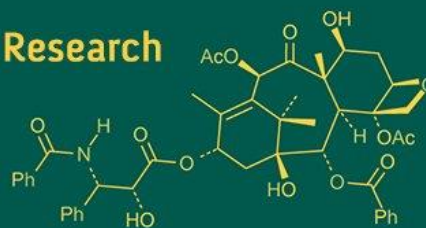


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Biogenic synthesis and characterization of sulphur nanoparticles (S-NPs) using different plant leaf extract

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Abstract

The Present investigation entitled "Biogenic synthesis and characterization of sulphur nanoparticles using different plant leaf extracts," was conducted at the Biotechnology Center, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, during Kharif 2023-2024. Using aqueous leaf extracts of *Azadirachta indica* (Neem), *Catharanthus roseus* (Vinca), *Mangifera indica* (Mango), and *Polyalthia longifolia* (Ashoka) the study sought to create a sustainable, economical, and environmentally friendly method for the manufacture of sulfur nanoparticles (SNPs). A light-yellow powder of nanoparticles was created by using sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) as the sulfur precursor and hydrochloric acid (HCl) as the reducing and acidifying agent. Depending on the plant extract utilized, the biosynthesized SNPs' surface charge and particle size varied significantly.

The particle size obtained were 86.2 nm for Neem, 98.69 nm for Vinca, 126.9 nm for Mango, and 187.3 nm for Ashoka extracts. These differences are attributed to variations in the phytochemical composition, including phenolics, flavonoids, and terpenoids, which influence the reduction rate and stabilization efficiency during nanoparticle formation. Neem and vinca extracts produced smaller nanoparticles, indicating higher reducing and capping capacities of their bioactive compounds. Zeta potential analysis confirmed that all synthesized SNPs were negatively charged, reflecting good colloidal stability. The respective zeta potential values were -21.8 mV (neem), -19.2 mV (vinca), -16.8 mV (mango) and -14.3 mV (ashoka) with neem-based SNPs exhibiting the greatest stability.

Overall, the study shows that a straightforward, environmentally friendly, and effective way to create stable sulphur nanoparticles is by biogenic synthesis utilizing plant extracts. *Azadirachta indica* was the most successful of all the plants studied it produced smaller, more stable nanoparticles that showed promise for use in nanobiotechnology and agriculture.

Keywords: Biogenic synthesis, sulphur nanoparticles, plant extracts, sodium thiosulfate, zeta potential, green nanotechnology

1. Introduction

The design, manipulation, and application of nanoscale materials are all part of the cutting-edge and innovative field of nanotechnology (Ali *et al.* 2014; Agrahari and Dubey 2020) ^[5, 2]. The Greek word "nano," which literally translates to "dwarf," is used to characterize materials that have at least one dimension of less than 100 nm. Nanotechnology is now present in every facet of daily life. It has increasingly turned to green or biogenic synthesis routes for nanomaterials, as these methods offer milder reaction conditions, reduced environmental footprint and the potential for biocompatibility when compare to the conventional chemical and physical methods (Antunes Filho *et al.*, 2023) ^[6]. In particular plant extract made synthesis of nanoparticles has become a preferred route because plant phytochemicals (flavonoids, terpenoids, alkaloids, polysaccharides) act both as reductants converting precursors into elemental or ionic nanomaterials, and as stabilisers or capping agents preventing aggregation (Huang *et al.*, 2007; Raj & Trivedi & Soni, 2022) ^[14, 20]. Nanotechnology has emerged as one of most dynamic areas in modern science due to its wide applications in medicine, agriculture, energy, and environmental remediation. While physical procedures emit harmful byproducts that harm the environment, chemical approaches use hazardous precursors to create nanoparticles. Therefore, it is imperative to employ eco-friendly, environmentally safe methods for the synthesis of nanoparticles (Bhardwaj *et al.*, 2020) ^[9].

