Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 9 September 2024

Research Article

Green Synthesis of Sulfur Oxide Nanoparticles Using *Syzygium cumini* Bark Extract: Impact on Tomato (Arka Vikas S-22) Seed Germination

Babulla S, Pallavi S, Rohith P and Muni Kumari A*

Department of Genetics and Genomics, Yogi Vemana University, Kadapa, India

***Corresponding Author:** Muni Kumari A, Associate Professor, Department of Genetics and Genomics, Yogi Vemana University, Kadapa, Andhra Pradesh, India.

DOI: 10.31080/ASMI.2024.07.1427

Received: July 23, 2024

Published: August 28, 2024

Abstract

Metal oxide nanoparticles have wide range of applications. Green synthesis of metal oxide nanoparticles involves the usage of plant extracts as reducing agents. It is a significant ecofriendly procedure for nanoparticle synthesis as this procedure reduces the usage of chemicals. In the present study green synthesis of Sulfur Oxide Nanoparticles (ZnO NPs) was carried out using *Syzygium cumini* Bark Extract. The synthesized Sulfur Oxide Nanoparticles were characterized using scanning electron microscope (SEM) coupled with energy dispersive X-ray spectrometer (EDAX). Fourier transform infrared (FT-IR) spectrophotometer. And the XRD pattern of the green synthesized ZnO NPs were carried out using X-ray Diffractometer. The effect of Sulfur Oxide Nanoparticles on Tomato (Arka vikas S-22) Seed Germination was tested. When compared with control seedlings it was found that these nano particles are effective in enhancing the root and shoot length of the tomato seed lings.

Keywords: Metal Oxide Nanoparticles; *Syzygium cumini*; Sulfur Oxide Nanoparticles

Abbreviations

ZnO NPs: Sulfur Oxide Nanoparticles; SEM: Scanning Electron Microscope; EDAX: Energy Dispersive X-ray Spectrometer; FT-IR: Fourier Transform Infrared Spectrophotometer; XRD: X-ray Diffractometer

Introduction

Nanotechnology is an emerging scientific field that has wield in varied fields such as energy, environment, electronics, etc. The extensive use of metal nanoparticles (particles smaller than 100 nm) is increasing day by day owing to their exclusive characteristics [1]. Physicochemical and biological methods are being used to synthesize nanoparticles (NPs). However, the cost of processing and ultimately detrimental to the ecosystem is the biggest consideration in preferring the green synthesis. In the recent years, seeds, leaves, barks, plant biomass and micro-organisms have become the economical and eco-friendly solution to synthesize nanoparticles. The aqueous bark extract serves as the surface capping and stabilizing agent in green synthesis with the help of the phytoconstituents present in it and determines the characteristics of the nanoparticles (NPs) paved the way for various biomedical applications [2]. Depending on the Phytochemicals such as tannins, saponins, alkaloids, terpenoids, flavanoids, etc., found in the aqueous bark extract is the major rationale for the fabrication of metal nanoparticles. Moreover; plants and its various parts are identified as better alternatives for the drugs to cure various diseases [3].

Plant based nanoparticles synthesis have a number of advantages over other methods, including ease of preparation, processing, cost-effectiveness, rapid development, reproducible, stable components, environmental friendliness, and the avoidance of harsh and toxic chemicals. Furthermore, the particle size influenced by the compounds like phenols, polyphenols etc., in the bark extract plays crucial role in reduction of ions to form stable NPs. There is a significant variation in chemical compositions of plant extract of same species when it collected from different parts of the world and may lead to different results. So these results indicate there is a need of in-depth study about the compounds present in the plant materials which play a crucial role in the synthesis of NPs. Green synthesized Nanoparticles with more consistent uniformity and drug loading and release capacity would be the further area of research [4].

Sulfur is an essential macronutrient; according to its chemical nature it plays important roles in various cellular metabolic processes. As an organic thiol it is incorporated in essential amino acids such as cysteine, methionine and different co-enzymes (biotin, coenzyme A, iron-sulfur proteins, ethylene, thiamine pyrophosphate and lipoic acid), and thioredoxins and sulfolipids are often responsible for the structure and biological activity of proteins. Recently, it has been noted that a deficiency of sulfur in soil is a problematic issue which leads to reduction in yield and quality of seeds [5]. Day by day the concentration of sulfur in soil is decreasing due to the increasing proportions of high analysis and sulfur-free fertilizers and the decreasing use of traditional organic manures in agricultural land. Sulfur is taken up by a plant's roots in the form of sulfate from the soil, which is also a transportable form in the plant system.

