

Research Article

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Analysis on Synthesis of Silica Nanoparticles and its Effect on Growth of *T. Harzianum* & *Rhizoctonia* Species



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Abstract

Silica nanoparticles have been prepared by sol-gel method and peptization process. The silica nanoparticles were obtained by hydrolysis of tetraethyl-orthosilicate (TEOS) in ethanol but variation in parameters improved particles size from 290 nm to 85 nm in sol-gel process and 190 nm by peptization process using nitric acid as electrolyte. Detailed study on these nanoparticles was done by characterizing nanoparticles using dynamic light scattering. Scanning electron microscopy, X-ray diffraction, FTIR analysis and UV-visible spectrophotometer. Antifungal effect of prepared nano silica was carried out in Potato dextrose agar (PDA) media using concentration of nanoparticle at 1wt% against *Trichoderma harzianum* and *rhizoctonia solani*.

Keywords: Silica nanoparticles; Temperature; TEOS; DLS; *Trichoderma harzianum*; *Rhizoctonia solani*

Introduction

Nanoparticles with size ranging between objects and microparticles have attracted much attention. These particles with various specialized functions not only deepen our understanding of nature but also serve the basic for development of new advance technology. Nanoparticles are characterized by size-dependent properties both size and surface effects are important. By controlling these, it is possible in principle to design materials of required optical, magnetic, elastic, chemical etc. properties. There is increasing interest in the design and synthesis of topological structures composed of monocrystals of various size and shapes [1]. The sol-gel methods are the most general method of synthesis of silica nanoparticles. Appetence in the sol-gel processing of ceramic and glass materials started in the half of 1800s by Ebelman and Graham's researches on silica gels [2]. The sol-gel technique is in expensive and the silica gels manufactured are non-poisonous matters [3-8]. Stober supplied monodisperse and nonporous silica spheres with the hydrolysis of tetraethyl orthosilicate (TEOS) in strongly basic medium. Stober and Fink promoted chemical reactions which checked up the growth of spherical silica particles [9]. Bogush and Zukoski procure monodispersed silica particles with controlled hydrolysis of TEOS in ethanol [10]. Sung Kyoo Park provided silica nanoparticles from TEOS in ethanol in order that controlled particle properties using a semi-batch process [11]. Ryu had prepared amorphous silica by oxidation of silicon [12-13].

In the present work, we suggested a novel method for preparing amorphous silica nanoparticles through the sol-gel techniques

at various temperature range from 78°C to 650°C to obtain best particle size even we prepared silica nanoparticles through peptization process also, using electrolyte nitric acid / hydrochloric acid / sulfuric acid for comparative study on particle size analysis to obtain best particle size below 100 nm. Another study on performed for fungal effect on human body. Fungi are heterotrophic organisms which are able to reproduce sexually as well as asexually. About 100 infectious fungal agents have been detected in man. The mycoses or fungal infection can be of various forms as:

- a) Superficial-seen on the skin, the hair, and nails.
- b) Subcutaneous-infection reaching dermis or subcutaneous tissue
- c) Systemic-internal organs infected deeply
- d) Opportunistic-infection in immunocompromised patients

Several works have been highlighted very high bactericidal efficiency on different microorganisms but there is no detail explanation for antifungal testing with silica nanoparticles against fungus that causing human disease. In this study, the effect of silica nanoparticle was observed against *Trichoderma Harzianum* and *Rhizoctonia Solani*.

Trichoderma Harzianum

Morphology: Colonies were originally hyaline darkening to white with green tufts in most species. The form was irregular which rapidly grew and merged forming green carpet like appearance.

The conidiophores were branched and hyaline. Phialides were divergent and flask-shaped. Conidia were generally green, smooth or roughened, ranged in shape from globose to ellipsoidal, and were produced in slimy heads. Conidiophores were highly branched and demonstrated a pyramidal arrangement.

