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Green Synthesis and Characterization of Zinc Sulphide Nanoparticles from Macro Fungi Pleutrous Florida

Suganya. P and Mahalingam P.U*
[1] *Department of Biology, The Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu, India.*
Email: Suganya12589@gmail.com
[2] *Department of Biology, The Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu, India.*
Email: pumahalingam.gri@gmail.com
Corresponding author: Mahalingam P.U

Abstract: *In the present study, an attempt was made on green synthesis and characterization of ZnS nanoparticles from edilble mushroom. The biosynthesis of ZnS was made from the aquous extract of edible mushroom, Pleurotus florida and authenticated using UV- Vis Spectroscopic analysis. ZnS nanoparticles were purified with aquous – ethanol precipitation method and obtained as crystalline materials. The pure form of ZnS nanoparticles was sequentially subjected for FTIR, XRD, SEM, EDAX, and TEM analysis for nanostructural characterization. In order to evaluate the biopotential properties of ZnS, a seed germination study was carried out with green gram (Vigna radiata) with different concentration of ZnS. This study reveals that ZnS nanoparticles positively influence the germination of green gram.*

Keywords: *ZnS nanoparticles, Pleurotus florida, green synthesis, green gram*

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I. Introduction

Zinc is one of the key microelement available on the earth having wider application in the diversed fields include engineering, sensors, solar batteries, cosmetics, chemical sciences and biological sciences (Senapati 2013). Zinc plays significant roles in biological system where it coordinates many metabolic processes through carbohydrate, lipid, nucleic acid, and protein synthesis as well as their degradation (Malarkodi and Annadurai 2013). Zinc is also an integral component of many enzymes and is the only metal to be represented in all six enzyme classes viz. oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Baishya and Sarkar 2011). In particular, zinc is one of the essential nutrients required for plant growth because it controls the synthesis of indole acetic acid (IAA), a phytohormone which regulates the plant growth. It is also necessary for the chlorophyll synthesis and carbohydrate formation. It enables the plants to lower air temperatures and helps in the biosynthesis of cytochrome, a pigment that maintains the plasma membrane integrity and the synthesis of leaf cuticle. Nanoparticles are the most attractive materials having many potential properties with wide range applications. The synthesis of nanoparticles can be achieved through, physical, chemical and biological methods. Biologically inspired nanoparticles synthesis is currently a rapid expending area of research because of viable, low cost and ecofriendly in nature (Waghmare *et al.* 2011). Further,biological methods for nanoparticles synthesis enabling at mild pH, pressure, temperature and cost effective (Hussian *et al.* 2003). Mushrooms are white rot fungi classified under macrofungi group of organisms having a heterotropic mode nutrition. *Pleurotus* sp is the third most common cultivated mushroom worldwide for food purposes because it considered to be rich of proteins, vitamins, aminoacids, minerals, and antioxidant compounds (phenolics, organic acid, alkaloids) and oxidation reductase enzymes (Ajith and Janardhanan 2007). These bioactive compounds may positively influence the green synthesis of gold and silver nanoparticles (Narasimha *et al.* 2011). Therefore, in the present study an attempt was made on green synthesis of ZnS from mushroom extract and to characterize its nanostructured properties and the biopotencial activities.

II. Experimental Section

PREPARATION OF MUSHROOM EXTRACT

The mushroom extract was prepared using standard procedure (Senapati and Sarkar 2014). Fresh and completely bloomed oyster mushroom sample of *Pleuotus florida* was directly procured from the mushroom farm, Gandhigram, Tamilnadu. India and thoroughly washed with distilled water till to remove all impurities adhered to the mushroom materials. Then, mushroom extract was obtained from 100 gram of chopped mushroom materials through boiling for 10 min in 500ml distilled water, cooled at room temperature and filtered with whatmann filter paper No.1. The mushroom extract, thus prepared was used as potential capping agent cum stabilizer for improved synthesis of ZnS nanoparticles.