Crop yield and seed quality assessment are closely related with the sulfur content of the seed. In different plants, the S-containing amino acids are an important determining and evaluating index for seed quality assessment. As mentioned previously, the S-limited condition reduces the proportions of S-containing amino acids in grains, whereas its application increases the content also. In addition to this, it increases the nutritional value of the grains as well [6].

Syzygium cumini (Java plum) is an evergreen tropical tree in the family of Myrtaceae. It is mostly present in the region of South America and Southeast Asia. Most part of the plant associates with multiple applications such as food, medicine, furnishings. However, without any effective use, the leaves are usually discarded as waste. Java plum leaves have a high proportion of ferulic acid, rutin, catechin, and limonene [7].

Tomatoes (*Solanum lycopersicum* L.) are widely distributed as a vegetable crop consumed in a different form. It is nutrient-enriched containing proteins, fibres, carbohydrates, calories, water, a large number of vitamins, and 37 minerals [8]. Additionally, tomatoes are considered one of the most effective and nutritious foods in the human diet. The fruit of a tomato is rich in phytochemical compounds such as carotenoids, vitamins, and phenolic compounds. These compounds have high antioxidant activity which is beneficial to human health [9].

Seed treatment is a process of treating seeds by any physical, chemical, biological or other agent(s) to destroy harmful seed-borne organisms or to protect the seeds against infection [10]. It is done to prevent germination failure and seedling infection, to destroy external and internal seed borne pathogens and to develop a protective zone around the seed in the soil which protects the germinating seed and seedling from the attack of certain soil borne pathogens. The biocidal properties of the NPs have significant practical relevance. Antibacterial and antifungal properties of metal NPs can be tapped to control bacterial and fungal organisms responsible for crop losses [11]. NPs enhance the plant growth and are involved in plant growth promotion [12]. However, it must be very clear that these NPs should not have any adverse effect in plant systems. Hence, in present investigation it was planned to study the influence of Sulfur NPs on seed germination in tomato.

Materials and Methods

Sodium thiosulphate ($Na_2S_2O_3$, HiMedia), Whattman No 1 papers, Germination towel papers, All other chemicals used are analytical grade and received from Merck, India. Throughout the experiment double distilled water was used.

Collection and Preparation of *Syzygium cumini*(SC) stem bark extract

Syzygium cumini stem bark was collected from the premises of Yogi Vemana University, Kadapa, India. Aqueous *Syzygium cumini* stem bark extract was prepared by a green process. Collected SC stem bark was washed several times with double distilled water to remove the dust and foreign particles on the surface of bark. 20 grams of bark samples were finely chopped and boiled in 100 mL double distilled water in 250 mL conical flask for 30 min. The

Syzygium cumini extract was filtered with Whattman grade No. 1 filter paper. The extract was centrifuged at 7500 rpm to remove the heavy molecular weight constituents. The collected extract was preserved at 4^0 C for further experimental analysis.

Green syntheses of Sulfur Nanoparticles using *Syzygium cumini* bark extract

10 ml of Aqueous *Syzygium cumini* stem bark extract was added into 90 ml of 0.1 M Sodium thiosulphate aqueous solution at room temperature. The reaction mixture was further heated on a hot plate at 60^0C for 2 h with continuous magnetic stirring. The solution was allowed to cool and centrifuged at 10,000 rpm for 15 min. The precipitate obtained was dried at around 60^0C in hot air oven over night. The obtained powder samples were used for further characterization.

Characterization of green synthesized Sulfur Nanoparticles

The characterization of green synthesized Sulfur NPs was analyzed in UV–Vis spectrophotometer in the range of 200–700 nm. The topographical structure and atomic composition were examined by scanning electron microscope (SEM) coupled with energy dispersive X-ray spectrometer (EDAX). The Fourier transform infrared (FT-IR) spectrophotometer was used to test the functional groups involved in the synthesized nanoparticles by adopting KBr pellet method [13] in the spectral range of 500- 4000 cm^{-1}. The XRD pattern of the green synthesized ZnO NPs were carried out using X-ray Diffractometer.