Pathogenicity: *Trichoderma harzianum* infections are opportunistic and develop in immunocompromised patients, such as neutropenic cases and transplant recipients, as well as patients with chronic renal failure, chronic lung disease, or amyloidosis. Peritonitis, pulmonary, perihepatic, and disseminated infections have so far been reported. A disseminated fungal infection was detected in the postmortem examination of a renal transplant recipient and confirmed in culture. The only other reported infection by this fungus caused peritonitis in a diabetic patient. *Trichoderma harzianum* were identified as causative agents of opportunistic fungal infections with increasing frequency. *Trichoderma harzianum* isolates are reported predominantly to cause health problems in humans ranging from localized infections to fatal disseminated diseases. Thus, it is very necessary to delete the negative pathogenic aspects of fungi by using proper treatment and care [14].

Rhizoctonia Solani

Human infections caused by *Rhizoctonia* are very rare, and first case report of keratitis by *Rhizactonia* was reported way back in 1977 [15]. *Rhizoctonia solani* is a most widely recognized strong saprophyte with a great diversity of host plants. It is a first ever case of extensive human mycosis caused by *Rhizoctonia solani* in a 65-year-old diabetic and hypertensive farmer, with a history of head injury caused by fall of mud wall. Necrotic material collected revealed septate fungal hyphae with bacterial co-infection and after mycological study & diagnosis it was confirmed the presence of *rhizoctonia solani* [16]. So, in this paper we tested anti-fungal effect of silica nanoparticles against these two fungi i.e. *trichoderma harzianum* and *rhizoctonia solani* in PDA media.

Experimental

Materials

Tetraethyl orthosilicate (TEOS) were purchased from Sigma Aldrich Chemicals Pvt Limited (India). Ethanol from Fischer Scientific (India), and Ammonium hydroxide from Qualikems Fine Chemical Pvt. Ltd. (India) was purchased. Potato dextrose agar (PDA) was purchased from HiMedia Laboratories Pvt Limited (India). Instruments-UV 1800 Shimadzu UV spectrophotometer, Malvern instruments-Zetasizer Nano S-90, Shimadzu 8400 spectrophotometer and Bruker D8 Focus X-Ray Diffractometer, Bruker Universal materials tester (CETR UMT-3) SEM-Zeiss microscopy.

Methods

Synthesis of Silica Nanoparticles through Sol-Gel Method

Silica nanoparticles were prepared according to the well-known Stöber method by hydrolysis and condensation of tetraethyl-orthosilicate. 8 ml of TEOS was added in a mixture of 100 ml ethanol with 35 ml of DI water. This solution was stirred

for 40 minutes and pH was maintained at 10 by adding ammonium hydroxide drop-wise. The synthesis starts with mixing and stirring of the components, requires a reaction time of about 2hr and is finished by centrifugation at 8000 rpm for 5-10 minutes and overnight of drying at temperatures at 100°C and calcinated at 650°C for 2 hours. Same process was used to obtain best particle size value of silica nanoparticle by changing in different parameters like temperature (78°C, 100°C, 650°C) and TEOS (4 ml, 8ml, 16ml) concentration.

Synthesis of Silica Nanoparticles through Peptization Process

In a typical peptization process, a specific amount of TEOS (8 mL) was added to 100 ml of ethanol and 35 ml of DI water under continuous stirring. After 20 minutes electrolyte i.e., nitric acid or hydrochloric acid or sulfuric acid was added to this solution and obtained silica gel containing salt. This silica gel was washed with DI water to remove salt and obtain a silica wet gel. After that DI water and NaOH was added to maintain pH at 10. Silica gel was formed, then heated for overnight at 150°C.

Antifungal Testing

Dissolved 39 gm of PDA in 1000 ml distilled water. Under stirring this solution was sterilized by autoclaving at 15 psi at 121°C for 15 minutes. Nanoparticles concentration was maintained in the media at 1%. Cooled it to room temperature prior to dispense. The fungi were inoculated on separate petriplates containing sterilized media with 1wt% of nanoparticles. After this placed these petriplates in BOD (Biochemical oxygen demand) chamber. Growth of fungus was observed for 7-10 days.

