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Green Synthesis of Zinc Oxide Nanoparticles using Eucalyptus Lanceolata Leaf Litter in the Control of Bacterial and Fungal Crop Diseases

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Abstract

Nanotechnology is a significant area of scientific inquiry that has garnered considerable attention from scholars in the contemporary period. Various plant materials are being explored as potential resources in green chemistry-based techniques for producing metal Nanoparticles (NPs). This is in response to the adverse impacts of synthetic chemicals, which contribute to significant abiotic climate change concerns in modern agriculture. The primary categories of crop diseases are those resulting from bacterial and fungal infestations. Once a plant becomes infected, it can pose a significant danger to the growth of crops, resulting in a decrease in yield and quality. Furthermore, such infections can adversely affect food safety, creating a health concern for humans. This work aimed to investigate the process of Green Synthesis and Characterisation of Zinc Oxide Nanoparticles (GSC-ZnO-NP) utilizing an extract derived from Eucalyptus lanceolatus (specifically, leaf litter). The paper intended to control bacterial and fungal crop diseases using the GSC-ZnO-NP in conjunction with Eucalyptus lanceolatus extracts. The sample's crystalline structure and surface characteristics have been evaluated using X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). The antimicrobial activity of the proposed GSC-ZnO-NP was evaluated against bacterial and fungal isolates from infected crops. The progressive augmentation in magnitude ranging from 10° to 30° in XRD signifies the emergence of ZnO-NPs characterized by distinct and well-defined crystalline configurations. Also, the antimicrobial efficacy of the Zn-NPs extract was comparable to or surpassed that of Fluconazole, suggesting the prospective use of this environmentally-friendly manufactured nanomaterial as a substitute or adjunct therapy for bacterial and fungal infections caused by these microorganisms in crops.

Keywords: *Green Synthesis, Zinc Oxide Nanoparticles, Eucalyptus lanceolatus, bacterial, fungal, crop diseases.*

Introduction

Bacterial and fungal pathogens that affect plants contribute to the decline in crops' quality and quantity, leading to substantial reductions in agricultural output and posing a severe threat to global food security. In the context of trade globalization, it is worth noting that harmful bacteria and fungi have the potential to be transmitted to different nations or areas through the exchange of crops. Consequently, this phenomenon contributes to the escalation of plant disease dissemination [1]. Using fungal diseases of plants as a case study, researchers have globally examined the 2009-2010 harvesting information for five significant crops (rice, wheat, soybean, maize, and potatoes). By making certain

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assumptions, these studies have demonstrated that if the consistently low incidence of fungal diseases in crops can be sustained, the resulting food production would be adequate to meet the nutritional needs of approximately 8.5% of the global population in 2011. If these five significant crops were to experience major epidemics concurrently, their yields would be inadequate to meet 40% of the global food demand [2].

Throughout history, individuals have engaged in persistent efforts to combat plant diseases. The historical documentation of rice blast in China dates back to the 17th century. The fungal disease has achieved global distribution and poses a significant threat to rice production, resulting in yield losses ranging from 10% to 35% globally. Bacterial wilt, induced by the bacterium Ralstonia spp., is a pervasive plant ailment with a global distribution that significantly harms economically significant crops like tomatoes and potatoes [3]. The prevailing methods for managing crop illnesses induced by fungal and bacterial pathogens encompass cultivating resistant cultivars, using chemical pesticides, and enhancing field management practices. The process of developing resistant types is frequently time-consuming, whereas the utilization of chemical pesticides has proven to be efficacious. However, it has also led to bacterial resistance, harmful emissions, and concerns regarding ecological and human well-being [4]. Field management procedures serve as a supplementary component in controlling pathogens and may include additional expenses related to human resources. Hence, there is a growing imperative to devise novel approaches for managing plant fungal and bacterial infections characterized by their environmental sustainability, efficacy, and dependability.