GREEN SYNTHESIS OF ZnS NANOPARTICLES

A study on green synthesis of ZnS nanoparticles was carried out by the standard method decribed by Senapati and Sarkar (2014). ZnS nanoparticles were synthesized by mixing1M of $ZnCl_2$, 1M of Na_2S and mushroom extract in equal volume of 1:1:1 ratio. Intially, 100ml of 1M $ZnCl_2$ was prepared in 250 glass conical flasks and placed on the magnetic stirrer with 70^{O} C, subsequently 100 ml of 1M Na_2S solution was added dropwise into $ZnCl_2$ solution and maintain a continuous stirring. Finally, mushroom extract was added dropwise to $ZnCl_2$ and Na_2S mixture under the continuous stirring at 70^{O} C. Then, the whole reaction mixture was incubated overnight at room temperatures at 27^{O} C that resulted in synthesis of ZnS as white precipitate. In order to authenticate ZnS nanoparticles, the whole reaction mixture was subjected to UV-Vis Spectroscopic analysis at 445nm (wavelength specific for ZnS) using Jasco –V/-3/3/d-UV-Vis Spectrophotometer. Subsequently, the whole reaction mixture was centrifuged at 2000 rpm for 10 min to obtain white precipitate as pellet and ultimately dried under Hot air oven (Yorco Hot Air sterilizer (oven)) at 120 ^{O} C for 2hrs. The dried ZnS nanomaterial was crushed with morter and pestle and obtained as fine powder and used for further study.

CHARACTERIZATION OF ZnS NANOPARTICLE

The ZnS nanoparticle was characterized for its structural properties through FTIR, XRD, SEM, EDAX, and TEM studies. In order to reveal various functional groups associated with ZnS nanoparticles, Fourier Transform Infrared Spectroscopic (FTIR) analysis was performed on the powdered ZnS nanoparticles using Perkin- Elmer Spectrum RXIFTIR System. X-ray diffraction crystalographic (XRD) technique is used to analyse metallic native of the nanoparticles (Narasimha *et al.* 2011). XRD analysis on powder sample of ZnS was carried out using X-ray diffractometer (BRUKER ECO D8ADVANCE) at wavelength of 1.54060 nm. The average crystallite size was calculated by the Debye Sherrer formula i.e., $D = K \lambda / \beta \cos\theta$ where K is the constant (K=0.9), λ is the wavelength of X-ray (λ=1.54060 A^0), β is the width of the XRD peak at half height, θ is the Bragg diffraction angle. Scanning Electron Microscopic (SEM) imaging on powder samples of ZnS sputter coated with gold palladium alloys was carried out using Scanning Electron Microscope (Vega 5 TESCAN 129ev) equipped with energy dispersive X-ray Spectroscopy (EDX) with SEM Bruker, nanoD-12480. Transmission Electron Microscopy (TEM) imaging on powdered ZnS nanoparticles with carbon coated was carried out using TEM (Philips CM 20 Biotwin 200Kv).

SEED GERMINATION STUDY USING ZnS NANOPARTICLES

The seed germination study was conducted under in-vitro condition by standard method (Manmathan *et al*. 2013). Ten healthy seeds of green gram (*Vigna radiata*) treated with various concentrations of ZnS nanoparticles includes 100ppm, 200ppm, 300ppm and 400ppm. The seeds were allowed to germinate under in-vitro by providing optimum conditions like temperature (30^{O}C), relative humitity (60%), and light (1200 lux). The germination and early growth of green gram in various treatments were observed upto 10 days.

III. Results And Discussion

UV-VIS SPECTROSCOPIC STUDIES ON ZnS NANOPARTICLES

The UV-Visible absorption spectrum was considered as a novel technique for preparation of nanoparticles. In this study, an absorption sharp peak obtained at 445 nm as shown in Figure 1 is confirmed the synthesis of Zinc Sulphide nanoparticles in mushroom extract of *P. florida*. Generally, absorption peaks exhibit rays blue shift and strong intensity. Such a strong peak is known to arise from quantum confinement effect, which occurs when the particle size becomes comparable with (or) smaller than the Bohr radius of excitation (Senapati and Sarkar 2014).