Characterization

Prepared nanoparticles were suspended in water and particle size was measured by Dynamic Light Scattering using Malvern instruments (Zetasizer Nano S-90). Fourier transform infrared spectroscopy of all prepared nanoparticles was performed with Shimadzu 8400 Spectro-photometer in powder form. X-Ray Diffraction was carried out of all nanoparticles using Bruker D8 Focus in powder form. UV visible spectrum of prepared nanoparticles have been reported by UV 1800 Shimadzu UV spectrophotometer suspensions form prepared in water and SEM was performed using Zeiss scanning electron microscope in powder form. Antifungal effect was observed in PDA (potato dextrose agar) media.

Results and Discussion

Particle size measurement was carried out through dynamic light scattering. Particle size of prepared silica nanoparticles through sol-gel method was observed as mentioned in Table 1 and particle size of prepared silica nanoparticles through peptization process by using different electrolyte like nitric acid or HCl or H₂ SO₄ is mentioned in Table 2. Particle size of nano silica at different concentration of precursor and at different temperature up to 650°C was measured. To obtain particle size below 100 nm silica nanoparticle was prepared through two different process- sol gel and peptization process. Through sol-gel process particle size of nano silica was observed 154 nm at 650°C by 8 ml of TEOS that is minimum particle size as compared to 78°C and 100°C because

at higher temperature gel structure breaks. After that we changed the concentration of TEOS, firstly reduced by $\frac{1}{2}$ (4 ml of TEOS), obtained particle size was 260 nm at 650°C. After increasing the

concentration of TEOS twice means 16 ml observed the particle size was 85 nm at 650°C.

Table 1: Particle size analysis of nano-silica prepared through sol-gel process.

TEOS concentration	Temperature	Nano-Silica particle size
4 ml	78°C	290 nm
4 ml	100°C	279 nm
4 ml	650°C	260 nm
8 ml	78°C	203 nm
8 ml	100°C	175 nm
8 ml	650°C	154 nm
16 ml	78°C	149 nm
16 ml	100°C	105 nm
16 ml	650°C	85 nm

Table 2: Particle size analysis of nano-silica prepared through peptization process.

Acid	Particle size
Nitric acid	190 nm
Hydrochloric acid	310 nm
Sulfuric acid	277 nm

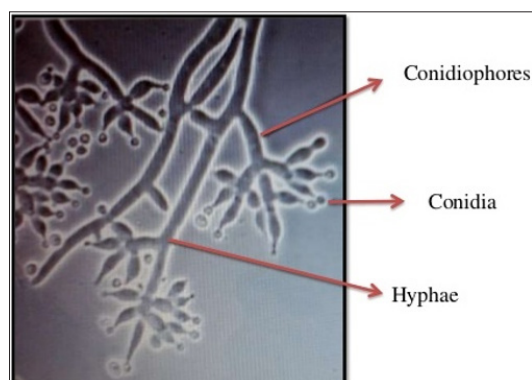


Figure 1: Morphological structure of Trichoderma.

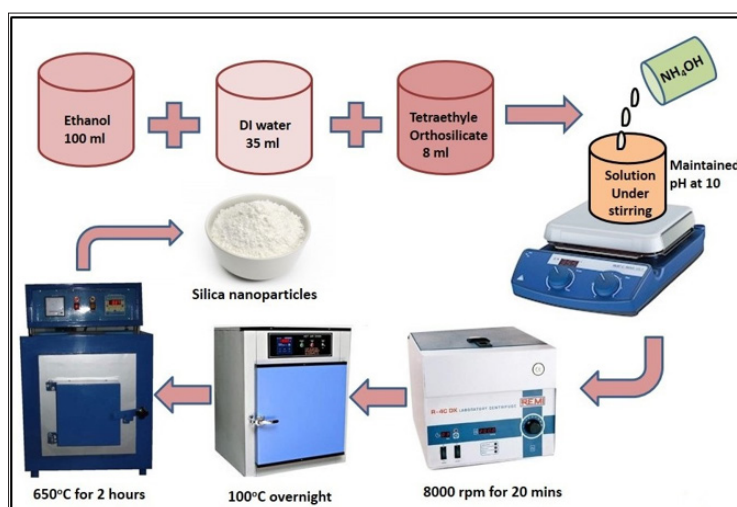


Figure 2: Synthesis procedure of silica nanoparticle through sol-gel process.

