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Bio-Coatings for Preservation of Fresh Fruits and Vegetables Using Green-synthesized Zinc Oxide Nanoparticles from Citrus limetta Extract

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***Abstract*—Zinc oxide (ZnO) Nanoparticles (NPs) are potentially useful in various fields The biological method (green synthesis) has recently been adopted as an expedient alternative to generate ZnO NPs with higher stability, as this approach is economical, eco-friendly and non-toxic and it includes plant extracts, microorganisms, etc. In this work, an aqueous extract of citrus limetta peel was used as the biological reduction agent for the synthesis of ZnO NPs from zinc sulphate. The nanoparticles were characterized by various techniques like TEM, FTIR, XRD and UV-Visible spectroscopy. Its anti-microbial activity was tested against bacterial species Escherichia coli, pseudomonas aeruginosa and Staphylococcus aureus and fungal species candia-auris, anaerobic fungi. The antibacterial activity of ZnO NPs was found to be significantly effective against Staphylococcus aureus. These NPs are employed as matrixes in Edible coatings and films (ECF), which are then applied to fruits to extend shelf life and improve storage quality. This paper presents an efficient use of ZnO NPs as a bio coating material with a wide range of potential applications, especially in the biomedical field.**

***Index Terms*—Zinc oxide nanoparticle, Stabilizing agent, Citrus limetta, Antibacterial activity, Edible coating, Antioxidant activity.**

I. INTRODUCTION

India is the world's second-largest producer of fruits and vegetables and is crucial to the nation's food, nutritional, and economic security. 102.481 million tons of fruit are produced on 69.3 lakh hectares of land, whereas 200.445 million tons of vegetables are produced on 102.60 lakh hectares of land (NHB, 2020). However, a significant issue with fruits and vegetables is their increased perishability, which results in short shelf life and postharvest losses. Due to the high moisture content (75–95%) of fruits and vegetables, which causes dehydration, mechanical damage, environmental stress, pathological breakdown, poor postharvest management, insufficient cold storage, processing facilities, etc., postharvest loss in India is estimated to be between 8 and 18% (APEDA, 2019) (Otoni et al., 2017). Nanoparticles contain special physical and chemical properties, as well as antioxidant and antibacterial properties that can be employed to keep fruits and vegetables fresh (Zafar and Iqbal 2024). Nowadays, nanotechnology is regarded as a proven state-of-the-art technology with many applications in the chemical, pharmaceutical, mechanical, and food processing industries. Additionally, nanotechnology has intriguing applications in the fields of environmental sciences, computers, power generation, optics, and medicine delivery (Ramsden J and William Andrew 2016).

By shielding fruit from physical, chemical, and microbiological deterioration, coatings increase postharvest life, boost marketability, and maintain nutritional and nutraceutical qualities (OlunusiSO et al., 2024). Citrus fruits are significant and diversified, but because of their high-water content and higher nutritional components, they are often attacked by phytopathogenic microorganisms and suffer harm during farming, ripening, harvesting, and commercialization. (Wang, Z et al., 2022). Due to their distinct physical, chemical, and biological characteristics that set them apart from their conventional counterparts, nanomaterials are currently gaining more and more attention. (Bhavyasree, P.G et al., 2022). Recent research has shown that a number of nanomaterials, notably zinc oxide, have antifungal qualities. (Djearamane, S et al., 2022).

Zinc oxide nanoparticles (ZnO NPs) are among the most promising nanomaterials that have been discovered. Because of their unique surface attraction capabilities and high physiochemical stability, ZnO NPs can be employed for antifungal, antibacterial, and other biomedical applications. According to the FDA-USA, ZnO NPs are the safest NPs (Dhanasegaran, K et al., 2021).

Edible coatings are sustainable and eco-friendly substitutes for plastic packaging materials. They can reduce the gas exchange between fruits and vegetables and the environment by forming a semi-permeable composite film on their surface through soaking, coating, or spraying (Faraj, A.M and Nouri, M 2024). An edible film (EF) is a prepared, thin layer of edible material that can be applied to or in between food components, whereas an edible coating (EC) is a thin layer of edible material created as a coating on a food product (McHugh, 2000). The antibacterial qualities, low production costs, and low toxicity of zinc oxide nanoparticles (ZnO-NPs) have drawn interest (Ren, Z. et al., 2021).

ZnO-NPs in various sizes and forms can be produced using a range of physical and chemical techniques. Using various plant extracts, environmentally friendly techniques (green synthesis) have recently been established for the manufacture and characterisation of chitosan–nano-ZnO composite films for cherry tomato preservation (Zhou, Y et al., 2021). In addition to preventing gas diffusion, the edible covering slows respiration, prevents water loss, preserves sensory qualities, delays fruit ripening, and prolongs storage life (Ibrahim Hmmam1 et al., 2023). Zinc oxide nanoparticles (ZnO-NPs) primarily work against microorganisms by producing reactive oxygen species (ROS) and interacting with Zn^{2+} on cell surfaces (Angelucia Gonçalves Parente et al., 2025).

The majority of the aforementioned disadvantages can be successfully managed by using biosynthesis-derived (green synthesis-derived) biomaterials for NP synthesis (e.g., plant materials/derivatives, microbes, biopolymers, algae, or other biomolecules), enabling environmentally friendly, simple-to-apply, economical, high-yield-producing, and controllable approaches (Mousa Abdullah Alghuthaymi 2025).

The development of antimicrobial food packaging is made possible by chitosan, a non-toxic, film-forming, biodegradable biopolymer with antibacterial qualities (Laila Al-Naamani et al., 2018). One species in the Solanaceae family is the cherry tomato. These fruits are distinguished by their small, oval, pink-red form. Among the most important crops in the Solanaceae family (Jianming Sun et al., 2025).

