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Green synthesis, optimization, and characterization of zinc oxide nanoparticle using *Lantana camara* L. leaf extract

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Abstract
In this study, zinc oxide nanoparticles (ZnO-NPs) were successfully synthesized using a green approach with *lantana camara* leaf extract. The biogenic zinc oxide nanoparticles were optimized on the basis of various physiochemical parameters such as pH, temperature, incubation time, precursor type and concentration of capping agent which revealed that the zinc oxide nanoparticles were best optimized by using zinc nitrate heptahydrate as precursor with ph 7 at a temperature of 40^{o}C for 4 hours and stabilized with the help of 2 molar sodium hydroxide. The synthesized nanoparticles were comprehensively characterized using modern analytical techniques. X-ray diffraction (XRD) analysis revealed the crystalline nature of ZnO-NPs with an average particle size of 9.6 nm. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images depicted rod-shaped nanoparticles with slight agglomeration, having a particle size ranging from 9.6 nm to 25.5 nm. UV-VIS spectroscopy results indicated significant absorption at 360 nm, corresponding to the ZnO-NPs. The results of Fourier transform infrared (FTIR) revealed certain functional groups such as alkenes, nitro compounds, aliphatic ethers and more present on the surface of zinc oxide nanoparticles. The study demonstrates the potential of ZnO-NPs synthesized from *lantana camara* leaf extract to enhance seedling growth, offering agricultural applications for improved seed germination and crop improvement.

Keywords: Nanoparticles, secondary metabolites, biogenic, characterization and optimization

Introduction

In last decade, anoparticles have emerged as versatile solutions for a range of challenges across diverse scientific domains including agriculture, engineering, medicine, water purification, and catalysis. Remarkable progress in this field has been documented by influential researchers such as Colvin *et al.*, (1994) [7], Chan and Nie (1998) [5], Cao (2004) [3], Goodsell (2004) [11], Klefenz (2004) [18], Pissuwan *et al.*, (2006) [24], Tan *et al.*, (2006) [32], Cai *et al.*, (2008) [2], and Lee *et al.*, (2008) [20]. Notably, metal nanoparticles have found a compelling application in enhancing seed germination and growth. The distinctive properties of nanoparticles, including optical, electrical, and mechanical attributes, are intricately linked to their size, composition, and structural configuration (Caruso, 2001) [4]. The refinement of synthesis methods has enabled optimization of these properties, as exemplified by the work of Cho *et al.*, (2013) [6]. Various metal nanoparticles such as copper iron silver titanium gold etc. have been synthesized by various methods and harnessed for enhancing the growth and development of crop plants. While nanoparticles have demonstrated superior efficacy compared to traditional metal salt-based approaches, commercially available nanoparticles are primarily synthesized using energy-intensive and expensive physical and chemical methods. The drawbacks of these methods lie in their energy consumption, toxicity, and limited environmental friendliness (Vijayaraghavan and Ashok kumar, 2017) [33]. The pursuit of biocompatible, non-toxic, and eco-friendly nanoparticle synthesis has led to the exploration of alternative routes, notably through microorganisms (Klaus *et al.*, 1999; Konishi *et al.*, 2007) [17, 20] and plant extracts (Shankar *et al.*, 2004; Ahmad *et al.*, 2011) [30, 1]. Among these options, plant extracts have gained prominence due to their wide availability, renewable nature, simplicity of the synthesis process, efficiency, stability of synthesized nanoparticles, and cost-effectiveness (Iravani, 2011; Vijayaraghavan and Ashokkumar, 2017) [14, 33]. This shift towards biologically mediated nanoparticles synthesis not only addresses the limitations of energy-intensive and chemically laden approaches but also aligns with sustainability goals and environmental stewardship.

By harnessing the power of natural materials, researchers are paving the way for greener and more effective applications of nanoparticles in various fields, contributing to a paradigm shift in modern science and technology.

