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Structural, Compositional, and Phytochemical Profiling of *Citrus Sinensis* Peel Waste-Synthesized Zinc Oxide Nanoparticles as a Potential Biocontrol Agent Against *Magnaporthe Oryzae*

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A B S T R A C T

Magnaporthe oryzae is the causal agent of rice blast disease that continues to threaten global rice production and food security. Conventional fungicides used against the pathogen have accelerated fungicide resistance and caused environmental harm. Hence, this study explored the potential of green-synthesized zinc oxide nanoparticles (ZnO NPs), derived from Citrus sinensis (sweet orange) peel extract, as an eco-friendly and sustainable biocontrol agent against M. oryzae. Orange peel extract was obtained and screened for phytochemicals, confirming the presence of flavonoids and other compounds capable of acting as reducing and capping agents. The extract was then used to biosynthesize ZnO NPs, and the resulting nanoparticles were characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Scanning Electron Microscopy paired with Energy-Dispersive X-ray Spectroscopy (SEM-EDS). UV-Vis analysis revealed near-UV absorption maxima, consistent with the optical behavior of zinc oxide. FTIR analysis confirmed strong Zn-O lattice vibrations, indicating a hydroxylated surface with possible residual phytochemical capping. SEM imaging revealed agglomerated, quasi-spherical nanoscale features, while EDS confirmed a zinc-dominant elemental composition. These physicochemical characteristics correspond closely to the properties reported in previous studies by Mazhar et al. (2023), Ghamari et al. (2022), and others documenting the antifungal mechanisms of ZnO NPs. These findings suggest that Citrus sinensis peel-mediated ZnO NPs possess the physicochemical profile associated with antifungal activity and therefore hold potential as a green inhibition agent against M. oryzae, warranting direct antifungal bioassays to validate this potential.

Keywords: Agricultural waste valorization; *Citrus sinensis*; Green synthesis; *Magnaporthe oryzae*; Zinc oxide nanoparticles

1. Introduction

Magnaporthe oryzae (anamorph *Pyricularia oryzae*) is a hemibiotrophic fungal pathogen that infects rice (Oryza sativa) at every developmental stage, causing rice blast disease. Yield losses typically range from 10 to 30% and can reach 100% under favorable humid, moderate-temperature conditions, underscoring the disease's threat to a crop that sustains over half of the world's population (Garcia et al., 2024). Beyond rice, *M. oryzae* has expanded its host range to wheat, ryegrass, maize, and finger millet, and is now reported in approximately 85 countries, including the Philippines (Younas et al., 2023).

Conventional fungicides have accelerated resistance in pathogen populations and leave residues that degrade soil health and disrupt surrounding ecosystems, motivating the search for sustainable alternatives. Nanotechnology-based interventions such as nano-pesticides and nano-fertilizers have shown promise for improving soil health, raising crop yields, and reducing chemical dependency while addressing phytopathogen resistance through green approaches (Subba et al., 2024). Existing countermeasures — resistant cultivars, clean seed, and gene-introgression breeding programs — remain costly or inaccessible to smallholder farmers, particularly in the Philippines, and do not fully counter the pathogen's ecological adaptability (Khadka et al., 2025). Among metal oxide nanomaterials, zinc oxide nanoparticles (ZnO NPs) stand out for their solubility, chemical stability across pH and temperature ranges, surface plasmon resonance-driven photocatalytic activity, and strong adhesive interaction with pathogenic cells (Islam et al., 2024). ZnO NPs have shown direct antifungal activity against *M. oryzae* by inhibiting conidiation: bio-fabricated ZnO NPs applied at 75 mg/mL produced strong inhibitory zones and achieved 98% disease control (Zhou et al., 2023), illustrating their potential as a viable alternative to synthetic fungicides.

Conventional ZnO synthesis routes — sol-gel processing, mechanical milling, and thermal evaporation — typically rely on organic solvents and toxic reducing agents such as hydrazine, posing environmental and phytotoxicity risks. Green synthesis addresses this gap: phenolic compounds and phytocompounds from plant extracts act as natural reducing and capping agents, yielding ZnO NPs that are less toxic, more biocompatible, and more environmentally friendly than their chemically synthesized counterparts (Shah et al., 2025; Zikalala et al., 2022).