Zinc oxide offers enormous potential for use in a variety of industries, including antibacterial agents, medication carriers, cosmetics, and biosensors. (Tu Uyen Doan Thi et al., 2020). Chitosan was selected because of its linear structure, which is made up of polymeric 2-amino-2-deoxy-β-d-glucose. Because of its excellent film-forming and thermal properties, low cost, hydrophilicity, biocompatibility, and good matrix for affinity ligand coupling, it has been widely used to prepare polymer membranes for use in a variety of fields, including food science, biochemistry, pharmaceuticals, medicine, agriculture, and others. (Madasamy Malini et al., 2015).

The US Food and Drug Administration (US-FDA) has accepted chitosan as a generally recognized as safe (GRAS) material after it was determined to be non-toxic. Fish processing waste and the exoskeletons of seafood, including crab, shrimp, locust, and others, are rich sources of chitosan. (Santosh Kumar et al., 2020). By preventing the growth of bacteria and fungi and lowering their respiration rates, chitosan can create a thin layer on pulp surfaces that may regulate fruit deterioration and preserve fruit quality (Yage Xin et al., 2019). The green approach of NP synthesis is an innovative and superior way to produce NPs. Because of their toxicity, NPs produced chemically and physically have limited applications in the clinical field. Green synthesis approaches are practical, economical, and environmentally friendly technologies with intriguing characteristics for the NPs (Zeba Azim et al., 2022)

The size, content, crystallinity, and shape of metal nanoparticles (NPs) such zinc oxide (ZnO), TiO_2, and silver are the main characteristics that define their intrinsic qualities. Their chemical, mechanical, electrical, structural, morphological, and optical properties can all be altered by shrinking them to the nanoscale. These altered characteristics enable the NPs to interact with cell biomolecules in a special way, which makes it easier for the NPs to physically enter the interior cellular structures. (Amna Sirelkhatim et al., 2015). The size, shape, and concentration of ZnO NPS as well as the kind of media utilized were the primary determinants of the toxicity mechanisms of ZnO NPs against bacteria or fungi. Higher antibacterial effects are typically associated with smaller particle sizes because they increase the surface area to volume ratio (Sinouvassane Djearamane et al., 2022). ZnO nanoparticles are regarded as safe materials for humans when compared to other metal oxides. ZnO is also frequently utilized as a zinc supplement in the food sector since it is less hazardous than other nanoparticles, such silver nanoparticles (Laila Al-Naamani et al., 2018).

Because of their small size, nanosized ZnO particles exhibit strong antibacterial properties. Once inside the bacterial cell, they can stimulate various bactericidal mechanisms, such as the bacterial surface or bacterial core, produce ROS (reactive oxygen species), release Zn2+, and even be endocytosed by cells. (Xian-Qing Zhou et al., 2023). ZnO-NPs are among the metal oxides used to treat materials because of their special qualities, which include stable chemical nature, non-toxic features, UV and antibacterial protection, strong photocatalytic activity, and outstanding visible wavelength spectrum clarity (Govind Kumawat et al., 2025).

Because of its broad-spectrum antibacterial capabilities, low toxicity, high stability, and biocompatibility, zinc oxide (ZnO) nanoparticles have been extensively researched. Reactive oxygen species (ROS), the production of Zn2+ ions, and direct interactions with bacterial cell membranes that cause oxidative stress and membrane rupture are some of the ways that ZnO nanoparticles have antibacterial effects (Naila Zubair et al., 2025). When added to materials like surface coatings, ZnO nanoparticles can be effective antimicrobial and antifungal agents (Reddy ARN, Srividya L 2018).

Fruits can be coated to slow down climacteric ripening, enhance their look, prevent moisture migration and volatile compound loss, and lower their respiration rate. Enhanced antibacterial efficacy and increased coating uniformity on fruit surfaces are two benefits of using nanoparticles into edible coatings (N. F. Rosman et al., 2024). It is significant that there is plenty of research supporting the beneficial benefits of ZnO NPs in food pack aging on fruit postharvest quality. For instance, ZnO NPs with chitosan oligosaccharide composites for tomato application (Xiaomeng Guo et al., 2024). While employing green-synthesised ZnO-NPs as a filter to strengthen the qualities of CS, the use of CS as a fruit coating material has been extensively investigated.

The effects of applying green-synthesised ZnO-NPs to the CS matrix on tomato postharvest (Nana Millicent Duduzile Buthelezi 2025).

NPs like zinc oxide (ZnO) and silver (Ag) can significantly increase the mechanical strength, structural integrity, and barrier qualities of polysaccharide coatings while also having antibacterial effects (S.Timilsina et al., 2024). A biodegradable polysaccharide with outstanding film-forming properties is chitosan. It comes from chitin, which is present in the exoskeletons of crustaceans, such as crabs and shrimp. By preventing the growth and spoiling of microorganisms, chitosan nanocomposites can be employed as a bioactive covering for food packaging materials to increase the shelf life of perishable goods. (Natalia Wronska et al., 2024)

II. REVIEW OF LITERATURE

One promising method for increasing the shelf life of fresh produce and lowering postharvest losses is edible coating. The postharvest life of a variety of fruits and vegetables has been successfully extended by adding plant extracts and essential oils to edible coatings. Edible coatings supplemented with plant extracts are gaining popularity due to their antioxidant effects, affordability, safety, and accessible availability. New techniques for incorporating these plant extracts into the edible coating matrix have been made possible by recent developments in postharvest technology. The use of nanoparticles in edible coatings greatly increases their shelf life. According to recent research, nanoparticles loaded with essential oils and plant extracts are essential to creating next-generation edible coatings with improved qualities for preserving fresh produce. (Praveen Gidagiri et al., 2025). This study shows that Applying CS coating enhanced with green-synthesised ZnO-NPs may be considered an efficient method to maintain postharvest quality and increase cherry tomato shelf life under ambient settings. In comparison to CS alone and the control, the current study demonstrated that adding ZnO-NPs (0.3%, 0.6%, and 0.9%) to CS (1.5%) coating delayed ripening and increased cherry tomato shelf life. In addition, CS 1 ZnO-NPs (0.6% and 0.9%) substantially decreased ripening indices and lipid oxidation and enhanced both antioxidant activity and antioxidant en- zymes of cherry tomato during shelf life compared with other treatments. Consequently, it is advised to add ZnO-NPs to the CS (1.5%) coating at concentrations between 0.6% and 0.9% in order to preserve cherry tomato quality throughout its shelf life. The study's findings might be used to improve cherry tomato fruit's postharvest quality, shelf life, and marketing, which might boost the export market. Future studies should study the incorporation of different inorganic nanoparticles into various edible coating materials which can perhaps lead to further enhancement of postharvest quality and shelf life of fresh product (Nana Millicent Duduzile Buthelezi 2025).