Materials and Methodology

Collection of Materials

The present investigation was carried out in the Plant Genomics Laboratory, Division of Plant Biotechnology, College of Biotechnology. The wild plants of *lantana camara* L. were taken from main campus of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. The required chemicals, including zinc acetate hexahydrate, zinc nitrate heptahydrate, zinc sulphate monohydrate and sodium hydroxide, were procured from Hi-media, while ethanol was obtained from Merck Germany PVT. Ltd.

Preparation of *Lantana camara* leaf extract

Plant material was collected and brought to the laboratory, where leaves were carefully separated and cleaned using both tap water and distilled water. Subsequently, the leaves were air-dried at room temperature for a span of 3 to 4 days. Following the thorough drying process, the leaves were finely powdered using a mortar and pestle. 5 grams of the powdered *Lantana camara* material was subjected to boiling in 100 ml of distilled water for duration of 15 minutes. The resulting mixture was then subjected to filtration using Whatman filter paper (No.1), aimed at removing solid residues and obtaining a clear extract. This process was meticulously repeated to ensure the purity of the extract.

Green synthesis of zinc oxide nanoparticles

A solution of 0.1 M zinc nitrate was created by dissolving zinc nitrate in 90 ml of deionized water. Once the zinc nitrate was fully dissolved, the solution-filled flask was subjected to heating on a water bath at 80°C for a period of 5 to 10 minutes. Subsequently, the heated zinc nitrate solution was blended with 10 ml of plant extract while maintaining continuous stirring. This amalgam of solutions was then maintained at a temperature of 100°C for duration of 5 hours, with rigorous stirring throughout the process.

Purification and concentration of zinc oxide nanoparticles

The resulting precipitate was removed by employing centrifugation at 7000 rpm for 15 minutes. Eventually, the precipitate underwent a transformation from brown to a solid pale yellow color. Subsequently, purification was carried out by sequential washing with de-ionized water and methanol, followed by air drying. Ultimately, this process yielded a final product in the form of white-colored powder.