By strengthening crop protection, boosting nutrient management, and fostering environmental sustainability, nanotechnology has enormous potential to transform agriculture (Sivarethinamohan and Sujatha, 2021) ^[125].

In order to solve these problems, nano-engineered sulfur, also referred to as nano-sulphur or NS and consisting of particles typically smaller than 100 nm, has emerged as a game-changing technology. Because of their high surface-area-to-volume ratio, improved solubility, and better soil-plant interface interactions, these nanoparticles facilitate more effective sulfur transport and assimilation than bulk forms of S (Wang *et al.*, 2022; Mahajan *et al.*, 2023) ^[27, 18]. For the production of proteins, lipids, vitamins, and compounds that improve plant flavor, sulfur is essential. Crop yield, seed quality metrics, and the amount of sulfur in seeds are closely related (Zenda *et al.*, 2021) ^[28]. Because of their special qualities, such as their large surface area and increased reactivity, nanomaterials are perfect for a variety of agricultural uses, such as controlling plant infections and releasing fertilizer slowly (Goswami *et al.*, 2022) ^[12].

In order to create nanoparticles that can lower metal ions, scientists are constantly investigating different bacteria, fungi, and plants (Bansal *et al.*, 2014; Duhan *et al.*, 2017) ^[8, 11]. The process is called "biofabrication of nanoparticles" when any biological material or its byproduct is used to create the nanoparticles. These make use of bacteria and their extracts, fungi and their cell-free extracts, plant tissue extracts, and plant materials and their crude and solvent-specific extracts, whether organic or aqueous. Different metal and nonmetal salts are used to test these extracts. The biofabrication process becomes somewhat uncontrollable when the biochemical content of extracts is derived from bacteria, fungi, animal tissue, or plants (Bansal *et al.*, 2014) ^[8]. Due to their distinct physicochemical and biological characteristics, including intense antibacterial activity, high surface reactivity, and prospective application in fertilizers, insecticides, and medication formulations, sulfur nanoparticles (SNPs) have garnered significant interest among the different nanomaterials. Sulfur nanoparticles are traditionally produced via physical and chemical processes that frequently need hazardous byproducts, significant energy consumption, and toxic reagents. Green or biogenic synthesis techniques employing plant-based biomolecules have been proposed as sustainable, economical, and environmentally friendly substitutes to address these shortcomings.

Target-specific and regulated release of nutrients and agrochemicals, plant-nanoparticle interaction, their reaction to soil science, uptake, localization, activity, disease resistance, crop production, and nutrient utilization are all made possible by nanotechnology (He *et al.* 2018). At first, sulfur (S) was not thought to be as important for crop nutrition as nitrogen (N), phosphorus (P), and potassium (K). But due to its significant function in plant metabolism and environmental resistance, it is currently receiving greater research (Abdin *et al.*, 2003; Turganbay *et al.*, 2019) ^[1, 26]. Modern agricultural intensification, cleaner emissions technologies, and the extensive use of high analysis sulfur-deficient fertilizers which were previously sufficiently supplied by atmospheric deposition and sulfur-rich fertilizers have all contributed to the global spread of S deficiencies (Zenda *et al.*, 2021; Alfalahi and Abdulqahar, 2021) ^[28, 4]. This issue is particularly serious in Indian

agriculture because of the underutilization of S-enriched amendments following the Green Revolution (Bhoyar *et al.*, 2019; Rakesh *et al.*, 2020) ^[16, 21].