This paper (ShahFaisal et al., 2025) tell us about Using aqueous fruit extracts of the medicinally significant plant M. fragrans, this research project primarily focuses on the environmentally friendly plant-based synthesis of biomedically significant ZnO-NPs. The crystalline structure of the produced NPs has been confirmed by XRD examination. An examination using Fourier transform infrared (FTIR) spectroscopy confirmed the presence of phytochemicals involved in the movement of metallic ions to nanoparticles. SEM and TEM examinations were used to evaluate morphologies and vibrational modes. Synthesized ZnO-NPs have demonstrated effective antioxidant and bacterial strain resistance. Bioengineered ZnO-NPs have a moderate ability to inhibit the enzymes α-amylase and α-glucosidase. Additionally, it was shown that synthesized ZnO-NPs were biocompatible with human red blood cells. The aforementioned biogenic ZnO-NPs can be used for various studies in illnesses, cosmetics, and cancer, according to the research findings.

This study (Angelucia Gonçalves Parente et al.,2025) demonstrates that a CS-based coating enhanced with ZnO-NPs is a viable natural method for preserving quality and lowering "Tommy Atkins" postharvest losses. mangoes. Quantitatively, the composite formulation inhibited Lasiodiplodia theobromae by 97.28% in vitro, provided the lowest AUDPC (17.9) among anthracnose treatments, decreased disease progression during cold storage by roughly 64.3%, and, in vivo, postponed the respiratory peak by 7 days at 9 °C.

This work (Sinouvassane Djearamane et al., 2022) showed a substantial dose-dependent growth-inhibitory effect of ZnONP on C. albicans using turbidity measurements and colony counting. The FTIR spectra of C. albicans treated with ZnONPs suggested that alcohol, amide A, alkynes, amides 1 and II, and phosphate groups might be involved in the surface interaction of ZnONPs with the fungal cells. Additionally, SEM micrographs showed the rupture of cell membranes, invagination, hyphae fracture, and pitting of the cell wall in C. albicans upon contact with ZnO NPs. Cell death may result from these alterations to the cell membrane. It is conceivable to conclude that ZnONP can be used as an efficient antifungal agent against C. albicans based on the results of this investigation.

The study's (Jianming Sun et al., 2025) findings show that this CTS-based composite coating has a lot of potential for preserving cherry tomatoes. It is anticipated to be used extensively in the preservation of fresh-cut fruits and vegetables, dairy products (cheese, yogurt, etc.), and fruits and vegetables with a high-water content and propensity to spoil (strawberries, blueberries, lettuce, etc.). Nanocomposite research is one area of future study. smart coatings (the creation of pH/humidity-responsive coatings for targeted release), multi-component synergy (the investigation of interactions with other natural antimicrobials to further enhance the preservation effect), and technology (the use of CTS nanoparticles to improve natamycin stability and controlled release). However, only one type of fruit and vegetable under particular humidity and temperature circumstances has been the subject of research to date.

This paper (Vıctor Falguera et al., 2011) tell us about When applied on fresh, minimally processed, and processed fruits and vegetables, edible films and coatings effectively prolong their shelf life while preserving their nutritional, sensory, and microbiological qualities. The capacity of certain formulations to suppress polyphenol oxidase activity and postpone browning responses has been the subject of particular testing. Within Additionally, by encapsulating bioactive components and creating novel goods with functional or nutraceutical effects, EF and EC are able to transport substances that have certain advantages for both the user and the food itself.

Microbiological stability, adhesion, cohesion, wettability, solubility, transparency, mechanical qualities, sensory appeal, and permeability to water vapor and gases are the most crucial attributes to assess in an edible coating. These characteristics allow for the prediction and optimization of their composition and behaviour.

This study (Dulce J. García-García et al., 2025) shows that Chitosan and nanostructured spaghetti-like ZnO nanostructures (Ch/ZnO-NPs) were used in this to create a nanocoating. For the preservation of strawberries, the coating shown significant improvements in microbiological and physicochemical properties. The wurtzite ZnO-NPs have a crystal size of 40 nm and resemble spaghetti. The strawberry surface was uniformly impregnated with the 2-3 μm thick Ch/ZnO-NPs coating, which is statistically independent of the immersion time. Strawberries' shelf life was extended by the nanocoating at both ambient temperature (25 °C) and refrigerated temperature (5 °C). For up to eight days, the physicochemical and textural characteristics of strawberries were enhanced by the uniform dispersion of the nanostructured ZnO within the chitosan matrix. Chitosan's antibacterial qualities were enhanced by the coating with nanostructured ZnO; a synergistic impact between these two substances was noted, lowering the microbial load. For strawberries kept at 5 °C. which suggest the potential of the Ch/ZnO-NPs coating in improving the preservation of fresh strawberries.