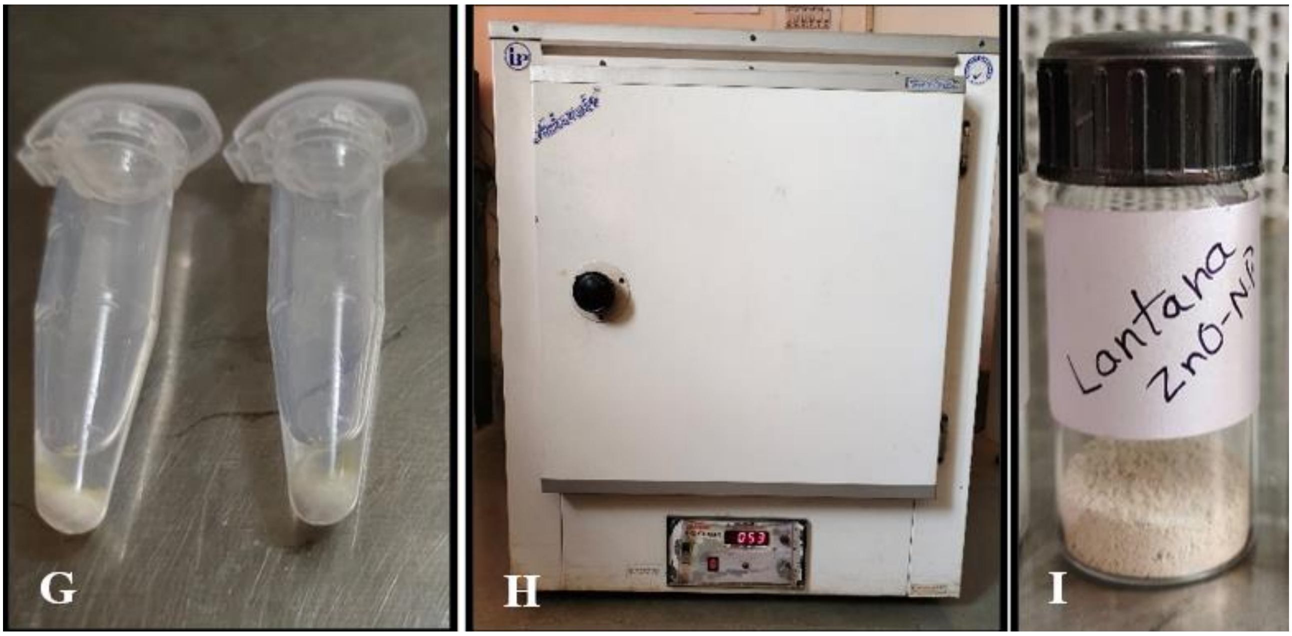

Fig 1: Procedure for the biogenic synthesis of ZnO-NPs from *lantana camara* leaf extract (A) Fresh leaves of *L. camara*, (B) Fine leaf powder, (C) Aqueous leaf extract, (D) Precursor and capping agent, (E) Incubation of leaf extract with precursor and addition of capping agent, (F) Centrifugation to obtain nanoparticles in pellet, (G) Purification of pellet, (I) Air drying of pellet suspended in deionized water and (H) Fine white colored ZnO nanopowder.

Optimization of ZnO nanoparticles

The production of metallic nanoparticles is influenced by a range of variables, including pH, temperature, capping agent concentration, incubation time, and precursor type. To attain the most optimal and effective yield of zinc oxide nanoparticles, a thorough optimization of four distinct physicochemical parameters was investigated for optimization.

pH value

Size and dissolution characteristics of the synthesized nanoparticles are intricately influenced by the pH of the solution. Under current study three distinct pH values: 6, 7, and 8, was investigated.

Incubation time

Contact time of reactants is also considered to be a major factor for the synthesis of a product. The synthesis of zinc oxide nanoparticles was done at four interval of time such as: 2 hours, 4 hours and 6 hours.

Temperature

It is the most important attribute among all the physiological parameters for biogenic synthesis of metallic nanoparticles as the whole reaction and reduction process of nanoparticles formation is depend on temperature. So generally the biogenic synthesis of nanoparticles requires 40 to 80ºC temperature for best and efficient synthesis and it may vary between different plants species used for reduction process.

Type of precursor

Generally in the literature certain salts of metals have been used for green synthesis of nanoparticles as they works as substrate on which the secondary metabolites of plants act and reduces its size to nanometers but not every salt goes for reduction process because of the conversion into oxide ions so in the case of potassium nanoparticles three types of substrates such as: potassium sulphate, potassium chloride and potassium acetate was used.

Conentration of capping agent

The capping agent such as sodium hydroxide can be used to stabilize the biogenic ZnO-NPs because the shelf life of green synthesized nanoparticles is less so to prevent agglomeration of nanoparticles, capping agents are used in the reaction.

Characterization of zinc oxide nanoparticles

The optical characteristics of the fabricated ZnO nanoparticles were validated via Ultra Violet-visible spectroscopy spanning the wavelength range of 200 to 400 nm. Functional groups present within the synthesized ZnO nanoparticles were analyzed employing a Fourier transform infrared (FT-IR) spectrometer. The FT-IR spectra were acquired across the spectrum of 4000 to 400 cm-^{1}, utilizing the KBr pellet method. The 2D structural attributes of the produced ZnO nanoparticles were elucidated through X-ray diffractometry, covering a 2θ range spanning from 20° to 80°. Further insight into the 3D structure of the ZnO nanoparticles was gained through scanning electron microscopy (SEM), while the size measurements were obtained utilizing transmission electron microscopy (TEM). These analytical techniques collectively offer a comprehensive characterization of the synthesized ZnO nanoparticles, shedding light on their optical, functional, and structural properties.

Results and discussion

Green synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles were successfully synthesized using an herbal plant leaf extract derived from *Lantana camara* L. These findings align with the outcomes of prior research by Elumalai and Velmurugan (2015) [36], who investigated the green synthesis of zinc oxide nanoparticles using *Lantana camara* leaf extract. Furthermore, the outcomes of this green synthesis approach for ZnO-NPs are consistent with the observations made by Parthasarathy *et al.*, (2016) [37]. In their study, they also explored the green synthesis of zinc oxide nanoparticles and demonstrated that plant extracts not only facilitate controlled synthesis but also play roles as stabilizing, capping, or hydrolytic agents. This convergence of results emphasizes the viability and potential of using plant extracts for eco-friendly nanoparticles synthesis.