Effect of green synthesised Sulfur Nanoparticles on Tomato seeds

This assay was conducted to investigate the effect Sulfur NPs on seed germination and seedling growth. Seeds of tomato plant (Arka vikas S-22) cultivar were collected to be further examined. The Sulfur NPs were diluted with distilled water to the concentrations of 0.00 (control), 30, 60, and 120 µg/ml. Ten seeds of tomato were placed on germination towel papers in Petri dishes (10-cm diameter), and three ml of each concentration were added per dish. Three replicates were used for each concentration such that the total numbers of tested samples were 30 samples/ treatment. All dishes were covered with a lid and were incubated at 25±2°C in the dark for 7 days; after which the root and shoot lengths were recorded. The dishes were monitored regularly during the incubation period to ensure they were kept wet. The relative elongation percentage, relative germination rate and germination index were calculated as follows:

$$\text{Relative germination rate} = \left[\frac{\text{Seeds germinated in tested samples}}{\text{Seeds germinated in control}}\right] \text{X } 100.$$

$$\text{Relative shoot/ root elongation} = \left[\frac{\text{Mean shoot or root length in tested samples}}{\text{Mean shoot or root length in control}}\right] \text{X } 100.$$

$$\text{Germination index} = \left[\frac{\text{Relative germination rate X relative root elongation}}{100}\right].$$

Results and Discussion

Collection and Preparation of *Syzygium cumini* (SC) stem bark extract

Syzygium cumini stem bark was successfully collected (figure 1) from the premises of Yogi Vemana University, Kadapa, India.

Figure 1: Plant from which the bark was collected.

Aqueous *Syzygium cumini* stem bark extract was prepared successfully (figure 2) by green process.

Green syntheses of Sulfur Nanoparticles using Syzygium cumini bark extract

Green synthesis of Sulfur Nanoparticles was successfully carried out in the experiment evidenced with a formation of ash coloured precipitate (Figure 3) in the bottom of the beaker and the precipitate obtained was dried at around 60^0C in hot air oven over night. The obtained powder samples were used for further characterization.

Figure 2: *Syzygium cumini* stem bark extract

Figure 3: Synthesised Sulfur NPs.

Characterization of green synthesized ZnO Nanoparticles

Uv-Spectrophotometer

UV–vis spectrophotometer (UV-1700 Spectrometer of Shimadzu) was used with a wavelength range of 240–720 nm. Sulfur NPs showed the strong absorbance peak at 274 nm (Figure 4). Similar findings have been documented by Tripathi., *et al*. [14]. Who reported that the absorbance spectra of Sulfur NPs were recorded between 260 and 280 nm.

SEM

The surface morphology of nanoparticles is one of the important parameters that impact on the biological efficiency. The nanoparticles were investigated by SEM image as shown in Figure 5. The SEM images show that the Sulfur particles were formed in a very uniform manner in the form of cubes and a few of them appeared spherically.

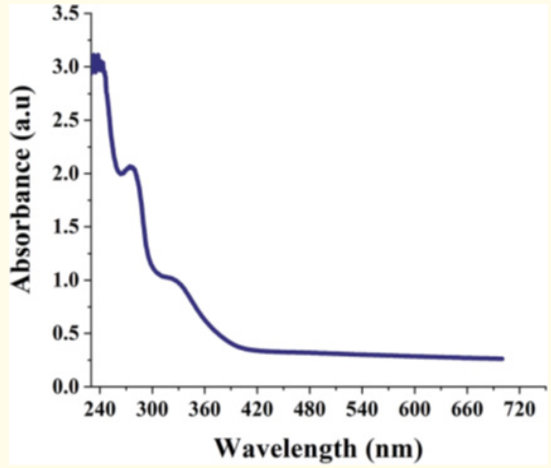


Figure 4: Uv absorption spectra of green synthesised Sulfur NPs.