Nanoparticles refer to substances that possess at least one dimension within the range of 1-100 nm, exhibiting a greater specific surface area and reactivity when compared to bulk materials of equal composition [5]. Nanoparticles include distinct characteristics such as surface and size, which have garnered significant focus and investigation due to their potential uses in several fields, including industry, biology, and consumer items. Simultaneously, the emergence of nanotechnology has been observed as a consequence of these advancements. Currently, nanomaterials find applications in several domains like electronic devices, communication, medical science, cosmetics, packaging of foods, and agrochemicals [6]. In recent years, there has been a growing interest in the utilization of nanotechnology within agriculture, particularly in plant protection. Extensive research efforts have been dedicated to investigating the possible application of nanomaterials to manage plant pathogens effectively. This area of study is now experiencing significant development and progress. Metallic elements such as silver (Ag), zinc (Zn), and iron (Fe), as well as metal oxides including zinc oxide (ZnO), cerium oxide (CeO2), and other iron oxides, have been extensively investigated for their potential in plant protection owing to their inherent antibacterial capabilities [7].

The discipline of nanotechnology has brought about significant advancements in several domains of science and technology, including its notable impact on the agricultural sector. The utilization of nanoparticles to mitigate agricultural diseases is an area that has garnered considerable attention. Among the many types of nanoparticles, ZnO-NPs have demonstrated significant promise owing to their notable antibacterial capabilities [8]. Nevertheless, producing these nanoparticles entails the utilization of toxic substances and hazardous procedures, hence giving rise to apprehensions regarding their impact on the environment and human well-being. This paper provides the utilization of green synthesis techniques that have arisen as a viable and environmentally conscious substitute. The utilization of Eucalyptus Lanceolata leaf litter for the green synthesis of ZnO nanoparticles has emerged as a noteworthy approach in the field. This method has garnered considerable interest due to its efficacy in managing bacterial and fungal crop diseases.

Related Works

The typical techniques employed in synthesizing ZnO nanoparticles need the utilization of hazardous chemicals and energy-intensive procedures, hence giving rise to apprehensions over their potential adverse effects on the environment and safety. Given these issues, "green synthesis" has become a viable and sustainable solution. The present methodology adopts an ecologically sensitive strategy by employing biocompatible and eco-friendly sources, specifically plant extracts, to reduce metal precursors into nanoparticles.

Tanuj et al. (2023) prepare zinc oxide nanoparticles using green synthesis. Rhododendron arboreum extract is used [9]. Bioactive chemicals from the plant are extracted to reduce and stabilize nanoparticles. Nanoparticles are characterized, and their photocatalytic degradation of cationic dyes, such as malachite green and Fuchsin basic dye, is proposed. This method is eco-friendly and resource-efficient. Plant extract composition may cause nanoparticle heterogeneity, which may have drawbacks.

Elsakhawy et al. (2022) recently published a mushroom-based nanoparticle synthesis method that promotes sustainable soil management [10]. Implementation involves cultivating specific mushrooms for nanoparticle production. Nanoparticle characterization and soil sustainability implications are the result values. This practice may improve soil quality and use renewable fungal resources. Fungal culture can be time-consuming, which is a drawback.

In their 2022 study, Maity et al. explored biosynthesized metal oxide nanoparticles for sustainable agriculture. Implementation involves making nanoparticles from plant extracts or microbial systems. Characterizing nanoparticles and their potential uses in crop production, protection, and management are output values [11]. The process's environmental sustainability and agricultural potential are advantages. However, this approach may struggle with large-scale production and uniformity.

In 2020, Selim et al. developed a green synthesis method for zinc oxide nanoparticles using Deverra tortuosa extract [12]. To synthesize nanoparticles, plant constituents are extracted and used. Nanoparticle cytotoxicity and characterization data are output. The renewable nature of plant extracts ensures a steady supply. However, the variability in nanoparticle attributes from these extracts may have drawbacks.

Mohammed et al. (2023) extensively describe a green synthesis method for zinc oxide nanoparticles using Cymbopogon citratus extract [13]. The implementation process involves extracting and using plant chemicals. The output includes nanoparticle characterization and antibacterial activity. This method is environmentally friendly and inhibits bacterial growth. However, scalability may be an issue.

The green synthesis of zinc oxide nanoparticles using Eucalyptus lanceolate leaf litter is described by Sharma et al. (2022). Leaf litter is collected and used to synthesize nanoparticles. Nanoparticle characterization, antibacterial testing, and maize agricultural efficacy evaluation are output values [14]. The use of leaf litter promotes sustainability and may benefit agriculture. Leaf litter composition variability may have drawbacks.