Optimization of zinc oxide nanoparticles

The selection of optimal conditions for the synthesis of green ZnO nanoparticles was driven by the spectral peak at 360 nm, which represented the highest intensity for ZnO-NPs. The pH optimization yielded a pH of 7, resulting in a concentration of 10.46 mg/ml and an absorbance value of 1.573 nm. Further, a synthesis time of 4 hours was determined to be optimal, resulting in a concentration of 11.91 mg/ml and an absorbance value of 1.750 nm. The temperature optimization identified 40°C as the best condition, leading to a concentration of 12.82 mg/ml and an absorbance value of 1.861 nm. Among the precursors tested, zinc nitrate heptahydrate was found to be the most suitable, yielding a concentration of 12.01 mg/ml and an absorbance value of 1.762 nm. Additionally, a concentration of 2 molar NaOH emerged as the optimal capping agent, resulting in a concentration of 11.13 mg/ml and an absorbance value of 1.655 nm. These optimized parameters collectively contribute to the efficient synthesis of ZnO-NPs using a green approach.

Table 1: Results of optimization of various physiochemical parameters for biogenic ZnO-NPs

Sample (pH value)	Absorbance at 360 nm	Concentration (mg/ml)
6	1.244	7.78
7	1.573	10.46
8	1.204	7.45
Sample (Incubation time)		
2 hours	1.378	8.87
4 hours	1.750	11.91
6 hours	1.690	11.42
Sample (Temperature)		
40	1.861	12.82
60	1.238	7.73
80	1.302	8.25
Sample (Precursor)		
Zinc acetate	1.709	11.57
Zinc nitrate	1.762	12.01
Zinc sulphate	1.647	11.07
Sample (Capping agent)		
1 molar	1.272	8.01
2 molar	1.655	11.13
3 molar	1.429	9.29

Characterization of zinc oxide nanoparticles

UV visible spectroscopy

The optical characteristics of ZnO nanoparticles (ZnO NPs) were evaluated through UV-Vis Spectrophotometry, encompassing absorbance measurements within the range of 300 to 400 nm. The obtained outcomes revealed that ZnO NPs exhibited a prominent peak at 360 nm, yielding an absorbance value of 0.765. These findings closely paralleled those of Gure *et al.*, (2021) [38], who investigated the optical attributes of zinc oxide nanoparticles via UV–Vis absorption spectroscopy within the 200–800 nm span. Similarly, Ramesh *et al.*, (2021) [39] scrutinized the optical features of their synthesized ZnO nanostructures utilizing UV–Vis spectroscopy, highlighting pronounced ultraviolet absorption across a range of approximately 200–900 nm. Remarkably, the optimal absorption wavelength for ZnO after annealing was noted at 365 nm.

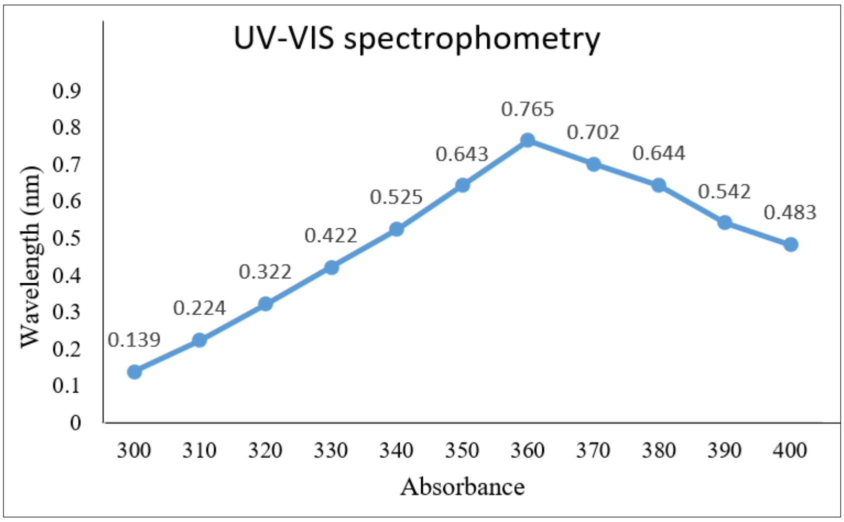

Fig 2: UV-VIS spectrophotometry results of green synthesized zinc oxide nanoparticles