From this paper (Tu Uyen Doan Thi et al., 2020) Orange peel extract was used as the reducing agent in this study's green synthesis approach, which allowed to successfully create ZnO NPs. The microstructural characteristics, shape, and bactericidal activity of the ZnO NPs against E. coli and S. aureus were shown to be significantly influenced by the annealing temperature and synthesis pH. While the bactericidal rate against S. aureus varied across the samples in the rather broad range of 89-98%, the bactericidal rate against E. coli was over 99.9% for all samples when UV light was not present. It is determined that the ZnO NPs' bactericidal activity is caused by the ROS mechanism, which interacts with the cell membrane and damages DNA and cell walls as a result. This study offers a straightforward and eco-friendly technique for the green synthesis of ZnO NPs using fruit peel, which could lower the cost of producing nanoparticles and the usage of hazardous chemicals.
In this paper (Santosh Kumar et al., 2020) it tell us how Cassia fistula fruit extract was used in green chemistry to create ZnO nanoparticles. The average diameter of the polyhedral nanoparticles was around 29 nm. The produced ZnO NPs were formed into a nanocomposite by reinforcing them into a chitosan-gelatine biopolymer matrix. hybrid movies. When compared to the control films, the created hybrid film demonstrated better elasticity, increased heat stability, and improved structural integrity. Furthermore, the produced nanocomposite films exhibited strong antibacterial action against E. coli. Chitosan-gelatine-ZnO nanocomposite films could therefore be a promising substitute active packaging technique to extend the shelf life of packed foods and an efficient antibacterial protection.

The characteristics of the primary edible-based coating materials were initially presented in this research. Next, the ECF created by other researchers using NPs like TiO2, Ag, and ZnO was examined. Researchers noticed that ECF with NPs had antibacterial action against the tested pathogen. A number of antimicrobial mechanisms are outlined and introduced, such as the photocatalytic reaction of NPs, the detachment of free metal ions, and the electrostatic contact between the cationic polymer or free metal ions and the charged cell membrane. The surface structure and mechanical characteristics of ECF with NPs, which are essential for its applications of fruits and vegetables during storage at room temperature or in a refrigerator, were then discussed. Additionally covered were the characteristics of ECF with NPs, such as gas modification, induction defence, and ion release. However, findings from other studies suggested that using ECF with various NPs could offer a practical and efficient means of preventing quality loss in postharvest fruits and vegetables. The antifungal mechanism induced by UV or visible light, as well as the interactions between nanosized particles and coating materials, are more crucial areas that need more research. Future work must also be done on the safety of these coatings as well as the prepared and used standards (Yage Xing et al., 2019).

Summary of different NPs and main edible-based materials used in coating films.

NPs	Main Edible Materials	Reference
TiO2	Shellac CS, WPI Potato starch SSPS WPI/CNFs CMC	Yemmireddy and Hung Canoetal,Xuetal,Zhang et al. Oleyaei et al., Teymourpour et al., Zolfi et al.AlizadehSani etal. Achachlouei et al.
Ag	CS Starch, PVA β-CD Agar, banana powder	Davoodbasha et al. Mathew and Kuriakose Lin et al, Kumar-Krishnan et Andrade et al Orsuwan et al.
ZnO	CS Sago starch FPI, FSG MC SSPS	Malini et al., Nafchi et al. Arfat et al Espitia et al Akbariazam et al.
Silicondioxide Others Silica CuO SNP	CS CS Kefiran, CMC CS	Yu et al, Sun et al. Shi et al. Song et al., Hasheminya et al. Shankar et al.
Ag-TiO2 MixedNPs TiO2-Ag Ag-Cu TiO2-SiO2	CS PLA FSG ZP	Lin et al., Chi et al., Arfat et al., Kadam et al.,

This paper shows that the ZnO nanoparticles have potential use in the food industries since they are biocompatible, nontoxic, and poisonous to microbes. Because ZnO nanoparticles break down cell membranes and increase membrane permeability, they significantly reduced the growth of bacteria. ZnO was recently listed as a safe substance by the FDA (Food and Drug Administration, USA). ZnO nanoparticles are more advantageous than other metal oxides since they are white, inexpensive to produce, and UV-blocking (Madasamy Malini et al., 2015).

Researchers saw the antibacterial activity of ECF with NPs against the tested pathogen. The surface structure and mechanical characteristics of ECF with NPs, which are essential for its applications in fruits, were then discussed. and veggies while being kept at room temperature or in a refrigerator. Additionally covered were the characteristics of ECF with NPs, such as gas modification, induction defense, and ion release. However, findings from other studies suggested that using ECF with various NPs could offer a practical and efficient means of preventing quality loss in postharvest fruits and vegetables. More significantly, more studies on the antifungal mechanism induced by UV or visible light and the interactions between nanoparticles and coating materials (Yage Xing et al., 2019).

Bio-coatings for preserving fruits and vegetables.

Bio-Coatings	Fresh Fruits and/or Vegetables	Coating Method	Main Results
Alginate	Green Mango	Dipping	Preserved the color of fresh-cut mangoes and increased the antioxidant potential of cubes
Alginate	Carrots	Dipping	Preservative tool to enhance the shelf life
Alginate	Apples	Dipping	Changes in eating quality parameters, appearance acceptance, and textural properties
Alginate	Pineapple	Dipping	Preservation

Alginate and Cassava starch	Pineapple	Dipping	Extending the post-harvest life
Alginate with Lemongrass essential oil	Apples	Dipping	Preserving the quality of fresh-cut apple

Bio-Coatings	Fresh Fruits and/or Vegetables	Coating Method	Main Results
Chitosan	Banana	Dipping	Increase the storage life
Chitosan	Zucchini	Dipping	Extending post-harvest life and enhancing the overall quality
Chitosan with Rice starch	Walnut	Dipping	Extend the shelf life of walnut
Chitosan with Lemongrass essential oil	Green bell pepper	Dipping	Maintaining the fruit quality
Chitosan with Eugenol and Aloe vera	Pineapple	Spraying	Preserved its quality and prolonged their shelf life
Chitosan with Beeswax and Pollen grain	Pears	Dipping	Decrease in weight loss, decay and rate of softening
Beeswax	Mandarin	Spreading	Maintaining various fruit quality parameters and sensory attributes
Beeswax with Sodium caseinate and Guar gum	Strawberries	Dipping	Reduce the respiration and transpiration rates of strawberries