FT-IR SPECTRUM ANALYSIS ON ZnS NANOPARTICLES

The FTIR spectrum of ZnS nanoparticles as shown in Figure 2 clearly indicates the characteristic functional groups which confirm the nanoscale properties of ZnS synthesized from mushroom extracts. The different peaks obtained at various points were compared with standard FTIR spectrum. Accordingly, the small peak observed at 3,448 cm^{-1} and corresponds to Hydrogen- bonded O-H stretch bending vibration of phenols and alcohol band; the set of small peaks present at 2,926 cm^{-1} and 2,856 cm^{-1} indicates the presence of C-H Stretch off C = O vibration of Aldehydes band and a small peak observed at 1,720 cm^{-1} represents C = O stretch correspond to Carboxylic acids band; a strong peak at 1,622 cm-1 represent C=O Strech corresponds to Amide band; a small peak at 1,562 cm^{-1} represent N-H stretch corresponds to amide; a small peak observed at 1,543

cm^{-1} represent N-H bend is the characteristic of Amines- Secnodry band; a small peak present at 1,517 cm^{-1} corresponds to N–O asymmetric stretch nitro compounds; a small peak corresponds to 1,460 cm^{-1} and 1,421 cm^{-1} are C–H bend and alkanes bond repectively; a small peak observed at 1,398 cm^{-1} indicate N=O bend; a small peak observed at 1,369 cm^{-1} indicates C–H rock alkanes; a long peak corresponds to 1,280 cm^{-1} is due to C–N stretch aliphatic amines bond; a strong peak observed at 1,120 cm^{-1} represent C–H wag ($–CH_2X$) alkyl haloides; a strong peak at 1, 016 cm^{-} is due to C–N stretch aliphatic amines; a broad peak observed at 9,16 cm^{-1} corresponds to O–H bend carboxylic acids; a small peak at 5,11 cm^{-1} corresponds to C–Br stretch alkyl halides bond and a strong peak observed at 4,70 cm^{-1} corresponds to C–Br stretch alkyl halides.

FTIR results of the present study on ZnS nanoparticles are in confirmily with FTIR spectrum obtained by Senapathi and Sarkar (2014) for ZnS nanoparticles synthesized from mushroom extract of *P.ostreatus*. Their spectrum indicates the presence of several strong peaks resresents C-OH vibrations (1,088 cm^{-1} and 1,111 cm^{-1}), O-H bending vibration (1,404 cm^{-1}), O=C=O strecting (2,358 cm^{-1}), phenol CO-H strecting (3,527 cm^{-1}), N-H strecting (3,482 cm^{-1}), alkenyl C-H stretch (3,010 cm^{-1}) and amide N-H strecting (3,742 cm^{-1}). Similar studies were also carried out by many researchers and characterized ZnS nanoparticles synthesized from fungal system using FTIR studies (Li et al. 2000., Sooklal et al. 1996).

XRD SPECTRUM ANALYSIS OF ZnS NANOPARTICLES

XRD patterns for ZnS nanoparticles synthesized using mushroom extract are shown in Figure 3. The X-ray pattern of ZnS nanoparticles shows sharper and stronger diffraction peaks at 25.879, 27.958, 31.449, 32.585, 33.324 and 33.324 units. These peaks correspond to cubic lattice structure of ZnS (Zinc blende) and assigned the planes (111), (220), (311). The different peaks were indicating high purity and well crystalline form ZnS. The presence of broad peaks in the XRD pattern implies presence of smaller particles (Palve and Garje 2011). Further, the shift in the 2θ peak values of ZnS nanoparticles may be due to presence of some protein molecule. The d-spacing values are in good agreement with JCPDS No.77-2100. These, protein molecule have nanoparticles represent the strong ability of binding to metal nanoparticles and formed a layer of ZnS nanoparticles to agglomeration. It has been earlier reported that proteins are binds to the nanoparticles through amide or aliphatic groups residue of the protein structures (Basavaraja *et al.* 2008).