Even silica nanoparticle was prepared through peptization process but obtained particle size was 190 nm, 310 nm and 277 nm using electrolyte nitric acid, HCl and H_2SO_4 . (Figures 1-7) So further for all characterization were proceed with silica nanoparticle prepared through 16 ml of TEOS due to smaller particle size and this particle size shows better anti-fungal effect as compared to others. XRD analysis was performed for silica nanoparticle prepared

through 16 ml of TEOS at temperature $78^\circ C$, $100^\circ C$ and $650^\circ C$. The X-ray powder diffraction pattern of the nanoparticles were recorded using $CuK\alpha$ ($1.5406 \text{ \AA}$) radiation at room temperature in the range 20 to 80° in 2θ scale. Amorphous nature of silica nanoparticle was confirmed by broad peak in XRD analysis at $2\theta=15^\circ$ ($hkl=100$) but XRD peak at $650^\circ C$ was best as compared to $78^\circ C$ and $100^\circ C$ as shown in Figures 8 & 9.

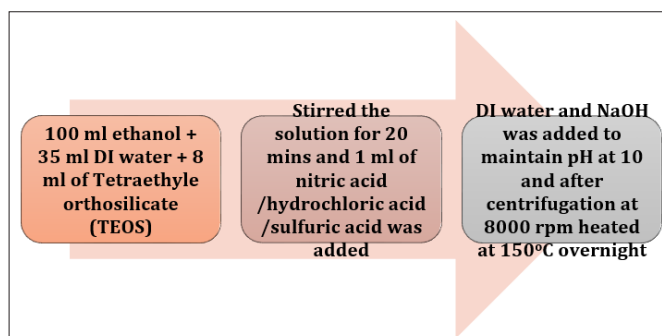


Figure 3: Synthesis procedure of silica nanoparticle through peptization process.

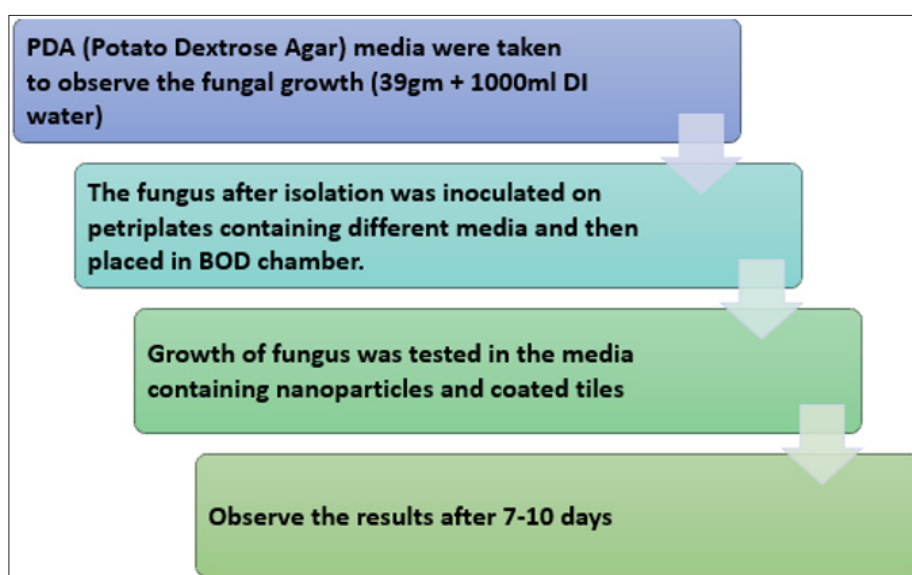


Figure 4: Flow chart for antifungal testing.