Bio-Coatings	Fresh Fruits and/or Vegetables	Coating Method	Main Results
Wax with Bavistin	Mandarin	Dipping	Prolongation of shelf life, as well as the quality of mandarin fruit
Carnauba wax	Sweet potatoes	Dipping	Preserve food quality and extend shelf life of sweet potato roots
Carnauba wax	Tomatoes	Dipping	Increased fruit gloss and improved tomatoes appearance
Carnauba wax and Grapefruit seed extract	Mandarin	Dipping	Can extend the post-harvest shelf life of mandarins
Cassava starch with Cinnamon essential oil	Guava	Dipping	Maintaining the quality of guava at room temperature and modified atmosphere, extending the useful life
Rice starch with Coconut oil and Green	Tomatoes	Dipping	Delayed ripening

tea leaf extract			effects on tomatoes
Arabic gum	Persimmon fruits	Dipping	Higher total phenolics, ascorbic acid, antioxidant activity, and titratable acidity

From this paper, we came to know different types of bio-coating methods, such as dipping and spraying, and different bio-coatings for different fruits and vegetables and their effects and results, where some bio-coating can increase their shelf life, some can increase their antimicrobial activity, and some can increase both and give a delayed ripening effect (Camelia Ungureanu et al., 2023)

III. MATERIALS AND METHODOLOGY

Citrus limetta peels were collected from fruit shops and prawn shells were collected from the fish shop and Distilled water, Zinc Nitrate, Acetic acid, Sodium Hydroxide were used. Equipment includes a tray dryer, UV–Vis spectrophotometer, Oil\Water bath, Bunsen burner, Muffle furnace.

IV. PROCEDURE

4.1 Zinc oxide Nanoparticle Preparation.

Citrus limetta fruit peels were collected, washed, dried and then ground into a fine powder and 5gram of powder was taken in a glass container with 250 ml of distilled water. And the sample is stirred for one hour. Then the mixture was filtered and stored in an argon atmosphere.

After that, collect the 50 ml extract and place it in a water bath for 45 minutes at 55-60℃. And add 5 grams of zinc nitrate in 25ml distilled water. The next step is to Heat the mixed solution at 55-60℃ for 45 minutes. After 45 minutes, a white solution will be prepared, which is the nanoparticle solution. The nanoparticle solution is dried on a silica crucible at a temperature of 150℃ using Bunsen burner.

The Nanoparticle solution is completely dried. The dried crude ZnO nanoparticles were washed with double distilled water to remove unreacted zinc nitrate. And the Resulting particles were washed with ethanol to remove unreacted phenolic compounds. The resulting particles were dried at 110℃ for 10-15 minutes. Then, the remaining particles are placed in a tray dryer at 100℃ for 2 days. Pure zinc oxide nanoparticles are obtained.

4.2 Chitosan preparation.

About 200g of crab shell or prawn shell waste was collected in fresh condition from the market and the shells were thoroughly rinsed thrice with tap water, desiccated at room temperature and subjected to size reduction followed by drying at room temperature. The shells were then anked in 1% sodium chloride and incubated for 24 hours at rotatory shakers. This saline treatment is carried out to remove dirt and other odd particles. After 24 hours, they were thoroughly washed with distilled water and air-dried.

4.3 Demineralization

Shells contain many inorganic components out of which calcium carbonate is the main. Diluted hydrochloric acid was used to remove calcium carbonate it also prevents the hydrolysis of chitin. The hydrochloric acid concentration ranging from 0.25 to 2N was used. They were soaked in HCl and kept for 24 hours at ambient conditions. The product mass was washed with distilled water to attain neutral PH and dried in the sunlight.

4.4 Deproteinization

Demineralized chitin samples were treated with 0.5 N sodium hydroxide solution at room temperature for 24 hours to remove proteins and other pigments. Then the samples were washed several times with distilled water until pH becomes neural, then dried under sunlight. After that, the dried sample was made into powder.

4.5 Phase-2 (Biocoating Preparation)

After obtaining the chitosan. Take 1.5g of chitosan and dissolve it in 150ML of distilled water containing 1% acetic acid, and stir the solution overnight until the solution becomes clear. The solution is stirred overnight for the complete dissolution of the chitosan. The next step is Plasticizing. In this, we add 0.75ml of glycerol and stir for 30 minutes. And then we added the ZnO nanoparticles, and we obtained the bio-coating material by green synthesis of Zinc oxide nanoparticles using Citrus limetta peel extract.

V. RESULT AND DISCUSSION

5.1 Extraction of Zinc Oxide Nanoparticles

Citrus limetta fruit peels were collected, washed in deionized water, dried and then ground into a fine powder. And the addition of deionized water, and powdered peel and the addition of zinc nitrate washing and drying it completely for obtaining pure zinc oxide.