SEM ANALYSIS ON STRUCTURAL CHARACTERIZATION OF ZnS NANOPARTICLES

Scanning Electron Microscope was used to detect the particle size and morphology of ZnS nanoparticles synthesized using mushroom extract *P.florida.* SEM micrograph as shown in Figure 4, clearly reveals that the presences of ZnS nanoparticles as spherical structure with particle size range from 10-20 nm. In order to confirm the element present in sample, EDAX was carried out, and the result reveals a strong presence of ZnS. (Figure 5). SEM micrograph for ZnS nanoparticle was reported by Senapathi and Sarkar (2014) and their SEM image reveals that ZnS appeared as cluster of tiny particles having spherical shapes. They also noted that the agglomeration of particles decreases as the amount of mushroom extract increased in their studies. They were also analysed the elements present on ZnS nanoparticles and the result reveales the presence of Zinc and sulphur in the ratio of 48.67:50.73 which are very close to the standard value.

TEM ANALYSIS ON STRUCTURAL CHARACTERIZATION OF ZnS NANOPARTICLES

A TEM micrograph showed a well distribution of ZnS nanoparticle as spherical particles (Figure 6A). The nanoparticles were oblate spherical with clear edge of crystal and lattice structure observed in HR-TEM micrograph as shown in Figure 6B, which supports the crystalline nature of ZnS nanoparticle. TEM histogram confirms the average particle size of ZnS as 1.7207±0.064 nm (Figure 6C). The polycrystalline nature of ZnS nanomaterials were determined using selected area diffraction (SAED) patterns (Figure 6D) and the results showed concentric rings around ZnS. Similar structural properties for nanoparticles was reported by Senapathi and Sarkar (2014) and they were obtained TEM micrograph for ZnS synthesized using mushroom extracts of *P.ostreatus.* They were also calculated average particle size of ZnS and found vary from 4.04 to 2.30nm in three different mushroom extract used for their study.