FTIR analysis

In the infrared spectrum (FTIR) of the synthesized green ZnO nanoparticles, several characteristic peaks are observed, revealing key information about their composition and structure. The spectrum displayed in Figure 3 demonstrates peaks within the range of 3300 to 500 cm^{-1}, which have been attributed to ZnO-NPs (Tas *et al.*, 2002) [40]. Specifically, the FTIR analysis reveals distinct bands at 426 cm^{-1} and 516 cm^{-1}, which correspond to metal-oxide (M-O) vibrations. Additionally, significant bands are identified at 3231.32 cm^{-1} and 1519.11 cm^{-1}, indicating the presence of O-H asymmetric stretching and N-O stretching vibrations, respectively. Notably, a prominent peak emerges at 1411.78 cm^{-1}, attributed to the S=O stretching of sulfate molecules within the ZnO nanoparticles. This finding underscores the role of sulfur compounds in the nanoparticles' composition. The functional groups responsible for the synthesis of ZnO nanoparticles are linked to nitro and ketone functionalities. These groups play a crucial role in the formation and stabilization of the nanoparticles during the synthesis process. This detailed FTIR analysis provides insights into the molecular components of the green synthesized ZnO nanoparticles and their potential applications.

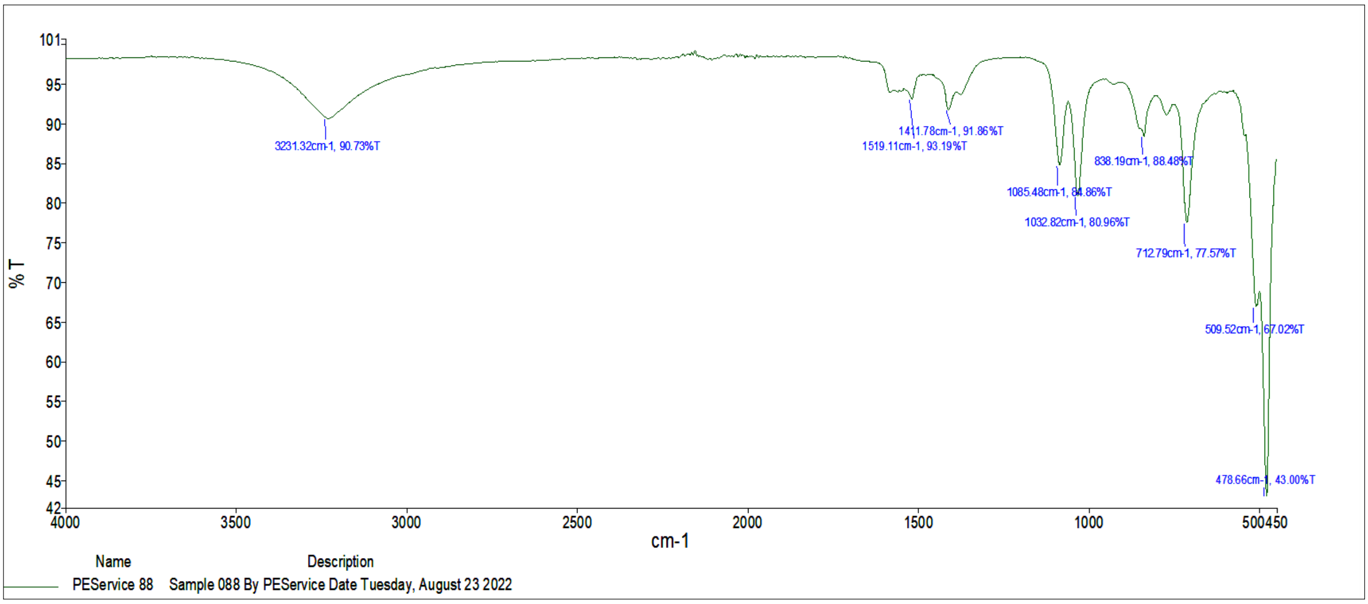

Fig 3: Graphical representation of FTIR analysis of ZnO-NPs synthesized from *L. camara*