Citrus sinensis (sweet orange) peel — typically discarded as agricultural waste — is rich in flavonoids, polyphenols, tannins, essential oils, and pectin, with reported pectin yields of 10–17% (Molla et al., 2026). Pectin's hydroxyl groups can be converted into aldehydic groups that reduce metal salts into nanoparticles, while its high surface charge limits nanoparticle aggregation, making it a sustainable, low-cost reducing agent that simultaneously converts an abundant byproduct into a valuable material.

The antimicrobial activity of ZnO NPs depends on intrinsic physicochemical properties — particle size, morphology, crystallinity, surface charge, and optical characteristics. ZnO nanoflowers exhibit greater photocatalytic activity than nanorods or nanospheres owing to their larger surface area, and smaller ZnO nanocrystals generally outperform larger ones (Da Silva et al., 2019). Despite these advantages, direct evidence linking orange peel-derived ZnO NPs to *M. oryzae* inhibition remains sparse: most citrus-waste ZnO NP studies remain confined to controlled laboratory conditions rather than field settings (Roopa et al., 2025), and related antifungal work has largely examined other pathogens or host plants (Subba et al., 2024).

This study addresses the gap by biosynthesizing ZnO NPs from Citrus sinensis peel extract and characterizing their physicochemical profile — through phytochemical screening, UV-Vis spectroscopy, FTIR spectroscopy, and SEM-EDS — to evaluate their theoretical potential as a biocontrol agent against *M. oryzae*. By providing a baseline for crop disease management and productivity, this study indirectly contributes to UN Sustainable Development Goals 2 (Zero Hunger) and 15 (Life on Land), advancing agricultural waste valorization and nanotechnology-based plant pathology as groundwork for future antifungal bioassays.

2. Methodology

This study followed a sequential protocol: collection and authentication of Citrus sinensis, preparation of orange peel powder, ethanolic extraction, phytochemical screening, green synthesis of ZnO nanoparticles, and characterization via UV-Vis spectroscopy, FTIR spectroscopy, and SEM-EDS. All procedures were conducted under laboratory safety protocols, with standard PPE observed throughout, and waste was segregated and disposed of according to Central Luzon State University (CLSU) laboratory guidelines.

2.1. Collection of Materials

Approximately 1.5 kilograms of oranges were collected from a local store in Buliran, San Miguel, Bulacan. Healthy, disease-free, and undamaged orange samples were bought to ensure the quality and consistency of the extract. The collected oranges were placed in clean polyethylene bags and stored in a refrigerator with a temperature of 4C, as guided by Kumar et al. (2020)'s methodology. Laboratory-grade 95% ethanol was bought from Philippine Medical Supplies. Zinc acetate dihydrate and other chemical reagents were purchased from the Center for Nanotechnology in Agriculture and Fisheries at Central Luzon State University. Necessary laboratory equipment, such as 1L beaker, test tubes, 250 mL Erlenmeyer flask, petri dish, and Whatmann No. 1 filter paper, were borrowed from the San Miguel National High School laboratory.

2.2. Authentication of Orange

The collected orange samples were brought to the University of the Philippines located in Diliman, Quezon, for authentication at the Jose Vera Santos Memorial Herbarium (PUH), Institute of Biology, College of Science. The authentication confirmed that the plant used by the researchers in this study is Citrus sinensis.

2.3. Preparation of Orange Peel Powder

The oranges were washed thoroughly with distilled water to remove dirt and impurities. It was then peeled thinly to obtain the peel and not the bitter white pith of the orange. The peels were cut into thin strips for easier dehydration. The strips were placed in an oven at 95°C for 4 hours to dry. Once dried, the peel strips were grounded by mortar and pestle into a fine powder, obtaining 50g of orange peel powder. The resulting orange peels were placed in a container to be stored at room temperature. The researchers referred to the process in Radulescu et al. (2025)'s study in the preparation of orange peels.

2.4. Orange Peel Extraction

50g of orange peel powder was mixed with 500 mL of ethanol. The maceration time is 72 hours, with frequent observation and stirring. After 72 hours, it was filtered with Whatmann no.1 filter paper to remove particles and obtain the ethanolic orange peel extract. The obtained extract was then stored in an amber glass jar to prevent sunlight from reaching the solution. To evaporate the ethanol solvent, a rotary evaporator from the Center for Nanotechnology in Agriculture and Fisheries at Central Luzon State University was used. The mixture slowly evaporated for 1 hour, producing a concentrated extract. The Citrus sinensis peel extraction method used in this methodology is based on the studies of Odetayo et al. (2024) and Doan Thi et al. (2021).

