**"Green Synthesis of Core-Shell ZnO Nanoparticles: A Sustainable Approach for Antibacterial Activity and Plant Pathogen Protection"**

**Abstract:**

The green synthesis of core-shell zinc oxide (ZnO) nanoparticles (NPs) has gained significant attention as a sustainable and eco-friendly approach for biomedical and agricultural applications. This study will synthesize ZnO NPs using **Citrus limetta** peel extract as a natural reducing and stabilizing agent. The biosynthesized nanoparticles will be characterized using **UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM)** to confirm their structural and morphological properties. The core-shell architecture will enhance the stability, biocompatibility, and antibacterial efficiency of ZnONPs.The antibacterial activity of these nanoparticles will evaluated against **common phytopathogenic bacteria**, including Pectobacteriumcarotovorum (soft rot pathogen) and Ralstoniasolanacearum (brown rot pathogen), using the agar well diffusion method. Results will demonstrate significant bactericidal effects, indicating their potential as an alternative to synthetic pesticides. The antibacterial action mechanism will involve **reactive oxygen species (ROS) generation**, leading to bacterial membrane disruption and cell death. Additionally, the biocompatibility of ZnO NPs will be assessed through phytotoxicity assays, revealing their safety for plant applications. This study will highlight the potential of green-synthesized core-shell ZnO NPs as an eco-friendly antimicrobial agent for sustainable plant pathogen protection. Their dual function as antibacterial agents and plant growth enhancers suggests a promising alternative to conventional agrochemicals, reducing environmental toxicity. Further research on field applications and long-term stability is recommended to advance their practical use in agricultural disease management.

**Keywords:** Green synthesis, ZnO nanoparticles, core-shell, antibacterial activity, plant pathogens, sustainable agriculture.

**1 Introduction:**

Nanotechnology has witnessed remarkable advancements in recent years, with zinc oxide (ZnO) nanoparticles gaining attention due to their unique physicochemical properties and diverse applications in fields such as medicine, agriculture, and environmental remediation [1]. ZnO nanoparticles exhibit remarkable antimicrobial properties, making them effective against a broad spectrum of bacterial pathogens. This has spurred interest in their potential applications for plant protection, particularly against diseases caused by bacterial infections that threaten agricultural productivity [1]

Zinc oxide nanoparticles (ZnO-NPs) are one of the metal oxide nanomaterials and a valuable and versatile inorganic compound due to its unique physical and chemical characteristics. They possess high chemical stability, a broadened radiation absorption spectrum, high electrochemical coupling coefficient, and high photostability with the molecular formula ZnO[2].ZnO-NPs have been widely manufactured and utilized in various commercial and additive products, including ceramics, cement, plastics, glass, ointments, lubricants, adhesives, sealants, pigments, batteries, ferrites, fire retardants, cosmetics, and sunscreens, as well as in foods as a source of zinc nutrient [3]. NanosizedZnO particles demonstrate significant antibacterial capabilities due to their small size, which can stimulate different bactericidal mechanisms once inside the bacterial cell, including the bacterial surface or bacterial core, generate ROS (reactive oxygen species), release Zn2+, and even be endocytosed by cells [4].Traditional methods of ZnO nanoparticle synthesis, such as sol-gel, chemical vapor deposition, and hydrothermal techniques, involve hazardous chemicals, high energy consumption, and the generation of toxic by-products. These methods not only pose significant environmental and health concerns but also increase production costs, limiting their large-scale application [5]. As a sustainable alternative, green synthesis methods have gained attention for their ability to minimize environmental impact. These methods employ plant extracts, microorganisms, or other biological entities as reducing and stabilizing agents, eliminating the need for harmful chemicals while reducing energy requirements [6].

Plant-based green synthesis, in particular, has proven to be an efficient and environmentally benign approach. The rich phytochemical composition of plant extracts, including flavonoids, alkaloids, and phenolic compounds, plays a crucial role in the reduction of metal ions and stabilization of nanoparticles. Moreover, this approach not only aligns with green chemistry principles but also offers cost-effectiveness and scalability, making it suitable for agricultural applications [7].

Core-shell nanostructures represent a significant advancement in nanoparticle design, enhancing stability, antimicrobial efficiency, and the controlled release of active agents. The core-shell architecture offers a protective barrier that can modulate interactions between the core and its environment, thereby enhancing the functional properties of the nanoparticles. In the context of plant protection, core-shell ZnO nanoparticles provide a dual advantage: combating plant pathogens through their antimicrobial action while reducing the need for synthetic pesticides, thus promoting sustainable agricultural practices [8].

This study aims to synthesize core-shell ZnO nanoparticles through a green synthesis approach and evaluate their antibacterial efficacy and protective effects against plant pathogens. By harnessing the potential of plant-derived bio compounds, the research seeks to contribute to the development of eco-friendly and cost-effective plant protection strategies that align with global sustainability goals.

**Motivation and Research Gap**

The growing threat of bacterial infections and plant pathogens has created an urgent need for sustainable and effective solutions. Zinc oxide nanoparticles (ZnO NPs) have shown remarkable antibacterial properties, but conventional synthesis methods often involve toxic chemicals and energy-intensive processes, posing environmental concerns. Green synthesis, using plant extracts, offers a safer, eco-friendly alternative that aligns with sustainability goals. Additionally, the development of core-shell ZnO NPs presents a promising approach to enhance stability, control release, and improve antibacterial efficacy. However, limited research has explored the green synthesis of core-shell ZnO NPs, particularly their application in plant pathogen protection. Investigating this area could pave the way for sustainable disease management strategies, reducing reliance on synthetic pesticides and minimizing environmental impact.