Using leaf extracts from four medicinal plants that demonstrated antibacterial activity with Na₂Sx, the SNP was synthesized. Na₂Sx combines with sulfuric acid in an acidic environment to produce visible yellow precipitates. (Paralikar *et al.*, 2017) ^[19]. Ingle *et al.* (2024) ^[15] used leaf extract from *Moringa oleifera* to create zinc oxide and SNP nanoparticles. The final nanoparticles were 26 nm and 78 nm in size, respectively. Using *Cannabis sativa* leaf extract, Dasauni, Nailwal, and Naik Nenavathu (2024) ^[17] demonstrated the biogenesis of sulfur nanoparticles with mixed rod and spherical morphology (20-30 nm diameter for spheres and 50-100 nm length for rods). In one study, Baloch *et al.*, (2023) ^[7] synthesized green SNPs using citrus limon leaves the resulting SNPs were spherical in shape and 40 nm in size. An aqueous extract of *Punica granatum* peels has been successfully used to create sodium thiosulphate SNPs (Salem *et al.*, 2016) ^[23]. The resulting SNPs were spherical in shape and roughly 50 nm in size. According to Akl M. Awwad *et al.* (2015) ^[3], sulfur nanoparticles were successfully made from sodium thiosulfate in the presence of *Albizia julibrissin* fruit extract at room temperature. The final product was described as highly crystalline sulfur with high purity, a spherical shape, an average particle size of roughly 20 nm, and a particle size distribution between 10 and 100 nm.

The environmentally friendly green synthesis of sulfur nanoparticles is receiving more attention due to growing environmental concerns (Dhand *et al.*, 2015) ^[10]. Using biological resources like plants and microbes, this green synthesis is an inexpensive method that can be carried out at room temperature. Compared to microorganisms, plant material has a shorter reaction time and contains certain chemical compounds that either act as reducing agents (phenolic compounds) or cover the nanoparticles (capping agents), improving their functionality and reactivity (Saxena *et al.*, 2012; Saikia and Lens, 2020) ^[24, 22].

Plant extracts serve as stabilizing and reducing agents in the biogenic synthesis method promoting the creation of nanoparticles in moderate environments. Flavonoids, terpenoids, alkaloids, tannins, phenols, and proteins are examples of phytochemicals found in plant extracts that are essential for the nucleation, growth, and stabilization of nanoparticles. In addition to reducing metal or non-metal ions, these bioactive substances cap the resultant nanoparticles, preventing agglomeration and regulating particle size. In addition to reducing environmental risks, this green synthesis method offers a sustainable way to produce functional sulfur nanoparticles that could be used in biomedicine, agriculture, and environmental preservation.

2. Materials and Methods

2.1 Materials

Hydrochloric acid (35% HCl) and sodium thiosulfate anhydrous Hi-ARTM (Na₂S₂O₃). Every chemical utilized was of analytical quality and didn't require any additional purification. Sulfur nanoparticles were prepared using sterile double-distilled water. Before being used, every glass container was cleaned with sterile double-distilled water and dried in a hot air oven. Aqueous extracts were prepared using sterile double-distilled water.

2.2 Collection and Preparation of Plant Extracts

Polyalthia longifolia (Ashoka), *Catharanthus roseus* (Vinca), *Azadirachta indica* (neem), and *Mangifera indica* (mango) all had fresh, mature, and healthy leaves that were gathered from the surrounding area. To ensure total cleanliness, the leaves were rinsed with sterile double-distilled water after being properly cleaned under running tap water to get rid of dust and debris. To make extraction easier, the cleaned leaves were dried and then chopped into little pieces.

A mortar and pestle were used to smash 20 g of the chopped leaves from each plant species in 400 mL of sterile double-distilled water in order to prepare the aqueous extract. To get rid of plant debris, the mixture was filtered through Whatman No. 1 filter paper. After that, the filtrate was centrifuged for five minutes at 1200 rpm to remove heavy biomaterials and produce a clear supernatant that was used as the plant aqueous extract. For later usage, these aqueous extracts were kept at room temperature.