Farooq et al. (2022) outline a green chemistry-based method for making zinc oxide nanoparticles from Calotropis gigantea plant derivatives. Implementation includes extracting and using plant constituents for nanoparticle synthesis [15]. Nanoparticle characterization and biological uses against bacterial and fungal infections are output values. Positives include using a plant derivative and possible biological applications, but negatives include extract composition changes.

Pursuing sustainable and ecologically sound remedies for crop illnesses holds significant significance in guaranteeing food security for an expanding worldwide populace. This literature review aims to provide a scholarly contribution by analyzing the green production of ZnO-NPs utilizing Eucalyptus Lanceolata leaf litter as an innovative approach to develop environmentally friendly and efficient techniques for crop protection.

Materials and Methods

Materials

The nanoparticles have been synthesized using zinc acetate and sodium hydroxide (NaOH) obtained from Sigma-Aldrich Chemicals Co. Ltd, USA. The leaf of Eucalyptus lanceolatus has been obtained from the vicinity of the Karaikudi area in Tamilnadu, India. Nine strains of bacteria and fungi were obtained from crops at the Agricultural University. These strains include four bacterial strains, namely Escherichia coli, Staphylococcus aureus, Pseudomonas multicide, and Bacillus subtilis, as well as four fungal strains, namely Aspergillus parasiticus, Fusarium solani, Aspergillus niger, and Rhizopus nigricans.

Preparation of Eucalyptus Lanceolatus Extract

Fig. 1 shows the preparation process of Eucalyptus lanceolatus extract. The leaves, stem, and blossoms of Eucalyptus lanceolatus were subjected to a cleaning process using water to remove any potential contaminants. Subsequently, they were air-dried at room temperature and pulverized into a powdered state. The powder of leaves was split into three sections and dissolved in acetone, methanol, or distilled water. The specimens were immersed within hermetically sealed plastic containers and afterward transferred, and the ensuing pollution was remediated. The plant parts were finely sliced, and a quantity of 15 g was added to a beaker containing 150 mL of distilled water. The combination was then heated to a temperature of 65°C and stirred intermittently for 50 minutes. Subsequently, the mixture was allowed to cool down to room temperature. The acetone and methanol extracts were subjected to evaporation by removing the bottle top. The plant extracts were carefully measured and combined in a solution containing 15% Di-Methyl Sulf-Oxide (DMSO) to prepare a concentrated solution, from which smaller quantities were subsequently derived.

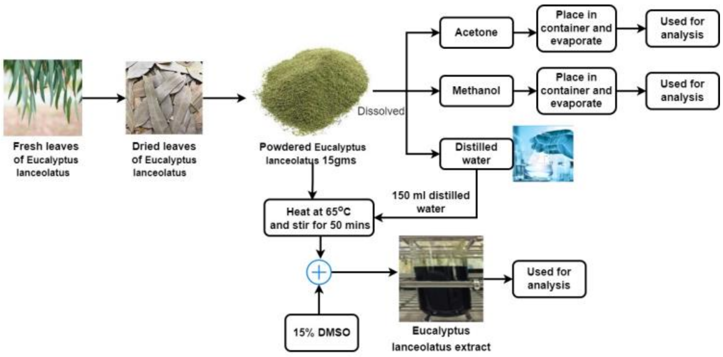

Figure 1: Preparation of Eucalyptus lanceolatus extract

Green Synthesis of ZnO-NP

All synthesized reagents showed sufficient efficacy for use in the research study, hence eliminating the need for further filtering. The compound zinc acetate [$Zn(C_4H_6O_4)$] was acquired from Sigma-Aldrich, boasting a purity level of roughly 99.5%. The use of triethylene glycol (TREG) as a polyol solvent was undertaken due to its capacity to effectively dissolve zinc acetate and its abundance of hydroxyl (-OH) groups that function as hydrolysis agents for zinc acetate. The researchers acquired finely ground plant material from Eucalyptus lanceolatus, including leaves, flowers, and stems. Distilled water was employed to create aqueous solutions. The zinc acetate powder was immersed in deionized water to create a standard zinc acetate solution with a concentration of 15 mM. This standard solution was then used to generate a series of concentrations ranging from 1 mM to 5 mM. The aqueous extract of fresh leaves from Eucalyptus lanceolatus was mixed with zinc acetate. The floral and stem extract was introduced into a flask at a volumetric ratio of 1.5 (volume/volume) to create a solution with a total volume of 55 mL. The flask was enveloped by an aluminum sheet and subjected to a heating process for 5 hours within a water bath maintained at a temperature of 65°C.