Table 2: Absorption peaks derived from FTIR graph for identification of compounds

Frequency Range	Absorption (cm-1)	Appearance	Group	Compound Class
3300-2500	3231.32	strong, broad	O-H stretching	carboxylic acid
1550-1500	1519.11	Strong	N-O stretching	nitro compound
1415-1380	1411.78	Strong	S=O stretching	sulfate
1150-1085	1085.48	Strong	C-O stretching	aliphatic ether
1070-1030	1032.82	Strong	S=O stretching	sulfoxide
850-550	838.19	Strong	C-Cl stretching	halo compound
730-665	712.79	Strong	C=C bending	alkene
600-500	509.52	Strong	C-I stretching	halo compound

XRD analysis of synthesized nanoparticles

X-ray diffraction analysis was conducted to verify the phase of the synthesized nanoparticles. The distinctive diffraction peaks confirmed the crystalline nature of ZnO-NPs. The presence of definite line broadening in the XRD peaks suggested that the prepared material consisted of particles within the nanoscale range. The diffraction peaks located at 31.84°, 34.52°, 36.33°, 47.63°, 56.71°, 62.96°, 68.13°, and 69.18° were accurately identified as characteristic of the hexagonal wurtzite phase of ZnO (Zhou *et al.*, 2007; Khoshhesab *et al.*, 2011) [25, 6]. Importantly, this analysis also affirmed the absence of impurities in the synthesized nanopowder, as there were no discernible XRD peaks other than those corresponding to ZnO-NPs.

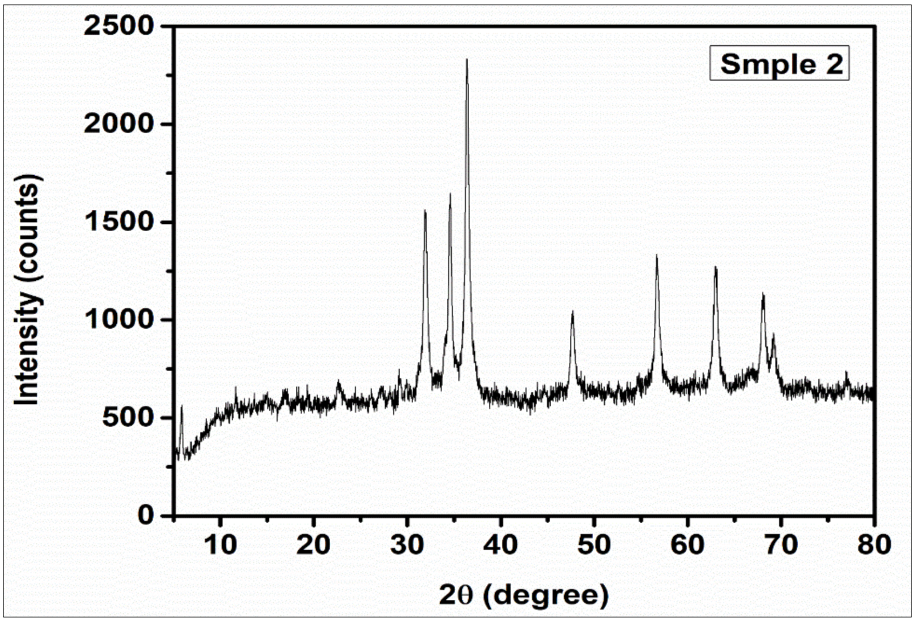

Fig 4: XRD pattern of ZnO-NPs nanoparticles

TEM analysis of zinc-oxide nanoparticles

Transmission electron microscopy (TEM) images clearly demonstrate that the ZnO nanoparticles produced through biosynthesis exhibit a distinctive rod-like morphology, characterized by a tightly constrained size distribution, as depicted in Figure 5. The diameter of these ZnO nanoparticles spans a range from 9.6 nm to 25.5 nm. Remarkably, the dimensions of the ZnO nanoparticles in this study closely mirror those obtained through biosynthesis using leaf extracts of *Parthenium hysterophorus* L, *Aloe barbadensis*, and *Poncirus trifoliate*. This consistency underscores the reproducibility of the biosynthesis method across diverse botanical sources and emphasizes the potential utility of this approach in generating nanoparticles with controlled and desirable properties.