2.3 Synthesis of Sulfur nanoparticles using plant leaf extract

For synthesis sulfur nanoparticles were used sodium thiosulfate as a sulfur precursor and hydrochloric acid as an acidifying agent. SNPs were synthesized as follows. 3.2M of 25 mL $\text{Na}_2\text{S}_2\text{O}_3$ solution was mixed with 50 mL of prepared plant aqueous extract in 250 mL beaker. To this mixture 25 mL of sterile double-distilled water was added and the solution is diluted to 100 mL and continues stirring on a magnetic stirrer for 5 minutes to ensure homogeneity. Afterwards HCl was added drop by drop with the mild stirring for allowing to sulphur precipitations uniformly. In plant extract and acidic solution ($\text{Na}_2\text{S}_2\text{O}_3$) undergoes through a disproportionation reaction to sulphur and sulfonic acid according to :



After being separated by centrifugation at 1000 rpm for five minutes, the suspended sulfur nanoparticles were repeatedly cleaned with sterile distilled water to get rid of any biological materials.

Following purification, the sulfur nanoparticles were vacuum-dried for two hours at 80 °C. It was a pale yellow powder.

2.4 Characterization of Sulfur Nanoparticles

The physicochemical characteristics of the dried sulfur nanoparticles were assessed. Zeta potential tests were carried out to assess the surface charge and colloidal stability of the produced nanoparticles, and the particle size distribution was examined using a particle size analyzer (Dynamic Light Scattering technique). The four plant extract-mediated syntheses were examined for variations in nanoparticle size and stability.

2.5 Characterization of Synthesized Sulfur Nanoparticles

The synthesized sulfur nanoparticles were characterized to determine their particle size and Zeta Potential.

2.5.1 Particle Size Analysis: Using a Dynamic Light Scattering (DLS) particle size analyzer, the average particle size and size distribution of the sulfur nanoparticles were ascertained using the Malvern Zeta sizer Nano ZS 90

device. To guarantee uniform suspension and avoid agglomeration, samples were ultrasonically dispersed in deionized water for ten minutes before analysis.

2.5.2 Zeta Potential: A Zeta Potential Analyzer (Malvern Zetasizer, nano ZS-90) was used to examine the stability and surface charge of the nanoparticles. To ensure accuracy and reproducibility, measurements were made at 25 °C and each sample was examined three times. Dynamic light scattering (DLS) measurements were performed in the usual range of + 100 mV to -100 mV. The electrostatic stability of the colloidal sulfur nanoparticles made with various plant extracts was evaluated using the zeta potential values. High levels of stability are usually exhibited by nanoparticles with Zeta potential values greater than +25 or less than -25; a negative value of -15 indicates the start of particle agglomeration.

3. Results and Discussion

When leaf extracts from *Azadirachta indica* (neem), *Catharanthus roseus* (Vinca), *Mangifera indica* (mango), and *Polyalthia longifolia* (Ashoka) are treated with sodium thiosulfate and acidic solution (HCl), a yellowish precipitate forms in the solution, indicating the formation of sulfur nanoparticles. The current study is founded on the green chemistry principle, which addresses the employment of safe, environmentally friendly technologies to lessen the impact of chemical manufacturing on the environment and human health. Plants are more effectively used in the biological manufacturing of nanoparticles since they are essential in reducing precursors. It demonstrated that, in comparison to other biological systems, nanoparticles made from plants, particularly leaf extract, are more stable. The bioreduction and stabilization of nanoparticles may be caused by a variety of biomolecules, including alkaloids, polysaccharides, flavonoids, terpenoids, etc.

3.1 Particle Size Analysis

Using the Malvern Zeta sizer Nano ZS 90 and nanoparticle tracking analysis (NTA) software, the resulting sulfur nanoparticles were further examined. According to NTA, the sulfur nanoparticles' respective sizes were 86.22, 126.9, 187.3, and 98.69 nm for *Azadirachta indica* (neem), *Mangifera indica* (mango), *Polyalthia longifolia* (ashoka), and *Catharanthus roseus* (vinca). (Fig.1). It shows the particle sizes of four plant leaf extract-mediated synthetic sulfur nanoparticles. The NTA approach examines individual nanoparticles and determines their sizes based on each particle's Brownian motion.