2.5. Phytochemical Analysis

The phytochemical analysis for phenolic compounds in the orange peel crude extract was conducted at the Center for Nanotechnology in Agriculture and Fisheries in Central Luzon State University.

2.6. Green Synthesis of Zinc Oxide Nanoparticles

Following the method utilized in Avdić et al. (2026)'s study, the orange peel extract was diluted with deionized water at a ratio of 1:10. In a separate beaker, a 0.1 M zinc acetate solution was prepared. Zinc acetate dihydrate solution was added to the beaker at a ratio of 1:1 extract to zinc acetate dihydrate solution with continuous stirring. The pH level of the mixture was adjusted to 12 and was then heated at 65 C. The mixture was stirred continuously until a pale-yellow color change is observed. It was then centrifuged at 5000 rpm (revolutions per minute) for 25 minutes and washed with deionized water twice to remove unreacted zinc ions and impurities. Once the precipitate is obtained, the product was oven-dried at 80 C for 12 hours until a light-yellow powder is left. The dried paste was ground with a mortar and pestle and calcinated in a furnace at 400 C for 1-2 hours to burn unreacted zinc ions.

2.7. Characterization of Zinc Oxide Nanoparticles

The resulting green-synthesized ZnO NPs were characterized by SEM-EDS, FTIR, and UV-Vis spectroscopy at the Center for Nanotechnology in Agriculture and Fisheries, Central Luzon State University. Fourier Transform Infrared (FTIR) spectroscopy was used to measure the absorption of frequencies by shining infrared light. Ultraviolet-Visible (UV-Vis) Spectroscopy was used to measure how much ultraviolet or visible light that the sample absorbed or transmitted. Scanning Electron Microscopy paired with Energy-Dispersive X-ray Spectroscopy (SEM-EDS) Analysis was used to create a high-resolution image of the sample and determined its elemental and chemical composition by measuring the X-rays emitted by the material.

3. Results and Discussions

3.1Phytochemical Properties of Citrus sinensis Extract

Qualitative screening confirmed the presence of tannins, saponins, flavonoids, coumarins, terpenoids, cardiac glycosides, and alkaloids in the ethanolic *Citrus sinensis* peel extract, each indicated by characteristic color changes or precipitate formation.

Table 1. Phytochemical Screening of Citrus sinensis peel extract.

Test conducted	**Test for Tannins**	**Test for Saponins**	**Test for Flavonoids**	**Test for Coumarins**	**Test for Terpenoids (Sterol)**	**Test for Cardiac Glycosides**	**Test for Alkaloids**
Name of Tests	Ferric Chloride Test	Froth Test	Ammonia Test	Sodium hydroxide Test	Salkowski Test	Keller-Killani Test	Maever's Test
Positive Result	Greenish-black precipitate	Stable Froth	Pale yellow precipitate	Yellow precipitate	Green coloration	Reddish-Brown ring at the interface	Flesh precipitate or yellow precipitate or turbidity
Sample	(+)	(+)	(+)	(+)	(+)	(+)	(+)

This multi-component profile indicates a chemically versatile extract rather than one dominated by a single metabolite class. Tannins and flavonoids, rich in phenolic hydroxyl and carbonyl groups, can coordinate with Zn^{2+} ions to anchor developing zinc nuclei; saponins act as amphiphilic stabilizers that limit agglomeration; alkaloids' nitrogen-donor groups serve as auxiliary ligands; and coumarins, glycosides, and terpenoids contribute additional oxygen-rich, surface-active compounds that regulate nucleation and capping. These observations are consistent with Molla et al. (2026) and Wondmnew and Tizazu (2024), who reported that extract concentration and precursor-to-extract ratio directly influence ZnO crystallite size, morphology, and optical properties. Overall, the C. sinensis extract functions as a source of multidentate ligands and protective capping agents that regulate particle nucleation, minimize agglomeration, and shape the final nanoparticle characteristics.