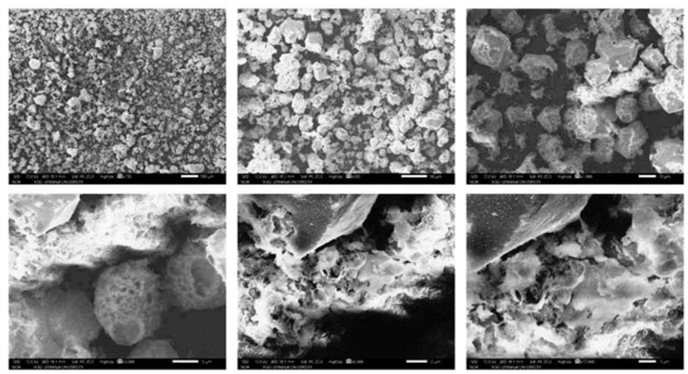


Figure 5: SEM images of green synthesised Sulfur NPs.

EDAX

The Energy Dispersive X-ray Diffractive (EDX) study was carried out for the synthesized Sulfur nanoparticles to know about the elemental composition. EDAX confirms the presence of Sulfur signals of Sulfur nanoparticles as shown in Figure 6 and this analysis showed the peaks that corresponded to the optical absorption of the produced nanoparticles. The elemental analysis of the nanoparticles, which proves that the produced nanoparticles are in its highest purified form.

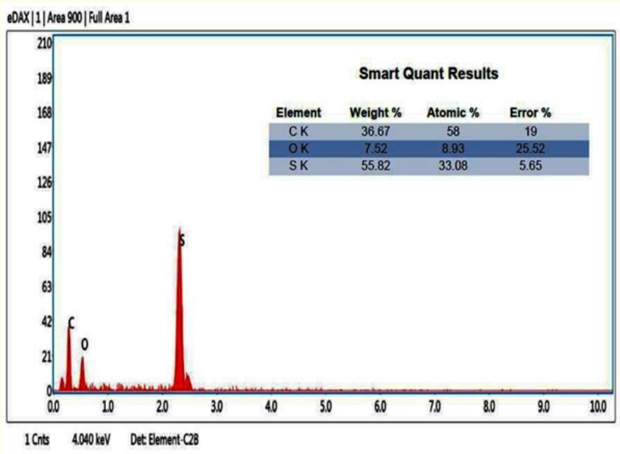


Figure 6: EDAX analysis of green synthesised Sulfur NPs.

XRD

Figure 7 shows that the XRD analysis of the prepared sulfur nanoparticles had broad peaks. The position and intensities of the diffraction peaks of all samples were compared with standard a-sulfur particle diffraction pattern. The average crystallite size of the as-prepared sulfur particles is about 5.7 nm, according to the Debye–Scherrer formula. It also confirms the synthesized nano powder was free of impurities as it does not contain any characteristics XRD peaks other than Sulfur peaks.

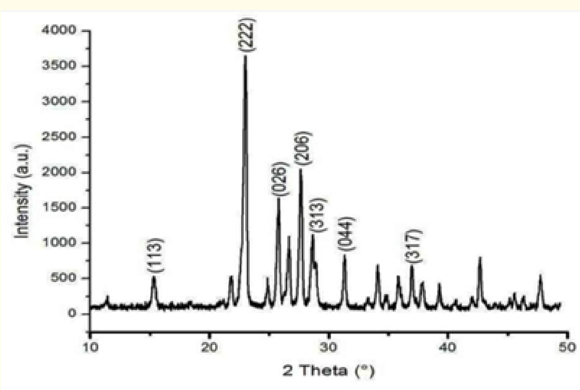


Figure 7: XRD pattern of green synthesised sulfur NPs.

FT-IR

Figure 8 shows a FTIR spectrum of SNPs. An intense peak at 2357 cm^{-1} corresponds to O=C=O bonds and the peak at 3737 cm_1 is due to the external isolated –OH groups.37 The symmetric stretching vibration at 1396 cm^{-1} shows the presence of COO– bonds. The peak occurring at 3606 cm^{-1} shows the N–H stretching vibration. The presence of O=C=O bonds, N–H bonds, and COO– bonds indicates that the biomolecules from *Syzygium cumini* leaf extract were present in the sample. The peaks at 667.9 cm^{-1}, 1056 cm^{-1}, 880.6 cm^{-1}, and 1539 cm^{-1} correspond to Sulfur. The peak positions showed slight shift from the characteristic peaks of Sulfur, 656 cm^{-1}, 1052 cm^{-1}, 876 cm^{-1}, 1513 cm^{-1}, respectively. The peak shift arises due to the interaction of SNPs with amine groups in the biomolecules. The results indicate that the biomolecules form the *Syzygium cumini* leaf extract proteins were bound onto the surface of as-prepared SNPs.