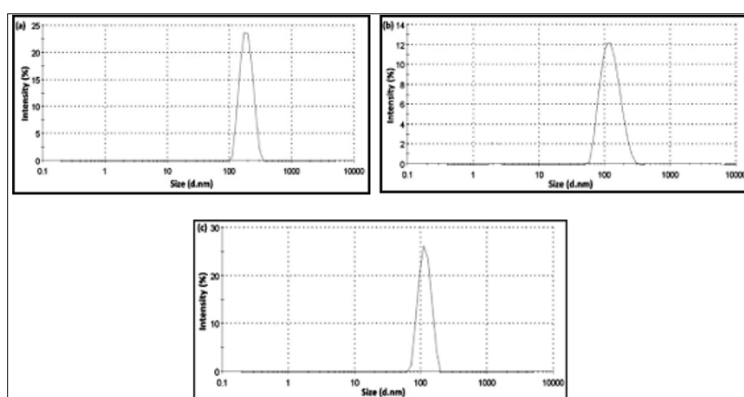


Figure 5: Particle size analysis of silica nanoparticles prepared through sol-gel method by 4 ml of TEOS at temperature (a) $78^\circ C$ (b) $100^\circ C$ (c) $650^\circ C$.

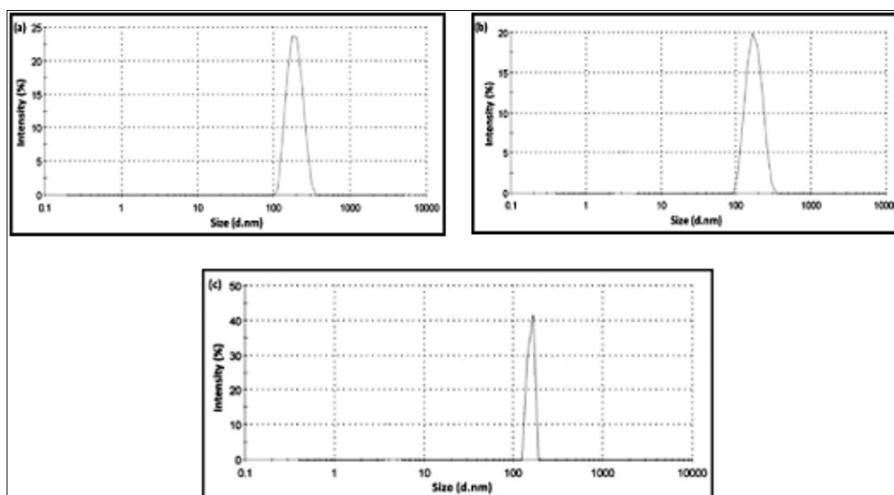


Figure 6: Particle size analysis of silica nanoparticles prepared through sol-gel method by 8 ml of TEOS at temperature (a) 78°C (b) 100°C (c) 650°C.

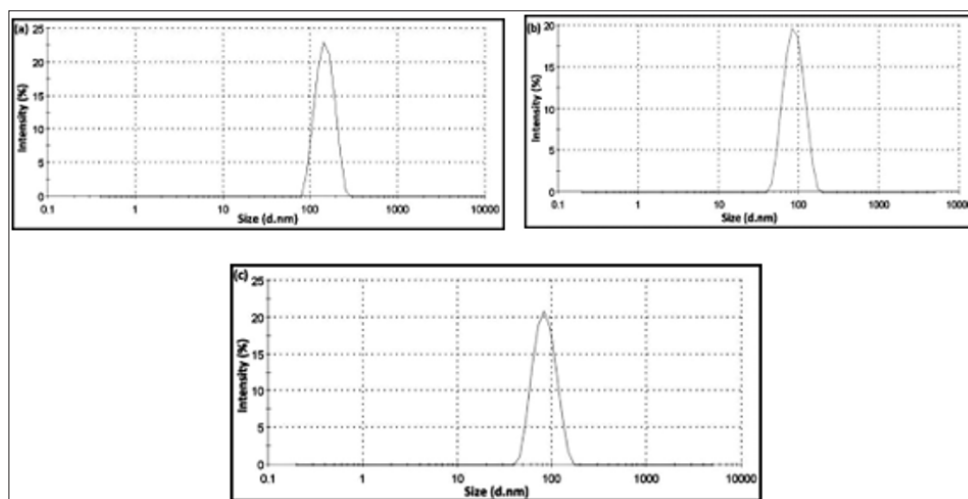


Figure 7: Particle size analysis of silica nanoparticles prepared through sol-gel method by 16 ml of TEOS at temperature (a) 78°C (b) 100°C (c) 650°C.