3.2. Physicochemical Characteristics of the Green-Synthesized ZnO Nanoparticles

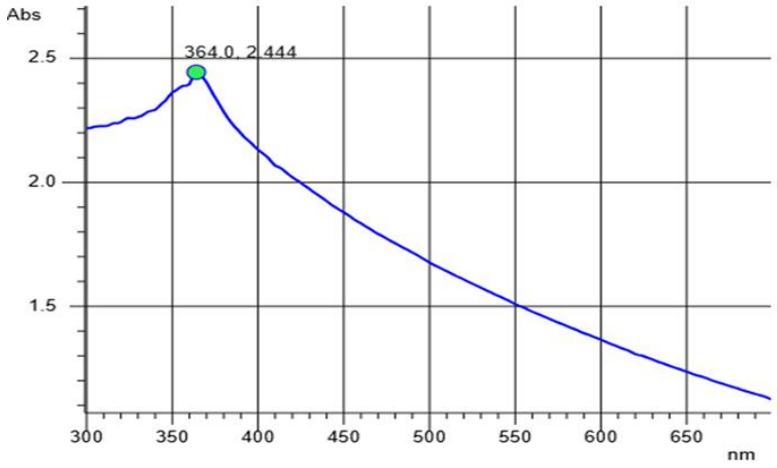
3.2.1. Ultraviolet-Visible (UV-Vis) Spectroscopy

Two independent wavelength scans (Hitachi UH5300 spectrophotometer) identified absorption maxima at 364.0 nm and 368.0 nm both within the near-UV region (350–380 nm) characteristic of crystalline zinc oxide. These correspond to band-gap energies of approximately 3.41 eV and 3.37 eV, consistent with a wide-bandgap ZnO semiconductor capable of absorbing UV light to generate reactive oxygen species (ROS) that can damage fungal cells. The recorded peaks closely match the ~370 nm peak reported by Molla et al. (2026) for orange peel-mediated ZnO, fall within the 365–380 nm range reported by Wondmnew and Tizazu (2024), and align with the ~360 nm absorption band reported by Okpara et al. (2020) for ZnO NPs mediated by orange, lemon, and grapefruit peels.

Table 2. UV-Vis Absorption peaks of Green-Synthesized Zinc Oxide Nanoparticles

Run	1	2
λmax (nm)	364.0	2.444
Absorption Peak	368.0	0.630

Fig. 1. UV-Vis Absorption Spectrum of Green-Synthesize Zinc Oxide Nanoparticles.



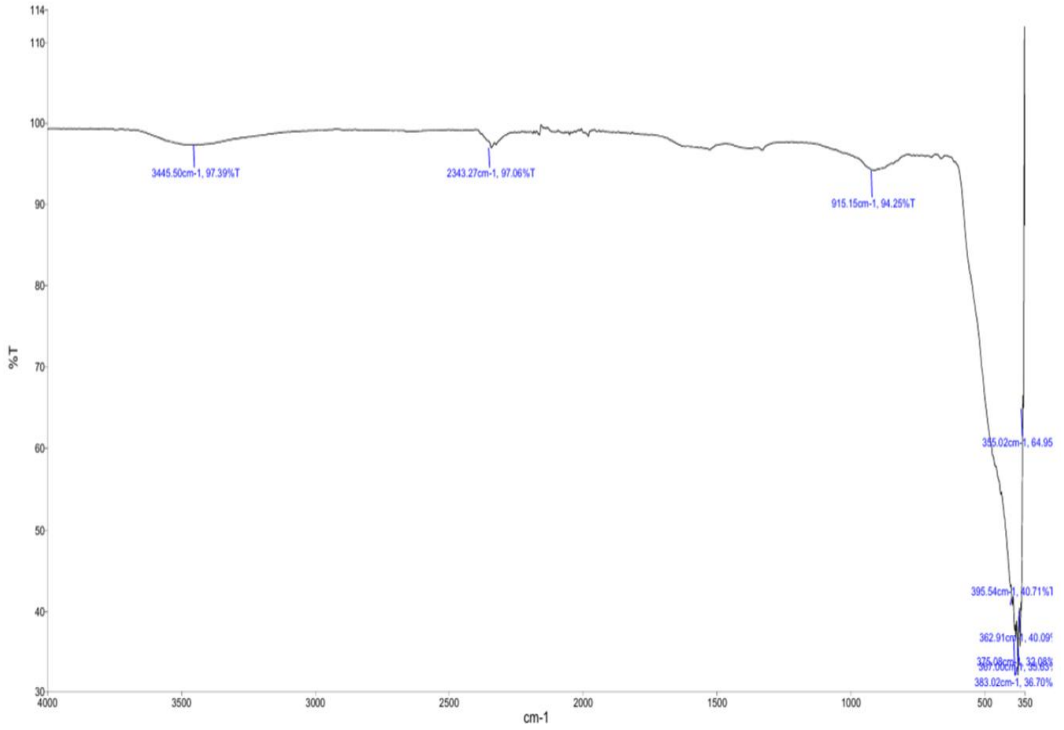
3.2.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis revealed a cluster of strong absorption bands between 395.54 cm^{-1} and 355.02 cm^{-1}, with the lowest transmittance (32.08%T) recorded at 375.08 cm^{-1}. Because metal-oxygen vibrations for zinc oxide typically occur below 600 cm^{-1}, this cluster provides direct evidence of Zn–O lattice bonding. A broad band at 3445.50 cm^{-1} corresponds to O–H stretching, indicating surface hydroxylation from adsorbed moisture and/or residual plant-derived hydroxyl groups, while a smaller peak at 915.15 cm^{-1} falls within the crowded molecular fingerprint region and is treated as tentative. These results align with the ZnO-associated bands reported by Wondmnew and Tizazu (2024; 436–450 cm^{-1}) and Okpara et al. (2020; ~553 cm^{-1}), and support a hydroxylated, phytochemically capped nanoparticle surface linked to ROS-mediated antifungal activity (Elamawi et al., 2019).