**Objectives:**

* To synthesize core-shell ZnO nanoparticles using plant extracts.
* To characterize the synthesized nanoparticles for structural, morphological, and optical properties.
* To evaluate the antibacterial activity of core-shell ZnO nanoparticles against plant pathogens.
* To assess the protective effects of the nanoparticles on plants against bacterial infections.

**2 Methodology:**

**2.1 Materials and methods:**

The materials will be required to include fresh plant material for extract preparation (*Citrus limetta peels*), zinc precursors such as zinc acetate or zinc nitrate, and sodium hydroxide (NaOH) for pH adjustment. Distilled water and ethanol will be used for washing and purification steps. Bacterial strains of Xanthomonascampestris and Pectobacteriumcarotovorum, known plant pathogens, will be utilized to assess the antibacterial efficacy of the synthesized nanoparticles. Nutrient agar for bacterial culture and sterile Petri dishes will be essential for antibacterial assays.

**2.1.1 *Preparation of Plant Extract:***\Fresh *Citrus limetta peels* will be washed thoroughly, dried, and ground into a fine powder. The powder will be boiled in distilled water for 30 minutes, followed by filtration to obtain the plant extract.

**2.2 Green Synthesis of ZnO Nanoparticles:**  
Zinc acetate dihydrate (or zinc nitrate hexahydrate) will be dissolved in distilled water and heated while stirring. The plant extract will be added dropwise as a reducing and stabilizing agent. The pH will be adjusted using NaOH or NH₄OH, leading to the formation of a precipitate. The mixture will be further heated, and the precipitate will be collected, washed, and dried.

**2.3 Core-Shell Formation:**  
For core-shell formation, the synthesized ZnO NPs will be coated with silica, chitosan, or alginate by dispersing the NPs in the coating solution, followed by centrifugation, washing, and drying.

**3.1 Characterization:**

**3.2 Surface characterization:**

***3.2.1 UV-Vis Spectroscopy:***

It will be used to analyze the optical properties and confirm the formation of ZnO nanoparticles by observing the characteristic absorption peak in the range of 350–380 nm.

* + 1. ***X-ray Diffraction (XRD):***

It will be used to determine the crystalline structure and phase purity of the nanoparticles. It will identify diffraction peaks corresponding to the wurtzite structure of ZnO, will confirm successful synthesis.

***3.2.3* *Scanning Electron Microscopy (SEM):***

It will Examine the surface morphology and particle size distribution. SEM images will provide insight into the uniformity and aggregation state of the nanoparticles.

***3.2.4 Transmission Electron Microscopy (TEM):***

It will Investigate the core-shell structure, nanoparticle size, and detailed internal morphology. TEM will allow for high-resolution imaging to confirm the formation of the core-shell architecture.

***3.2.5 Fourier-transform Infrared Spectroscopy (FTIR):***

It will Identify functional groups involved in the reduction and stabilization of ZnO nanoparticles. Characteristic peaks will correspond to hydroxyl, carboxyl, and other phytochemical groups from the plant extract are analyzed.

* + 1. ***Dynamic Light Scattering (DLS):***

It will measure the hydrodynamic size and polydispersity index of the nanoparticles and will provide insights into their stability and dispersion in aqueous solutions.

***3.2.7 Zeta Potential Analysis:***

It will evaluate the surface charge of the nanoparticles, which is crucial for understanding colloidal stability and predicting interactions with bacterial cell membranes.

**3.2.8 Antibacterial Activity:**

The antibacterial activity of core-shell ZnO nanoparticles will be evaluated using the agar well diffusion method to assess their effectiveness against plant pathogens. Bacterial cultures will be inoculated on nutrient agar plates, and wells will be created to introduce varying concentrations of ZnO nanoparticles. The plates will be incubated at 37°C for 24 hours, allowing the nanoparticles to interact with the bacterial strains. The formation of clear inhibition zones around the wells will indicate antibacterial activity, with larger zones suggesting greater efficacy. The mechanism of action is attributed to the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, and interference with metabolic processes. The core-shell structure is expected to enhance these antibacterial properties by providing a controlled release of active components and increasing nanoparticle stability, resulting in prolonged antimicrobial action. This evaluation will help determine the potential of green-synthesized core-shell ZnO nanoparticles as effective agents for plant pathogen protection, offering a sustainable alternative to synthetic antibacterial agents.

***3.2.8.1 Agar Well Diffusion Method:***

It will be used to evaluate the antibacterial activity against plant pathogens by measuring the zone of inhibition.

***3.2.8.2 Minimum Inhibitory Concentration (MIC)***

It will be used to determine the lowest concentration of nanoparticles required to inhibit bacterial growth.

***3.2.8.3 Minimum Bactericidal Concentration (MBC):***

It will be used to identify the minimum concentration required to kill bacteria.

**3.2.12 Plant Protection Assay:**

The plant protection assay will evaluate the effectiveness of core-shell ZnO nanoparticles in safeguarding plants against pathogenic infections. Plants will be inoculated with bacterial pathogens known to cause disease, followed by treatment with varying concentrations of ZnO nanoparticles. The health of treated plants will be monitored over time, assessing parameters such as disease progression, lesion size, and overall plant vitality. Comparisons between treated and untreated plants will help determine the nanoparticles' protective efficacy. The anticipated protective mechanism involves the nanoparticles' antimicrobial properties, which inhibit pathogen growth and enhance the plant’s natural defense responses.

***3.2.12.1 In vitro Pathogen Growth Inhibition:***

It will be used to test the effect of ZnO nanoparticles on plant pathogen growth in controlled environments.

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