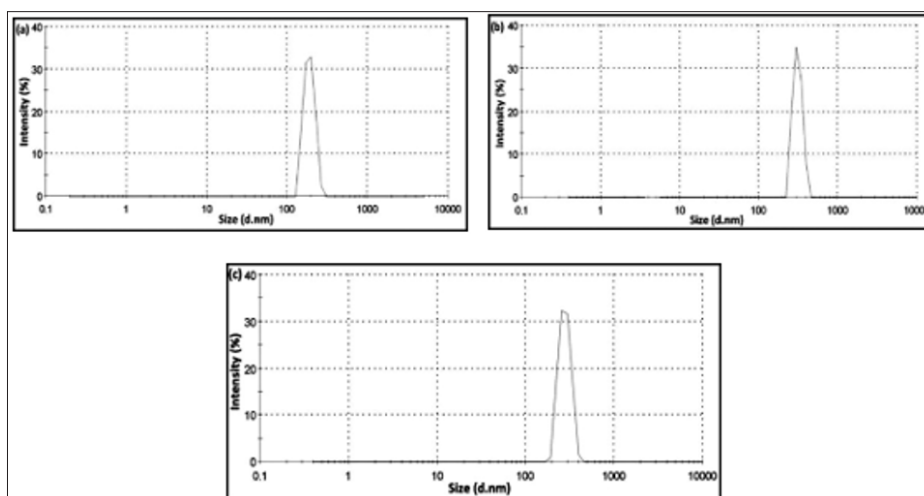


Figure 8: Particle size analysis of silica nanoparticles prepared through peptization process using (a) Nitric acid (b) Hydrochloric acid (c) Sulfuric acid.

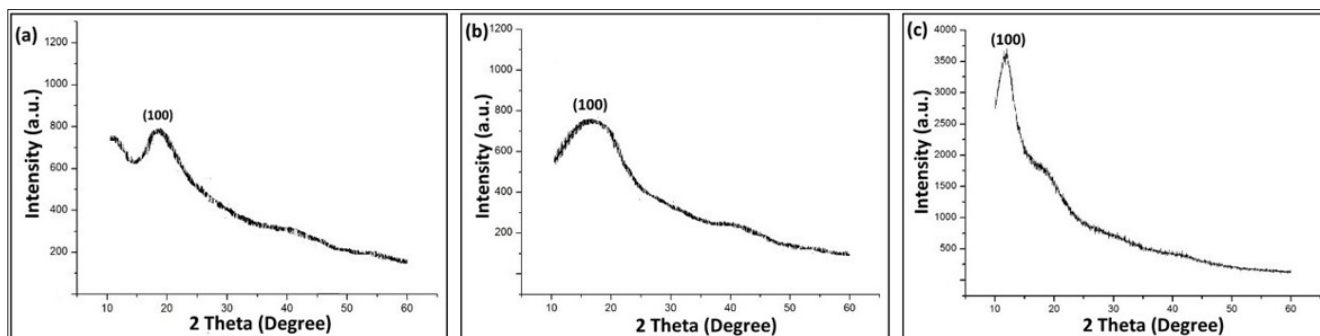


Figure 9: XRD analysis of silica nanoparticles prepared through sol-gel method by 16 ml of TEOS at temperature (a) 78°C (b) 100°C (c) 650°C.

In SEM image of silica nanoparticle have shown prepared by 16 ml of TEOS at 650°C. Scanning electron microscopy shows the spherical shape of silica nanoparticles. UV visible analysis was carried out in the region of 200-700 nm prepared by 16 ml of TEOS at 650°C and obtained absorption peak $\lambda_{\max}$ at 270 nm as shown in Figures 10 & 11. In FTIR analysis, shows the FTIR spectra of silica nanoparticles prepared through sol-gel process using 16 ml of precursor at 650°C. In the spectra of silica, the band around 1070 cm^{-1} corresponds to asymmetric stretching vibration of Si-O-Si

bond whereas 3300 cm^{-1} and 1640 cm^{-1} bands have appeared for H-O-H stretching and bending of absorbed water. Another peak at around 910 cm^{-1} corresponds to Si-OH bond. Antifungal testing of silica nanoparticle was carried out in potato dextrose media against fungus- *trichoderma harzianum* and *rhizoctonia solani* as shown in the Figures 12 & 13. The presence of silica nanoparticle (1wt%) reduced the growth of *T. harzianum* up to 80% as shown in Figure 13(ii). And growth reduction was observed up-to 70% in *Rhizoctonia solani* as shown in Figure 14.