Table 3. FTIR Peak positions and interpretation for orange peel-mediated ZnONPs.

Wavenumber (cm-1)	%T	Spectral region	Interpretation
3445.50	97.39	O-H stretching region	Adsorbed water/surface hydroxyls and possible residual hydroxyl-containing plant-derived groups.
2343.27	97.06	Atmospheric CO2/background region	Commonly associated with CO2 absorption; not treated as primary evidence of phytochemical capping.
915.15	97.06	Fingerprint / surface-hydroxyl region	May reflect residual C-O/C-H-related vibrations or surface Zn-OH; assignment is tentative.

Fig 2. FTIR Spectrum of Green-Synthesized Zinc Oxide Nanoparticles



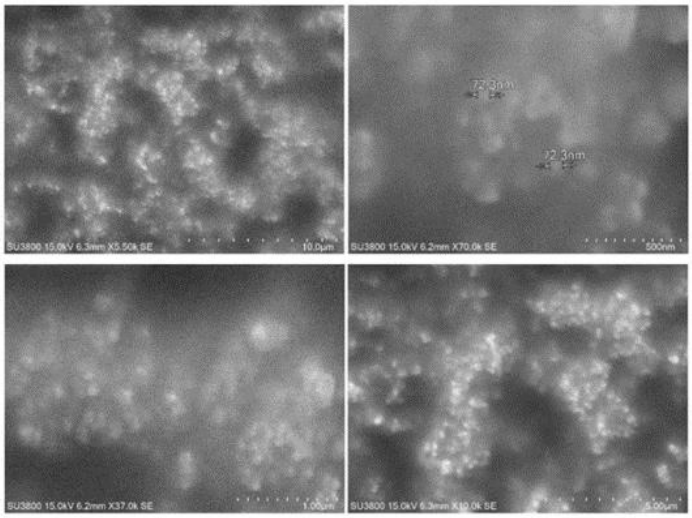
3.2.3. Scanning Electron Microscopy–Energy-Dispersive X-ray Spectroscopy (SEM-EDS)

SEM imaging at increasing magnification revealed heavily agglomerated, rounded-to-irregular, quasi-spherical nanostructures rather than distinct rod-shaped frameworks — a morphology typical of biogenic ZnO powders, which possess high surface energy and tend to aggregate during precipitation, centrifugation, drying, and calcination. A localized measurement confirmed at least one nanoscale feature of 72.3 nm.

Table 4. SEM observations for the Green-Synthesized Zinc Oxide Nanoparticles.