Figure 5.1.1: Collected Peels

Figure 5.1.2: Washed and dried peels

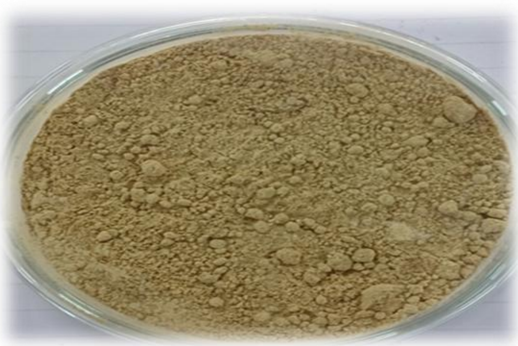


Figure 5.1.3: Powdered particles

Figure 5.1.4: Addition of peel powder and deionized water

Figure 5.1.5: Filtered and stored solution

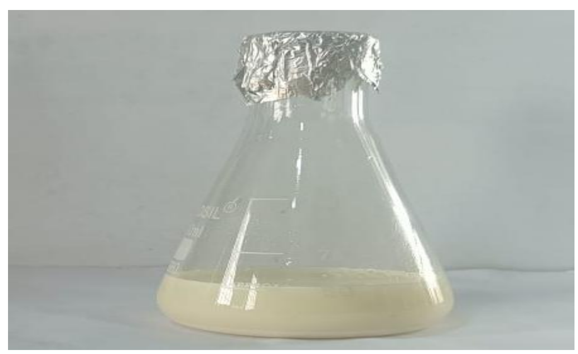


Figure 5.1.6: Addition of Zinc Nitrate

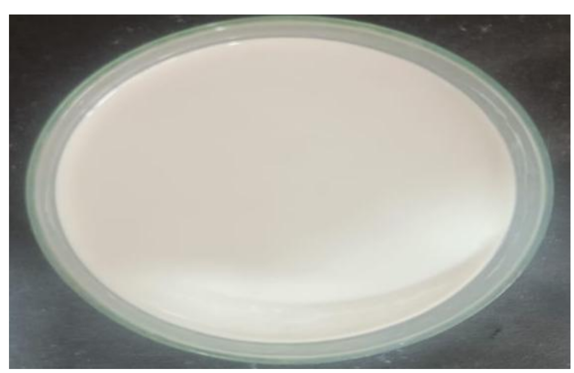


Figure 5.1.7: Dried and washed zinc oxide nanoparticle solution

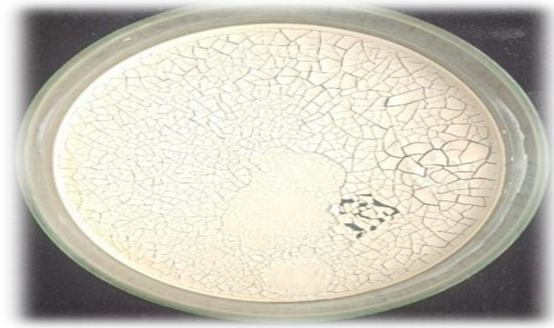


Figure 5.1.8: Pure zinc oxide nanoparticles

5.1.1 Characterisation of ZnO Nanoparticles

5.1.1.1 UV-Visible spectroscopy

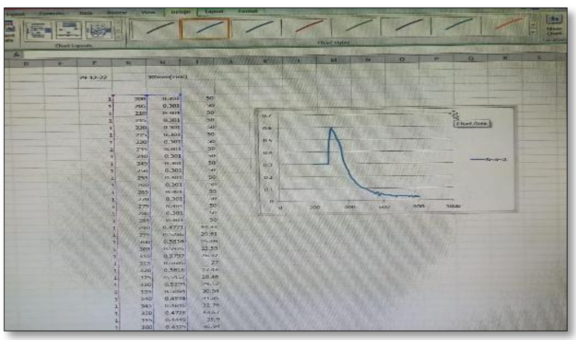
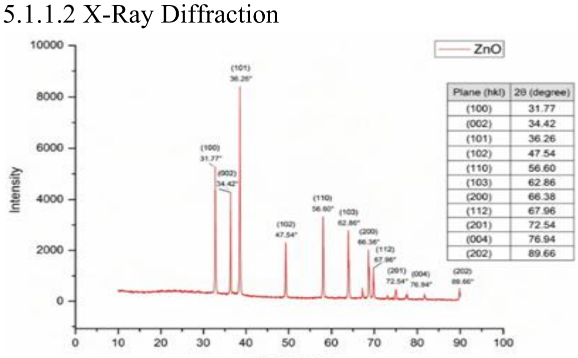


Figure 5.1.9: Observed the peak value of ZnO NPs at 305nm.

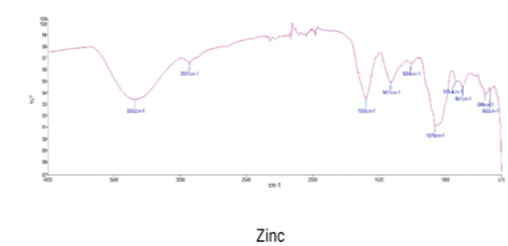
Formation of white precipitate and characteristic absorption peak (305 nm) in UV-Vis spectra confirmed ZnO nanoparticle formation. The presence of phytochemicals such as flavonoids, citric acid, and ascorbic acid in Citrus limetta peel extract acts as a natural reducing and stabilizing agents. These compounds reduce zinc ions (Zn^{2+}) to ZnO nanoparticles and prevent their aggregation. The UV-Vis absorption in the 305nm region is a well-documented indicator of ZnO nanoparticle formation.

5.1.1.2 X-Ray Diffraction



The X-ray diffraction pattern of zinc oxide nanoparticles shows definite line broadening of the X-ray diffraction peaks, showing that the prepared particles were in the nanoscale range, as shown in the diffraction peaks located at 31.77, 34.42, 36.26, 47.54, 56.60, 62.86, 66.38, 67.96, 72.54, 76.94 and 89.66 °. The diameter of zinc oxide crystallites was calculated by the Debye−Scherrer formula. more intense peaks corresponding to 101 planes located at position 36.26°, the crystallite size is about 27.9 nm (ShahFaisal et al., 2021).

5.1.1.3 Fourier Transform Infrared Spectroscopy (FTIR)



We performed FTIR in the range of 4000-575 cm^{-1} the figure shows a low absorption peak observed at around 3342cm^{-1} which can be hydroxyl (OH) groups also, some other bands were observed at around 2931, 1599, 1411, 1076 and more. A major change in IR spectra was noticed in this range. A deep absorbance band at 1500 indicates the presence of carbohydrate (C−O), (C=C) rings (polysaccharides, pectin, and cellulose). Both primary and secondary metabolites are present in the plant body abundantly. (ShahFaisal et al., 2021).

5.1.1.4 Antimicrobial Activity

5.1.1.4.1 Antibacterial Activity

ZnO NP-based bio-coating showed higher antibacterial activity against E. coli than control coating. ZnO nanoparticles produce reactive oxygen species (ROS) such as hydroxyl radicals and superoxide ions, which disrupt bacterial cell membranes and DNA. In this, we have done antibacterial activity for zinc oxide nanoparticles against Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli (Xian-Qing Zhou et al., 2023).