The findings unequivocally show that while catharanthus extract produced comparatively larger particles, neem leaf extract created the tiniest and most homogeneous nanoparticles. The variations in the phytochemical makeup of each plant extract are responsible for this diversity in particle size. Flavonoids, terpenoids, and azadirachtin, which are abundant in neem extract, function as potent reducing and capping agents that effectively stabilize the nucleated sulfur particles and stop them from aggregating. On the other hand, lower concentrations of stabilizing biomolecules in the aqueous extracts of mango, ashok, and catharanthus may be the cause of the somewhat bigger particle sizes produced with these extracts.

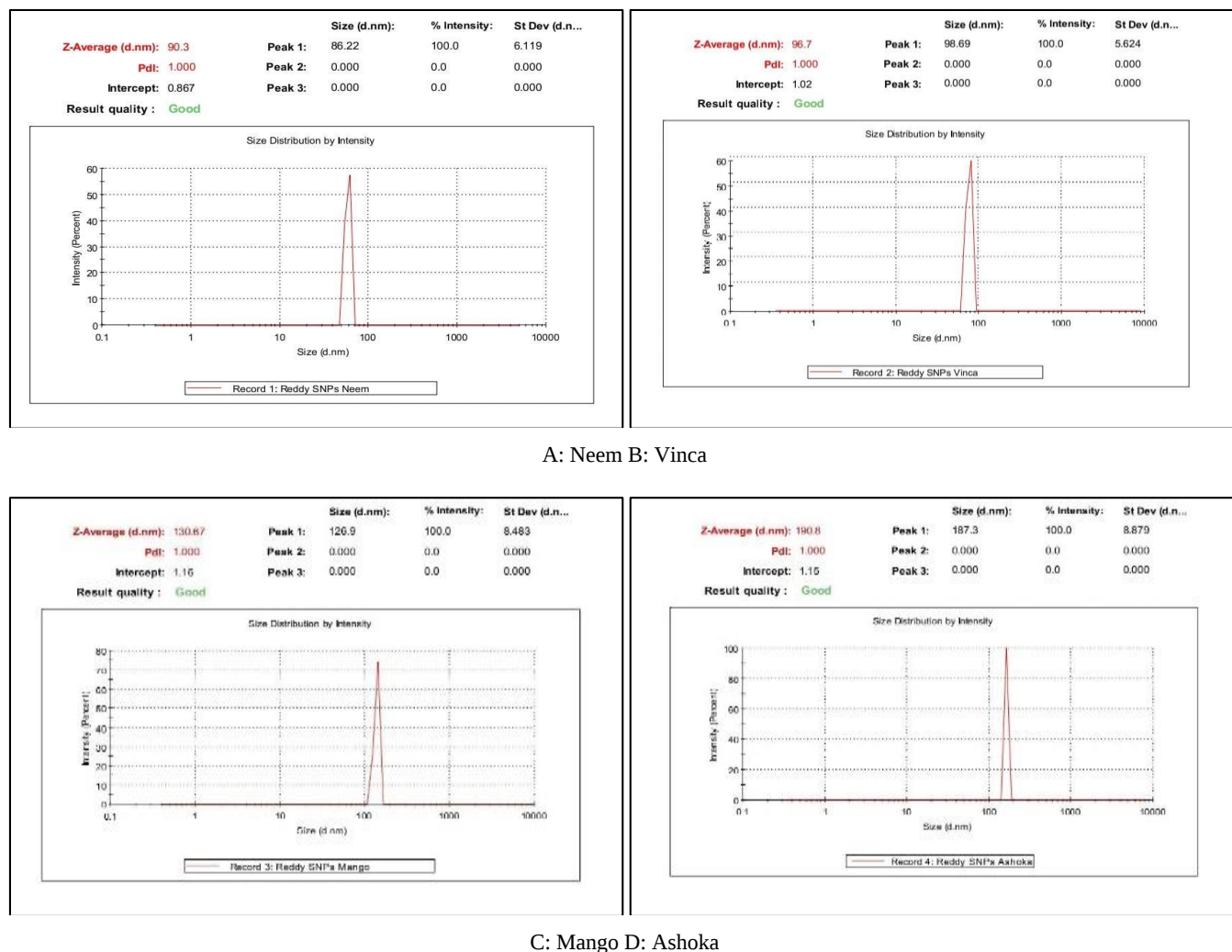


Fig 1: Characterization of sulphur nanoparticles synthesized using different plants leaf extract of Particle size (NTA)