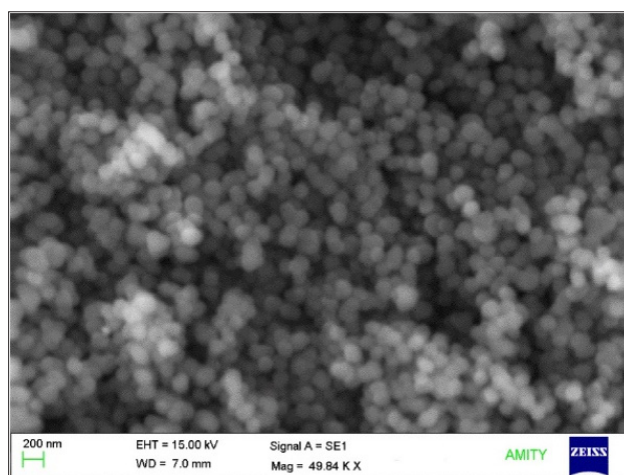


Figure 10: SEM image of SiO_2 nanoparticle.

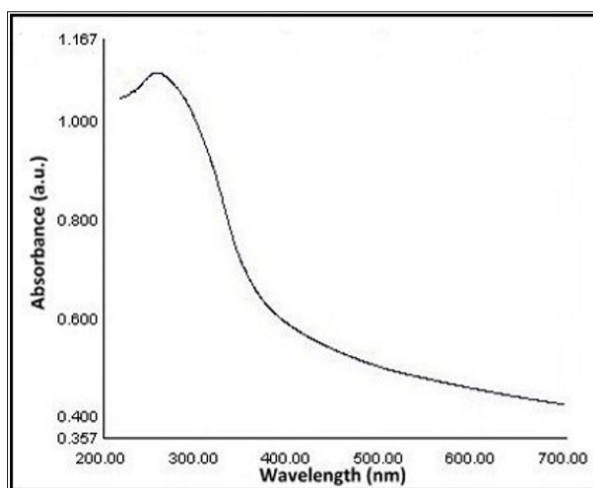


Figure 11: UV visible spectrum of silica nanoparticles.

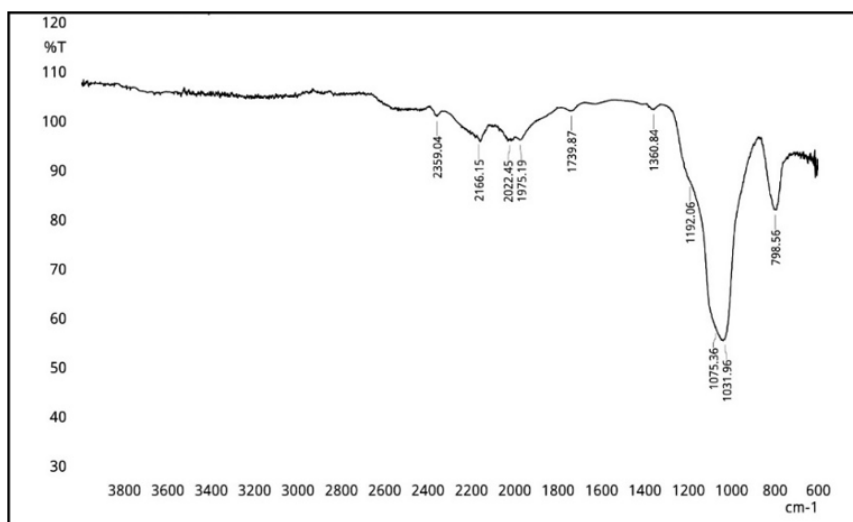


Figure 12: FTIR analysis of silica nanoparticles.