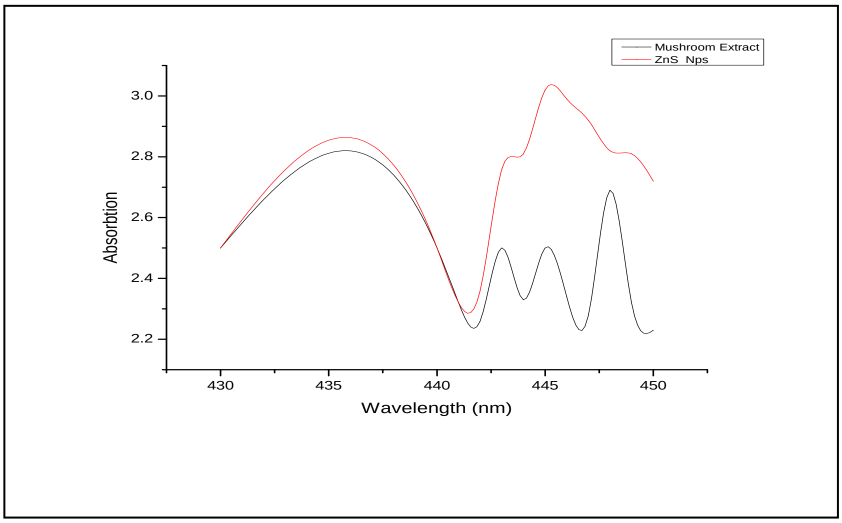

FIG. 1 UV-VISIBLE SPECTRUM OF ZNS NANOPARTICLES SYNTHESIZED USING MUSHROOM EXTRACT P.FLORIDA

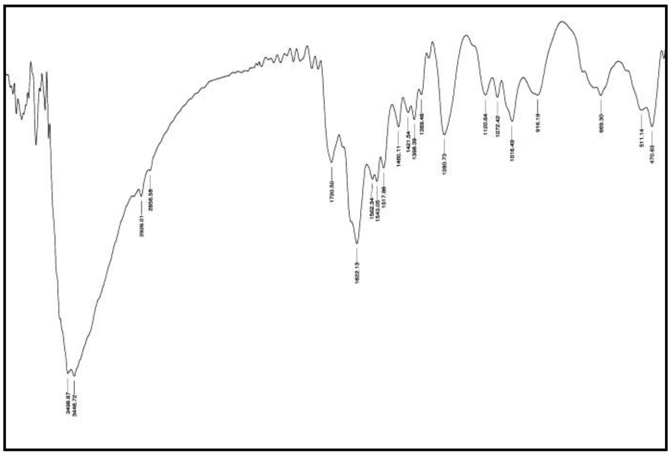

FIG. 2 FTIR SPECTRUM OF ZNS NANOPARTICLES SYNTHESIZED USING MUSHROOM EXTRACT OF P.FLORIDA

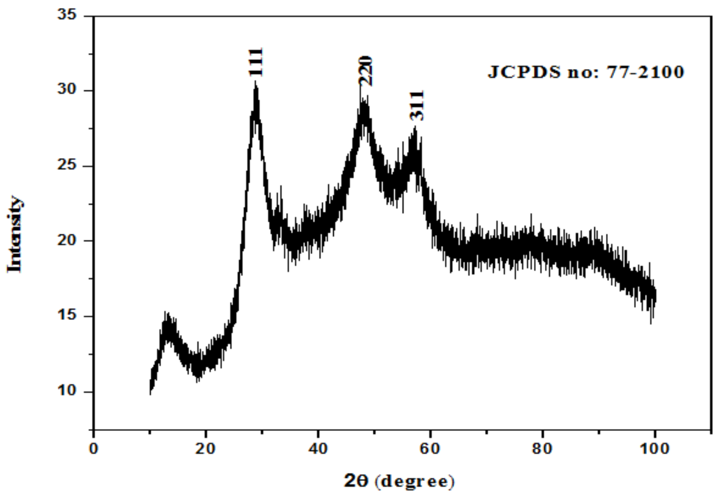

FIG. 3 XRD PATTERN OF ZNS NANOPARTICLES SYNTHESIZED USING MUSHROOM EXTRACT OF *P.FLORIDA*

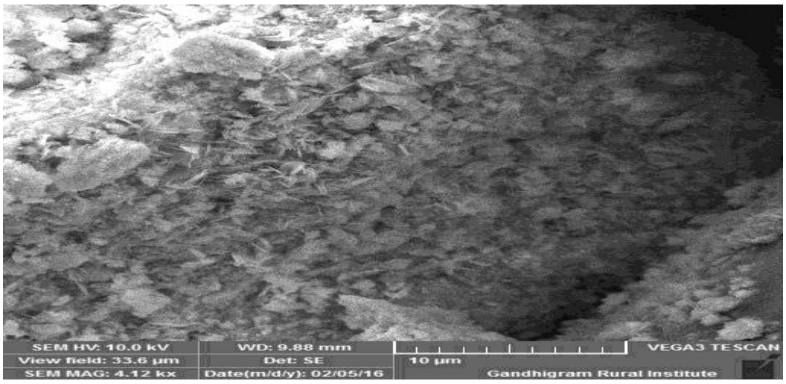

FIG. 4 SEM MICROGRAPH OF ZNS NANOPARTICLES SYNTHESIZED USING MUSHROOM EXTRACT OF P.FLORIDA

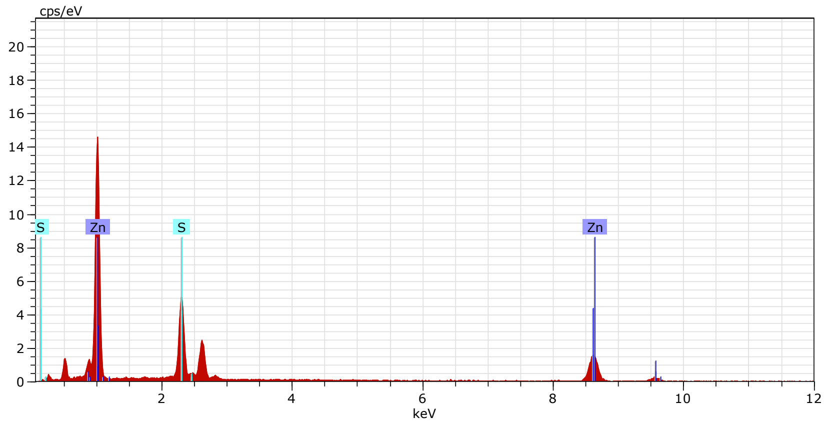

FIG. 5 EDAX PATTERN OF ZNS NANOPARTICLES SYNTHESIZED USING MUSHROOM EXTRACT OF P.FLORIDA

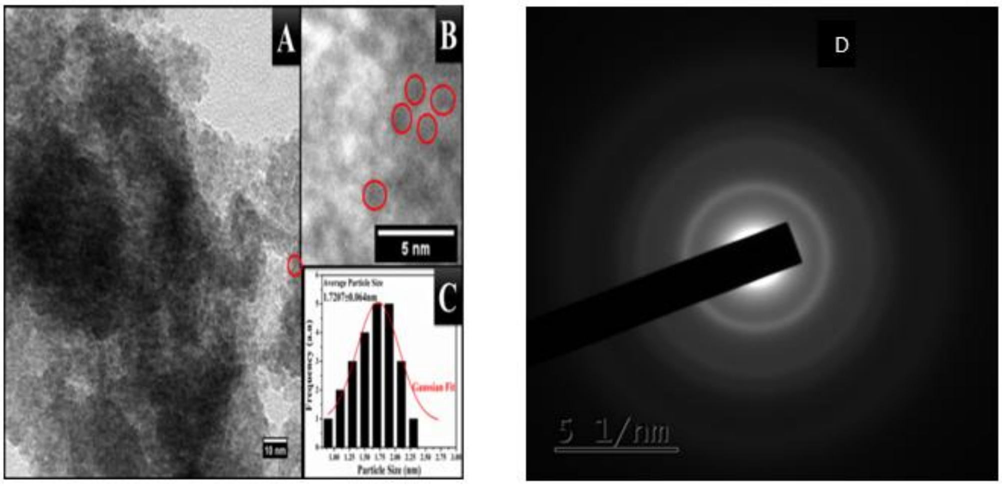

FIG. 6 A TEM MICROGRAPH OF ZNS NANOPARTICLES, B- NANOPARTICLES, C- HISTOGRAM AND D- SELECTED AREA ELECTRON DIFFRACTION (SAED)

SEED GERMINATION STUDY

In order to evalute the biofertilize potential of nanoparticles, an in-vitro seed germination study was conducted with green gram (*V. radiata*) using different concentrations of ZnS nanoparticles synthesized from mushroom extract. The seed germination test clearly reveals that ZnS nanoparticles positively influence the seed gerrmination and the early growth of green gram under the controlled conditions (Plate1).

PLATE. 1 SEED GERMINATION AND THE EARLY GROWTH OF GREEN GRAM (V. RADIATA) SUPPLEMENTED WITH VARIOUS CONCENTRATIONS OF ZNS NANOPARTICLES

IV. Conclusion

In conculsion, the mushroom extract is become a cost effective and ecofriendly material for the green synthesis of ZnS nanoparticles. Futher, the biologically synthesized ZnS nanoparticles were found suitable as plant nutrition by supporting better germination and early growth in green gram. Therefore, this intervention opens new door for fertilizer industries to produce ‘‘Bionanofertilizer’’ for improved crop production towards manage future demand on supply of Agricultural products.

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