Antimicrobial Activity of GSC-ZnO-NP Utilizing an Extract Derived from Eucalyptus Lanceolatus

The antibacterial and antifungal properties of the proposed GSC-ZnO-NP with the extract of Eucalyptus lanceolatus have been assessed in this work. The analysis considered several bacterial strains, including Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Bacillus subtilis, as well as fungal strains, such as Aspergillus parasiticus, Fusarium solani, Aspergillus niger, and Rhizopus nigarican. The laboratory equipment and growth medium underwent a disinfection process using autoclaving at a temperature of 120℃ and a pressure of 20 psi for 35 minutes. The disc diffusion method was employed to evaluate the antibacterial efficacy. A bacterial stock was prepared for replicating and rejuvenating bacteria through inoculation using culture media containing E. coli, S. aureus, P. multocida, B. subtilis, Aspergillus parasiticus, Fusarium solani, Aspergillus niger, Rhizopus nigarican. The bacteria were inoculated into a 5 mL nutrient agar solution and placed in an incubator set at 38℃ for 24 hours. Preparing research bacteria involved the introduction of a single cultured bacterial inoculation loop into a 6 mL NaCl solution with a concentration of 0.21%.

Furthermore, the solution was diluted, and its fertility was adjusted to 0.45 using the McFarland turbidity method, which involved the addition of artificially cultivated medium to achieve a concentration of 185 colonies forming units per milliliter. The technique of inhibition was employed to assess the antimicrobial efficacy. A 25 mL volume of nutritional agar combination was introduced into a petri dish, which was subsequently subjected to a disinfection process lasting fifteen minutes. This process resulted in solidifying the nutrient agar solution within the petri dish. Next, a volume of 0.2 mL of the microbial solution was introduced into the nutrient broth culture of the standard medicine (Rifampicine) to assess its antibacterial activity against the antifungal agent (Fluconazole) to control fungal infections. Subsequently, the sample was incubated at a temperature of 38℃ for 24 hours before its utilization to determine the apparent zone diameter.

Results and Discussion

The present study successfully employed an economically viable approach for the environmentally friendly production of ZnO-NPs. This was accomplished by utilizing an aqueous extract derived from the leaves of E. lanceolatus. In contrast to currently employed techniques for the biosynthesis of ZnO-NPs, our proposed method demonstrates simplicity, cost-effectiveness, and environmental sustainability characteristics. The methodology employed in this study effectively circumvented the utilization of poisonous organic solvents, elevated temperatures, increased pressures, dangerous substances, and excessive energy consumption. The phytochemicals included in the plant extract served as both reducing and capping agents during ZnO-NP manufacture. The study found the quantities of plant extracts and aqueous Zn solution as the operational factors that controlled the production characteristics of ZnO-NPs.

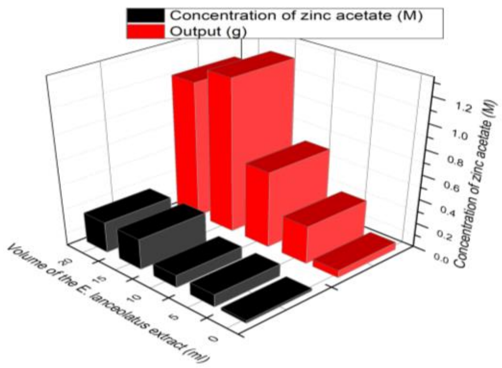

Figure 2: Optimization parameters employed in the GSC-ZnO-NPs utilizing the leaf extract derived from Eucalyptus lanceolatus

Fig. 2 presents the optimization parameters employed in the GSC-ZnO-NPs utilizing the leaf extract derived from Eucalyptus lanceolatus. The optimization method revealed that the production of GSC-ZnO-NPs was most efficient (Maximum output=1.24g) when using a 15 ml concentration of plant extract and a 0.25 M solution of aqueous zinc acetate dihydrate. Consequently, these conditions were subsequently shown to be optimal for achieving a higher yield of ZnO-NP, as indicated in Fig. 2.