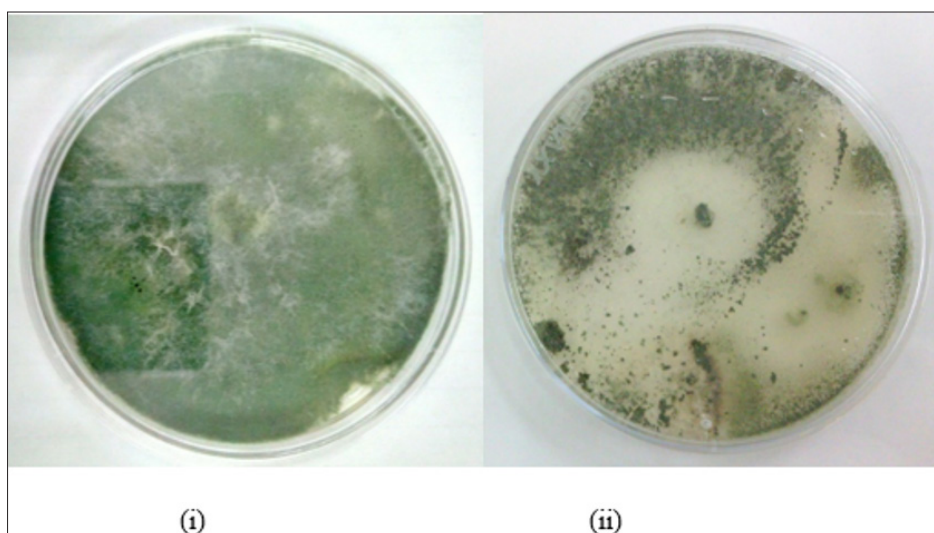


Figure 13: Growth of *Trichoderma Harzianum* in (i) control (ii) media containing silica nanoparticles.

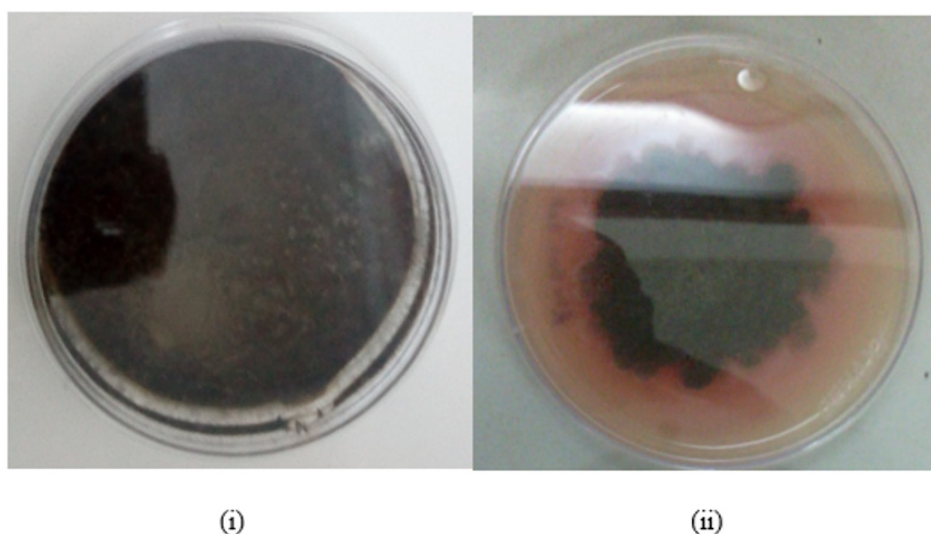


Figure 14: Growth of *Rhizoctonia Solani* in (i) control (ii) media containing silica nanoparticles.

Conclusion

We successfully prepared silica nanoparticles using sol-gel synthesis and peptization process. Characterization like DLS, UV spectroscopy, X-ray Diffraction, SEM was successfully performed. Presence of silica nanoparticle shows significant growth reduction of both type of fungus- *trichoderma harzianum* and *rhizoctonia solani*. This nanoparticle can be incorporated in various nano-coating for fungal growth reduction. These coatings can be used at various places like hospitals, toilets etc.

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