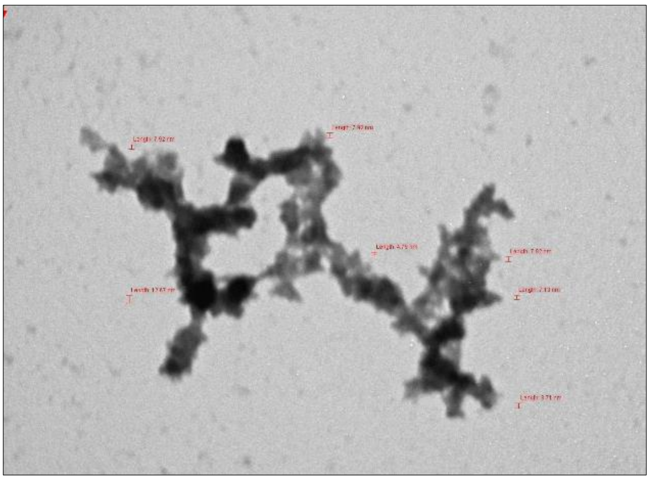

Fig 5: TEM images of ZnO NPs obtained by green synthesis using *L. camara*

SEM analysis of zinc-oxide nanoparticles

The morphology and structure of ZnO-NPs were examined through SEM analysis, as illustrated in Figure 6. The results revealed that the ZnO-NPs exhibited a crystalline structure and displayed a distinctive crystal-like shape, with minor instances of aggregation.

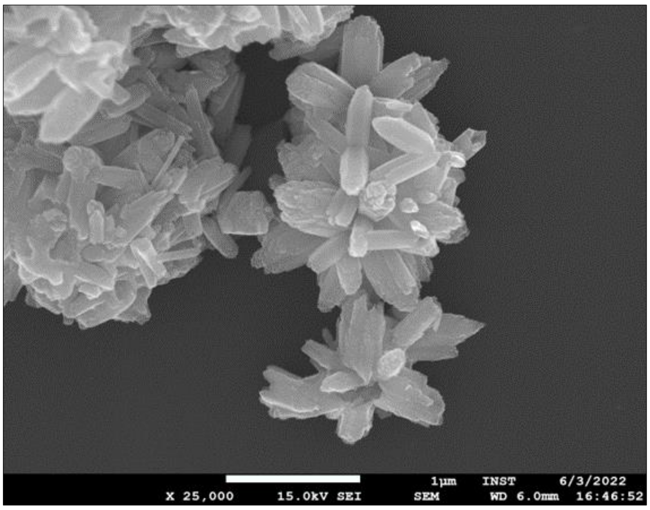

Fig 6: The Scanning electron micrograph of ZnO-NPs synthesized from *lantana camara* leaf extract.

Conclusion

In the present study, zinc oxide nanoparticles (ZnO-NPs) were successfully synthesized using *lantana camara* leaf extract and subjected to a comprehensive characterization employing modern analytical techniques. X-ray diffraction (XRD) analysis unequivocally confirmed the crystalline nature of the ZnO-NPs, revealing an average particle size of 9.6 nm. Further insights from transmission electron microscopy (TEM) elucidated that the nanoparticles exhibited a rod-like morphology with agglomeration, displaying particle sizes ranging around 25.5 nm. UV-VIS spectroscopy provided corroborating evidence, affirming the optical properties of the ZnO-NPs with an observed absorption peak at 360 nm. These compelling findings hold significant promise for potential agricultural applications aimed at enhancing seed germination rates and superior capacity for enhancing seedling growth and overall yield. By utilizing these findings, agricultural practices could be further advanced to contribute to improved crop production and sustainable agricultural practices.

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