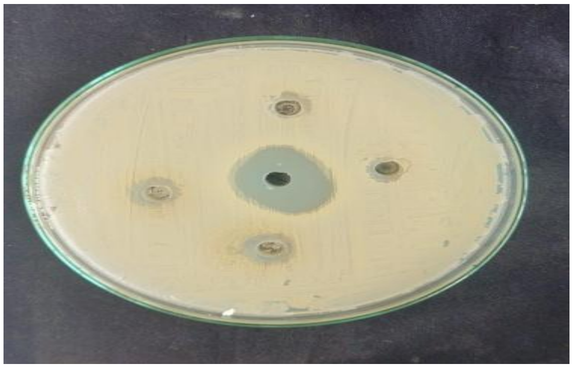


Figure 5.1.10: Pseudomonas aeruginosa

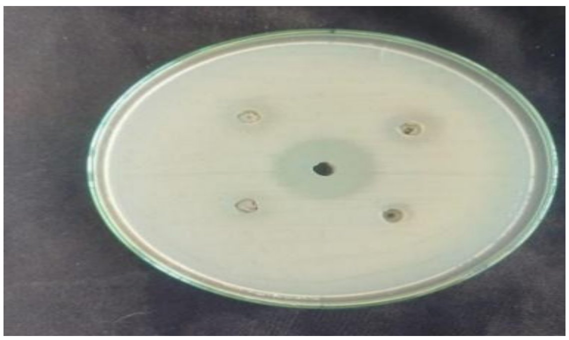


Figure 5.1.11: Staphylococcus aureus

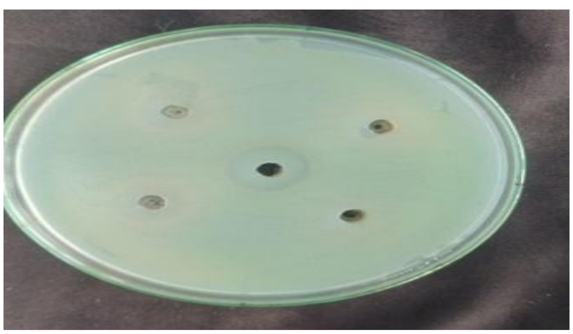


Figure 5.1.12: Escherichia coli

5.1.1.4.2 Antifungal Activity

In this, we have done antifungal activity for zinc oxide nanoparticles against Candida albicans and Aspergillus Niger. Because of their small size, nanosized ZnO particles exhibit strong antibacterial properties. Once inside the bacterial cell, they can stimulate various bactericidal mechanisms, such as the bacterial surface or bacterial core, produce ROS (reactive oxygen species), release Zn^{2+}, and even be endocytosed by cells. (Xian-Qing Zhou et al., 2023).

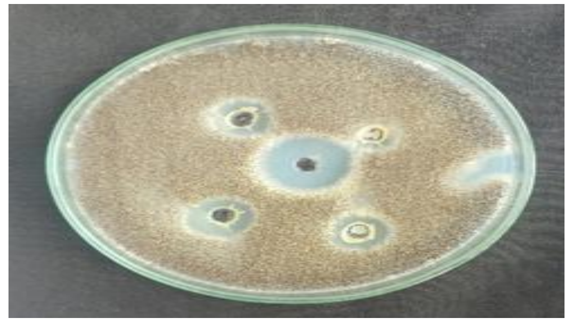


Figure 5.1.13: Candida albicans

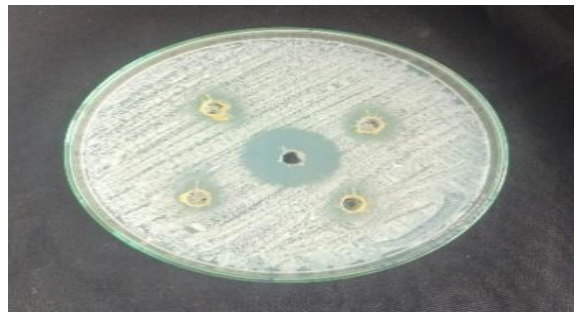


Figure 5.2.6: Aspergillus Niger

5.2 Chitosan Preparation

Prawn shell waste was collected, washed, and dried thoroughly, followed by demineralization, deproteinization, and we obtained the chitosan for bio-coating preparation

Figure 5.2.1: Sample collection

Figure 5.2.2: Demineralization

Figure 5.2.3: Deproteinization

Figure 5.2.4: Chitosan obtained

5.3 Biocoating Preparation

Addition of chitosan in 150ML of distilled water containing 1% acetic acid and stir the solution overnight until the solution becomes clear. The solution is stirred overnight for the complete dissolution of the chitosan.

The next step is Plasticizing. And zinc oxide nanoparticles addition to this solution, we obtain the bio-coating material by green synthesis of Zinc oxide nanoparticles using Citrus limetta peel extract.

Applying CS coating enhanced with green-synthesised ZnO-NPs may be considered an efficient method to maintain postharvest quality and increase cherry tomato shelf life under ambient settings. (Nana Millicent Duduzile Buthelezi 2025).

Figure 5.3.1: Biocoating Polymer

5.3.1 Characterisation for Biocoating

5.3.1.1 Antimicrobial activity

We performed antimicrobial activity for our bio-coating against Candida albicans and Escherichia coli, which showed us a good result and the zone formation, which helped us to confirm the high effectiveness of our bio-coating.