3.2 Zeta Potential Analysis

For every sample made from four distinct plant extracts, zeta potential analyses were performed. The surface charge obtained by synthesizing from various plant extracts was measured in this study, which revealed a significant negative zeta potential. The values of the zeta potential fell between -14.3 and -21.8. *Azadirachta indica* (neem) -21.8 mV, *Mangifera indica* (mango) -16.8, *Polyalthia longifolia* (Ashoka) -14.3, and *Catharanthus roseus* (Vinca) -19.2 (Fig. 2). These numbers verified that the nanoparticles had stabilized. *P. longifolia* leaf extract produced sulfur nanoparticles with a lower negative zeta potential value, whereas *A. indica* leaf extract produced sulfur nanoparticles with the highest negative zeta potential. These results were consistent with the earlier investigation, which also showed that sulfur nanoparticles had a negative potential value. The great stability of the sulfur nanoparticles made from *A. indica* was demonstrated by their significant negative zeta potential value.

The surface charge and colloidal stability of the produced sulfur nanoparticles were evaluated using zeta potential measurement. In comparison to other plant extracts, the neem-mediated sulfur nanoparticles had the greatest negative zeta potential value across all samples, suggesting stronger electrostatic repulsion and improved stability in aqueous solution. Strong adsorption of negatively charged phytochemicals, such as flavonoids and phenolic acids, on the surface of sulfur nanoparticles is suggested by the significantly negative zeta potential, which prevents agglomeration through repulsive forces. Sulfur nanoparticles made with ashok, catharanthus, and mango extracts, on the other hand, displayed smaller negative zeta potential values, suggesting a comparatively lower surface charge density and moderate colloidal stability. Smaller nanoparticles (derived from neem extract) showed increased surface charge and improved stability, which was consistent with the trend of zeta potential values.