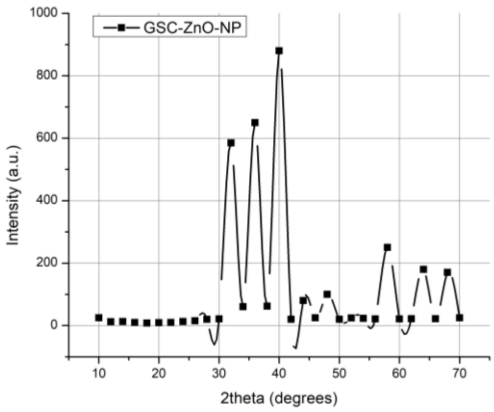

Figure 3: XRD analysis of GSC-ZnO-NP at a scanning angle of 2θ

Fig. 3 employs the XRD technique in examining GSC-ZnO-NP at a scanning angle of 2θ yields significant findings about the crystalline arrangement of the produced nanoparticles. The pattern in Fig. 3 has discernible peaks indicative of individual crystallographic planes associated with the ZnO-NPs. It is worth noting that the intensity values corresponding to each angle of 2θ provide information on the corresponding proportion of the crystalline facets. Based on the results presented, it is evident that the peaks seen at angles of 32°, 36°, and 40° exhibit notably high intensities of 585, 650, and 880 arbitrary units (a.u.), respectively. This observation implies that these crystallographic planes are mostly present in the manufactured ZnO-NPs.

Moreover, the progressive augmentation ranging from 10° to 30° signifies the emergence of ZnO-NPs characterized by distinct and well-defined crystalline configurations. This study's X-ray diffraction (XRD) research highlights the importance of utilizing Eucalyptus Lanceolata leaf litter as a sustainable approach for synthesizing crystalline ZnO-NPs. This method can potentially improve the antibacterial characteristics of ZnO-NPs, hence offering a promising solution for managing bacterial and fungal crop diseases.

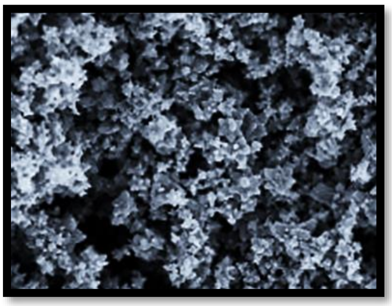

Figure 4: SEM analysis of GSC-ZnO-NP using Eucalyptus lanceolatus

Fig. 4 depicts the SEM analysis of GSC-ZnO-NP using Eucalyptus lanceolatus. The surface morphology of the produced zinc oxide nanoparticles (ZnO-NPs) was investigated by the utilization of scanning electron microscopy (SEM). The SEM images commonly exhibit many agglomerated particles characterized by uneven spherical shape, with a mean size ranging from 80 to 100 nanometers. The surface chemical composition of the produced ZnO nanoparticles was determined using energy-dispersive X-ray spectroscopy.

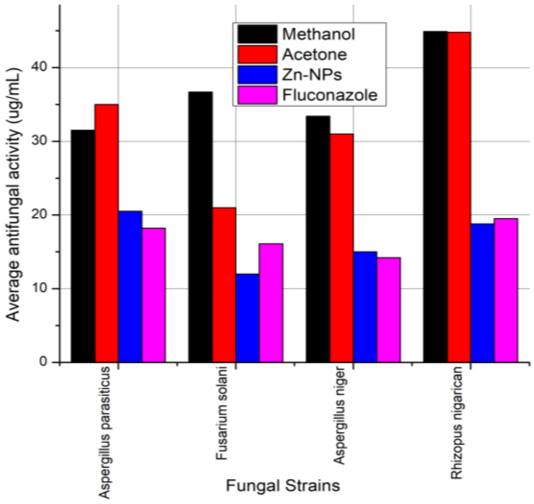

Figure 5: Average antifungal activity (μg/mL) of fungal strains using the extracts of Eucalyptus lanceolatus with nanoparticles