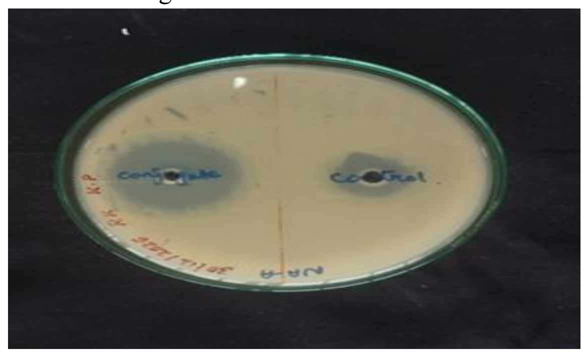


Figure 5.3.2: Candida albicans

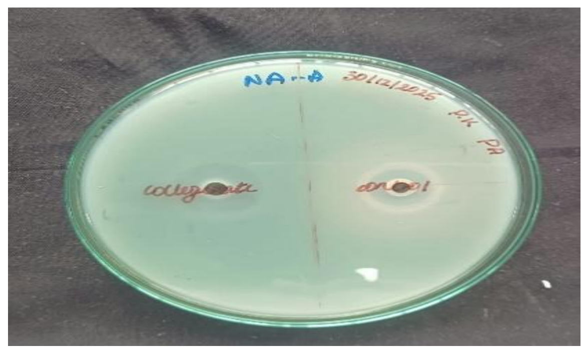


Figure 5.3.3: Escherichia coli

5.3.1.2 Cytotoxic Effect

The cytotoxic effect of the sample was tested against L929 cell line by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983). The cells were seeded in 96-well microplates (1 x 10^4 cells/well) and incubated at 37°C for 48 h in 5% CO_2 incubator and allowed to grow 70-80% confluence. Then the cells were treated with different concentrations of sample and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37ºC in the dark for 4 h. The formazan crystals were dissolved in 100 µL DMSO and the absorbance was read spectrometrically at 570 nm. Percentage of cell viability was calculated using the formula, Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100.

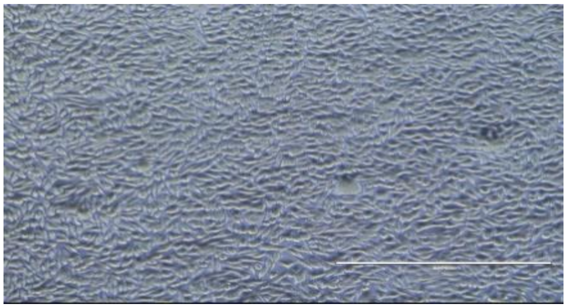


Figure 5.3.4: Control

Figure 5.3.5: Concentration 12.5

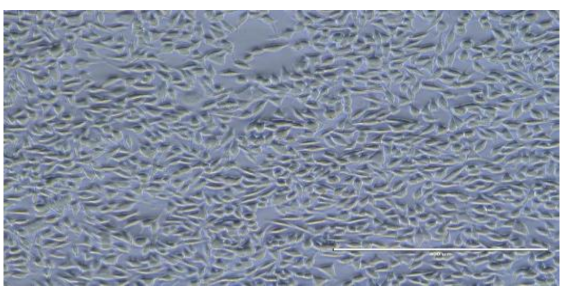


Figure 5.3.6: Concentration 25

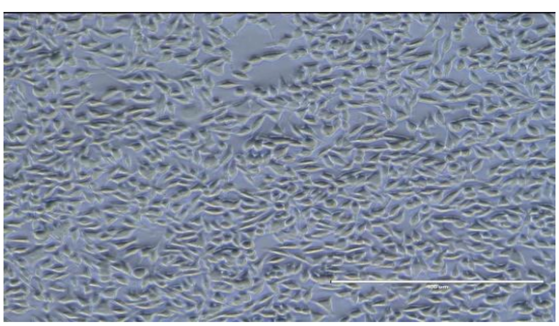


Figure 5.3.7: Concentration 50

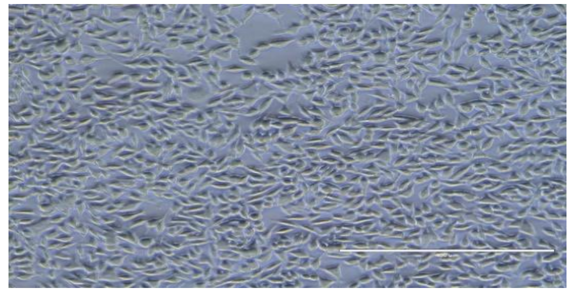


Figure 5.3.8: Concentration 100

Table 5.1: Different concentrations and cell viability for cytotoxic effect

Concentrations (µg /ml)	Cell Viability (%)
Control	100
12.5	97.00103413
25	86.65977249
50	77.71458118
100	68.30403303

5.3.1.3 Green Synthesized ZnO Nanoparticle-Based Edible Coatings for Fruit Preservation

We used tomatoes and strawberries for this study to demonstrate the effectiveness of our bio-coating. We used the dipping method; we dipped the tomatoes and strawberries. fig shows the Observation Day 1 of tomatoes and strawberry and fig and fig shows the Observation Day 15 of tomatoes and strawberry

Figure 5.3.9: Observation Day 1

Figure 5.3.10: Observation Day 1

Figure 5.3.11: Observation Day 15

Figure 5.3.12: Observation Day 15

VI. CONCLUSION

This study successfully formulated the bio-coating from green synthesized zinc oxide nanoparticles along with chitosan which is extracted from the prawn shell and we have done characterization for conformation of zinc oxide nanoparticles Characterisation such as UV-Visible spectroscopy, in which we observed the peak at 305 nm and FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY), in which we observed a deep absorbance band 1500 cm^{-1} and XRD (X-Ray Diffraction)101 planes located at position 36.26° and we have done antibacterial and antifungal against Psudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicans and Aspergillus Niger and we have done characterization for bio-coating such as cytotoxic effect in different concentration which showed less toxicity level and antimicrobial activity against Candida albicans and Escherichia coli which showed a good zone formation and the fig shows the effectiveness of our bio-coating compared with day one and day fifteen in general the bio-coating is the upcoming biotechnology invention which can help to reduce the problem related to food industries.

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