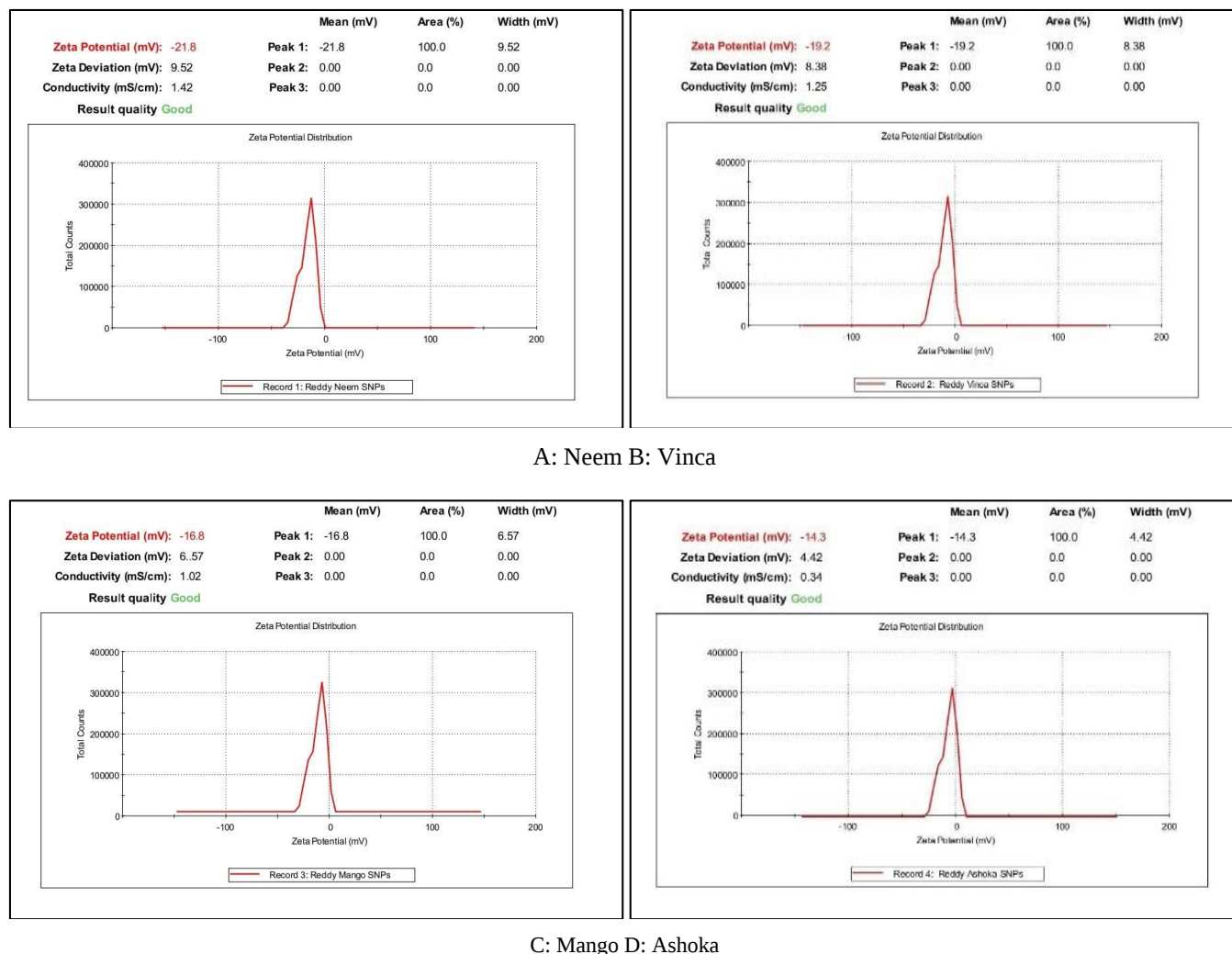


Fig 2: Characterization of sulphur nanoparticles synthesized using different plants leaf extract of Zeta potential

4. Conclusion

The current study, "Biogenic synthesis and characterization of sulfur nanoparticles using different plant leaf extracts," which was carried out in Kharif 2023-2024 at the Biotechnology Center, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. PDKV), Akola effectively demonstrated a sustainable and environmentally friendly method for the synthesis of sulfur nanoparticles (SNPs) using a variety of plant leaf extracts. The reduction of sodium thiosulfate in the presence of hydrochloric acid was successfully aided by the application of extracts from *Azadirachta indica* (neem), *Catharanthus roseus* (vinca), *Mangifera indica* (mango), and *Polyalthia longifolia* (ashoka) to create fine, light-yellow sulfur nanoparticles.

The results showed that the size, stability, and surface charge of the produced nanoparticles were significantly influenced by the phytochemical makeup of each plant extract. Neem-mediated SNPs showed the highest negative zeta potential (-21.8 mV) and the smallest particle size (86.2 nm), suggesting better stability and lowering power. Vinca (98.69 nm; -19.2 mV), mango (126.9 nm; -16.8 mV), and Ashoka (187.3 nm; -14.3 mV) extracts came next. Different bioactive substances, such as flavonoids, phenolics, and terpenoids, may contribute differently to the nucleation, growth, and stability processes during nanoparticle production, according to the found difference among extracts.

Overall, the study demonstrates that employing plant extracts for the green production of sulfur nanoparticles is a viable, straightforward, and non-toxic substitute for traditional chemical techniques. *Azadirachta indica* shown its promise as a superior biological source for nanoparticle synthesis by being the most effective among the examined plants in manufacturing smaller and more stable nanoparticles. These biogenically produced SNPs have the potential to be an environmentally benign substance with uses in nanobiotechnology, agriculture, and plant protection.

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