Fig. 5 illustrates the mean antifungal efficacy (expressed in μg/mL) of several fungal strains when treated with Eucalyptus lanceolatus extracts in conjunction with zinc nanoparticles (Zn-NPs), in comparison to the antifungal medication Fluconazole. The data elucidates the efficacy of various therapies in combating certain fungus strains. Significantly, the combination of Zn-NPs with extracts from Eucalyptus lanceolatus demonstrated enhanced antifungal efficacy in the case of Aspergillus parasiticus, Fusarium solani, and Aspergillus niger, surpassing the antifungal activity of methanol and acetone extracts. This observation implies a cooperative interaction between the plant extracts and Zn-NPs, suppressing fungal strain expansion. Furthermore, the antifungal efficacy of the Zn-NPs extract was comparable to or surpassed that of Fluconazole, suggesting the prospective use of this environmentally-friendly manufactured nanomaterial as a substitute or adjunct therapy for fungal infections caused by these microorganisms. The antifungal activity of Rhizopus nigarican was seen when treated with the extract of Zn-NPs. However, its effectiveness was slightly lower than Fluconazole, indicating its potential as an environmentally friendly antifungal agent.

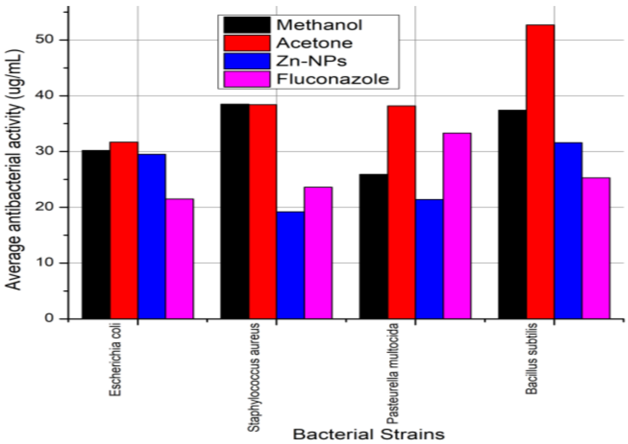

Figure 6: Average antibacterial activity (μg/mL) of bacterial strains using the extracts of Eucalyptus lanceolatus with nanoparticles

Fig. 6 illustrates the mean antibacterial efficacy (measured in μg/mL) of several bacterial strains when treated with extracts of Eucalyptus lanceolatus in conjunction with Zn-NPs, compared to the antibacterial agent Fluconazole. The data elucidates the efficacy of various medicines in combating particular bacterial strains. Significantly, the antibacterial activity of Zn-NPs mixed with extracts from Eucalyptus lanceolatus surpasses that of methanol and acetone extracts for Escherichia coli, Staphylococcus aureus, and Pasteurella multocida. This finding suggests a potential synergistic impact between the plant extracts and Zn-NPs in their ability to limit bacterial growth. Furthermore, the extract derived from Zn-NPs has a comparable or superior antibacterial effect to Fluconazole. This finding indicates that these nanoparticles, created using environmentally benign methods, can serve as a sustainable substitute or supplementary therapy for bacterial infections caused by these specific strains. Bacillus subtilis has notable antibacterial activity when exposed to the extract of Zn-NPs. However, its effectiveness is slightly lower compared to that of Fluconazole. This finding underscores the promising potential of employing this environmentally benign method to treat bacterial infections in crops.

Conclusion

This work aimed to examine the Green Synthesis and Characterisation procedure of Zinc Oxide Nanoparticles (GSC-ZnO-NP) using an extract obtained from Eucalyptus lanceolatus (particularly leaf litter). Furthermore, this study aimed to investigate the efficacy of GSC-ZnO-NP in combination with Eucalyptus lanceolatus extracts for managing bacterial and fungal crop diseases. XRD and SEM techniques assessed the sample's crystalline structure and surface features. The study aimed to assess the antibacterial efficacy of the proposed GSC-ZnO-NP against bacterial and fungal strains obtained from affected crops. The gradual increase in intensity observed in XRD patterns, ranging from 10° to 30°, indicates the presence of ZnO-NPs with clearly defined and unique crystalline structures. Additionally, the antimicrobial effectiveness of the Zn-NPs extract exhibited comparable or superior results to Fluconazole, indicating the potential application of this environmentally friendly synthesized nanomaterial as an alternative or supplementary treatment for bacterial and fungal infections caused by these microorganisms in crops.

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