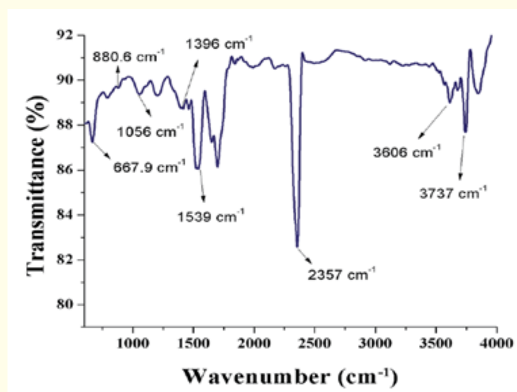
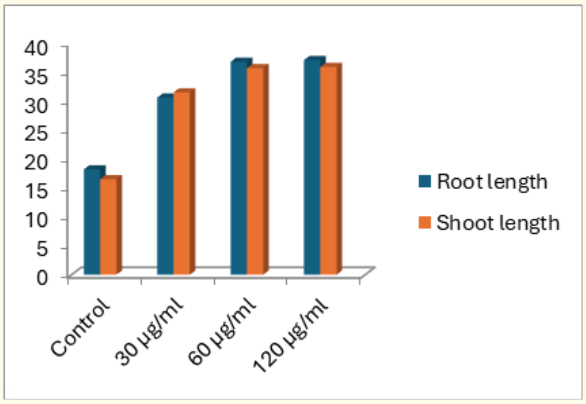


Figure 8: FTIR of green synthesised Sulfur NPs.

Effect of green synthesised Sulfur Nanoparticles on tomato (Arka vikas S-22) seeds

Graph 1 show the respective average root and shoot lengths of the germinated tomato seeds (incubated in the petri dishes at 25±2˚C in the dark for 7 days) after their exposure to Sulfur NPs. Relative to the control, Sulfur NPs significantly enhanced the root and shoot lengths of tomato seedlings when applied at the concentrations of 30µg/ml, 60µg/ml and 120µg/ml. At these concentrations, the Sulfur NPs had a stronger stimulant effect than control



Graph 1: Showing respective average root and shoot length of tomato seeds.

on the root and shoot length of seedlings. In this respect, the root and shoot length were promoted by 68.1, 102.1 and 103.8% of root length and 90.9, 116.3 and 117.5% of shoot length relative to the control at 30µg/ml, 60µg/ml and 120µg/ml of Sulfur NPs. The germination rate of tomato seeds were recorded 150, 216 and 225% respective to 30µg/ml, 60µg/ml and 120µg/ml of Sulfur NPs and finally germination index was 252.1, 436.5 and 458.5% respective to 30µg/ml, 60µg/ml and 120µg/ml of Sulfur NPs.

Furthermore, various studies with different crops emphasized that the supply of Sulfur in varying levels affects the extraction rates of wheat flour, quality of gluten and baking properties [15]. In addition, Sulfur also affects the Nitrogrn fixation in legume crops. The primary uptake mechanism of sulfate takes place via roots, and afterwards, it is trans located to various locations within the plant body through xylem. Assimilation of sulfur is occurring in the form of Cysteine; it acts as a precursor/donor of reduced sulfur mostly for other several organic sulfur compounds present in plants. Glucosinolates (GSLs) in response to defense against herbivores and other pathogens [16]. Sulfur deficiency leads to reduction in sulfate as well as S-containing compounds like Cysteine (Cys), methionine (Met) and GSH; protein synthesis was suppressed and the pools of soluble N, such as nitrate and amides, were also increased. Sulfur helps in seed growth and propagation [17]. Similarly our report suggests that sulfur is enhancing the root and shoot length of seed lings. Which can be applicable in agriculture for promoting the seedling growth. However further studies must be carried out in field trials for testing and promote the nanoparticles to field.

