisoned food technique was used to assess the relative effectiveness of biosynthesized silver nanoparticles under *in vitro* conditions at various concentrations of 150, 250, 350, 450, 550, 650, and 750 µl (Grover and Moore, 1962). In the current work, silver nanoparticles (AgNPs) made from mushrooms using green synthesis were tested on *R. solani* to see if they had any negative effects on mycelial growth. The data show that as in compared to the control treatment; AgNPs considerably reduced the mycelial growth of *R. solani* at various doses (Table 1, Figure 5). The most effective therapy out of the seven was biosynthesized mushroom AgNPs at a concentration of 750 µl, which inhibited *R. solani's* growth by 49.42 per cent. The following successful treatment was 650 µl of biosynthesized AgNPs, which gave a growth inhibition of 29.81 per cent, followed by AgNPs at a concentration of 550 µl, which gave a growth inhibition of 29.02 per cent. According to the data, there was a proportional rise in the amount of pathogen growth inhibition as AgNP concentration increased.

Table 1: Effect of different concentration of mycosynthesized mushroom nanoparticles on growth inhibition of *R.solani*

Trt. No.	Treatments	Concentration (Microlitre)	Mycelial growth (mm)	Growth inhibition (%)
T_1	Synthesized nanoparticles (2.5 ml extract + 75.5 ml 0.1 mM $AgNO_3$)	150 µl	83.00	2.36
T_2		250 µl	74.66	12.16
T_3		350 µl	72.33	14.90
T_4		450 µl	65.66	22.75
T_5		550 µl	60.33	29.02
T_6		650 µl	59.66	29.81
T_7		750 µl	42.99	49.42
T_8		Control	85.00	0.00

4. Discussion

Silver nanoparticles were created using a biosynthetic technique. Extract from *P. sajor-kaju* was used to create the silver nanoparticles. By adding the two mushroom extract concentrations to a silver nitrate solution, the ideal volume yielding the best results was discovered. The extract and silver nitrate solution were incubated together in the dark for up to 72 h. Pale yellow to reddish brown coloration changed as silver nitrate was broken down into silver nanoparticles. Just 72 h after incubation, the colour changed. As a control, a continuous silver nitrate solution exhibited no colour change.

Mulvaney (1996), has shown the effect of incubation period in the reaction mixtures of AgNPs' is due to surface plasmon resonance (SPR), which results in a colour change. The size, shape and dispersion of the particles in the aqueous suspensions have a major impact on the position and shape of the SPR band of AgNPs. AgNPs of *P. cornucopiae* var. *citrinopileatus* exhibited absorption peaks with a maximum absorption band between 420 and 450 nm.

Tomaszewska *et al.* (2013), like the present study, used the DLS approach relies on how light interacts with particles. This technique is useful for measuring narrow particle size distributions, especially between 2 and 500 nm. According to Murdock *et al.* (2008), the DLS technique is used to produce a particle's hydrodynamic radius, or diameter, polydispersive index and counts rate. Zeta potential, which is important for a range of applications, was used to examine the stability of mushroom silver nanoparticles. The requirements for NP stability were evaluated by Zhang *et al.* (2008), when the zeta potential values ranged from greater than + 30 mV to – 30 mV. The particles' behavior makes it obvious that they have substantial electric charges on their surfaces that are intended to prevent aggregation. Higher zeta potential nanoparticles tend to inhibit the development of aggregates, whether negative or positive (Rai *et al.*, 2015). Therefore, the more zeta potential there is, the more stable nanoparticles will be in solution (Raheman *et al.*, 2011). Zeta potential data on the negative side show how successfully the capping materials stabilize nanoparticles by producing strong negative charges that keep the particles away from one another (Haider and Mehdi, 2014). Senthilkumar and Sivakumar (2014), have shown that mushroom extract contains bimolecules that were crucial in the production and stability of nanoparticles, and FTIR is a highly effective method for identifying these molecules. Mushrooms are excellent suppliers of minerals including potassium, phosphorus and magnesium as well as vitamins like thiamine, riboflavin and niacin. They are also rich in proteins and amino acids, polysaccharides/oligosaccharides complexes (Cho *et al.*, 2013) and minerals (White *et al.*, 2002). By using UV-visible spectroscopy, FTIR, a particle size analyzer and SEM, silver nanoparticles were created and analyzed by (Sudha *et al.*, 2020). According to the findings, *S. ficaria's* crude keratinase enzyme is a potent bioreductant. Wali *et al.* (2019), the findings of his research demonstrated an understanding of the phenolic and flavonoid content of *S. officinalis* leaves, which may be responsible for the plant's potential antiradical and antibacterial effects on gram-positive pathogens. According to Thakur *et al.* (2020), *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Escherichia coli* could all grow more slowly when the Mahamanjishthadi kadha formulation was used. Malik and Mandan (2020) enhanced antimicrobial activity of as synthesized nanoparticles using natural antioxidants of plants origin. In case of antifungal activity, it was Bahera which showed the highest activity against the available test organisms followed by Amla, Harad and Triphala powder.

5. Conclusion

Utilizing mushroom extract and dark incubation, a straightforward, quick, and environmentally benign approach for the manufacture of silver nanoparticles was created. Additionally, UV-visible spectroscopic examination was carried out and the results are similar to the observed peak at 480 nm. Using a zeta sizer to measure the size of green synthetic silver nanoparticles, 72 h of dark incubation at a 2.5 ml concentration of mushroom extract were determined to be ideal. Additionally, the current study successfully exhibits the impact of mushroom extract against *R. solani*. The antifungal activity of both mushroom extract and mushroom AgNPs is enhanced by the antifungal capability as mushroom AgNP concentration increases.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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