Conclusion

In this study, we successfully demonstrated the green synthesis of sulfur nanoparticles (SNPs) using *Syzygium cumini* bark extract as a reducing and stabilizing agent.

This eco-friendly synthesis approach leverages the natural phytochemicals present in the bark extract, offering a sustainable alternative to conventional chemical methods.

The synthesized SNPs were characterized by various techniques, confirming their size, morphology, and elemental composition. The impact of these biologically synthesized sulfur nanoparticles tomato germination was investigated, revealing significant enhancements in germination rate, root length and shoot length compared to untreated controls. The SNPs likely provided essential nutrients and exhibited antimicrobial properties, reducing pathogen stress and promoting healthier seedling growth. Overall, the green synthesis of SNPs using *Syzygium cumini* bark extract not only presents an environmentally benign method for nanoparticle production but also shows promising potential in agricultural applications. This method aligns with sustainable agricultural practices, enhancing crop productivity while minimizing the use of harmful chemicals. Future research could further explore the mechanisms behind SNPs' positive effects on plant growth and extend their application to other crops.

Bibliography

1. Ibrahim Khan., *et al*. "Nanoparticles: Properties, applications and toxicities". *Arabian Journal of Chemistry* 12.7 (2019): 908-931.

2. Manikandan Arumugam., *et al*. "Green synthesis of zinc oxide nanoparticles (ZnO NPs) using Syzygium cumini: Potential multifaceted applications on antioxidants, cytotoxic and as nanonutrient for the growth of Sesamum indicum". *Environmental Technology and Innovation* 23 (2021): 101653.

3. Nasim N., *et al*. "Plant-derived natural products for drug discovery: current approaches and prospects". *Nucleus (Calcutta).* 65.3 (2022) 399-411.

4. Patra JK., *et al*. "Nano based drug delivery systems: recent developments and future prospects". *Journal of Nanobiotechnology* 16.71 (2018).

5. Mondal S., *et al*. "Sulfur in Seeds: An Overview". *Plants (Basel)* 11.3 (2022): 450.

6. Roa GA., *et al*. "Increasing Wheat Protein and Yield through Sulfur Fertilization and Its Relationship with Nitrogen". *Nitrogen* 5 (2024): 553-571.

7. Ruan ZP., *et al*. "Evaluation of the antioxidant activity of Syzygium cumini leaves". *Molecules* 13.10 (2008): 2545-2556.

8. Alseekh S., *et al*. "Canalization of tomato fruit metabolism". *Plant Cell* 29 (2017): 2753-2765.

9. Ali MY., *et al*. "Nutritional Composition and Bioactive Compounds in Tomatoes and Their Impact on Human Health and Disease: A Review". *Foods* 10.1 (2020): 45.

10. Gilbert GS., *et al*. "Seed Disinfestation Practices to Control Seed-Borne Fungi and Bacteria in Home Production of Sprouts". *Foods* 12.4 (2023): 747.

11. Slavin YN and Bach H. "Mechanisms of Antifungal Properties of Metal Nanoparticles". *Nanomaterials (Basel)* 12.24 (2022): 4470.

12. Khan S., *et al*. "The impact of silver nanoparticles on the growth of plants: The agriculture applications". 9.6 (2023): 16928.

13. Lucimara A Forato., *et al*. "Protein Structure in KBr Pellets by Infrared Spectroscopy". *Analytical Biochemistry* 259.1 (1998): 136-141.

14. RM Tripathi., *et al*. "Green synthesis of sulfur nanoparticles and evaluation of their catalytic detoxification of hexavalent chromium in water". *RSC Advances* 8 (2018) 36345-36352.

15. Mondal S., *et al*. "Sulfur in Seeds: An Overview." *Plants (Basel)* 11.3 (2022): 450.

16. Rubel MH., *et al*. "Glucosinolate Profile and Glucosinolate Biosynthesis and Breakdown Gene Expression Manifested by Black Rot Disease Infection in Cabbage". *Plants (Basel) 9*.9 (2020): 1121.

17. Mondal S., *et al*. "Sulfur in Seeds: An Overview". *Plants (Basel)* 11.39 (2022): 450.