SEM view	Observed morphology	Interpretation
5.50k; 10 um scale	Large clustered/agglomerated regions composed of finer bright granular features.	Shows substantial aggregation at the micrometer scale.
10.0k; 5 um scale	Dense clusters with many fine particle-like features.	Supports agglomerated powder morphology.
37.0k; 1 um scale	Finer granular particles remain grouped rather than fully isolated.	Indicates nanoscale/very fine constituents within larger agglomerates
70.0k; 500 nm scale	Approximately rounded to quasi-spherical features; two annotations read 72.3 nm.	Confirms at least one measured nanoscale dimension below 100 nm; not an average size distribution.

Fig 3. Morphological Characterization of Green-Synthesized Zinc Oxide Nanopartcles.

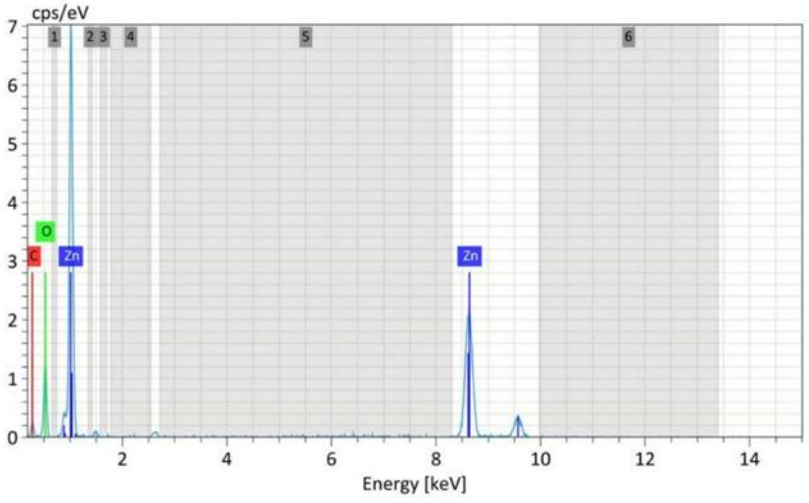


EDS analysis identified zinc, oxygen, and carbon as the dominant elements within the analyzed field. Zinc accounted for 84.62 mass% (90.91% normalized; 68.09 normalized atomic%), oxygen for 4.70 mass% (5.05% normalized), and carbon for 3.76 mass% (4.04% normalized). The strong Zn and O signals confirm successful conversion of the zinc precursor into a metal-oxide product, while the detected carbon — absent in pure ZnO — is attributed to residual organic material (e.g., pectin, flavonoids) from the C. sinensis extract that survived the 400°C calcination step, forming a natural capping layer that limits agglomeration. Because EDS is a highly localized surface technique, these values are not treated as an exact formula for the bulk powder; observed variation may reflect detector sensitivity, sample geometry, and preparation method.

Table 5. Energy-Dispersive X-Ray (EDX) Elemental Composition of Green-Synthesized Zinc Oxide Nanoparticles.

Element	Atomic no.	Net counts	Mass %	Norm. mass %	Norm. atomic %	1σ mass %	1σ rel. %
Carbon (C)	6	775	3.76	4.04	16.46	0.17	4.63
Oxygen (O)	8	4330	4.70	5.05	15.45	0.11	2.41
Zinc (Zn)	30	21849	84.62	90.91	68.09	3.63	4.30
Sum			93.07	100.00	100.00		

Fig 4. EDX Elemental Spectrum of Green-Synthesized Zinc Oxide Nanoparticles.



According to Elamawi et al. (2019) and Ghamari et al. (2022), zinc-rich ZnO NPs serve as an active reservoir for Zn^{2+} ionic dissolution — one of the primary mechanisms driving ZnO nanoparticle antifungal action against localized crop pathogens such as *M. oryzae*.

3.3. Correspondence with Reported Antifungal Characteristics

The characterized ZnO NPs share several structural hallmarks with ZnO NPs reported as active against *M. oryzae* in the literature: a nanoscale, quasi-spherical morphology; a zinc-dominant composition capable of sustaining Zn^{2+} dissolution; confirmed Zn–O lattice bonding consistent with ROS-generating surface chemistry; and near-UV optical absorption. Elamawi et al. (2019) reported that ZnO NPs applied at 10–200 mg/L significantly reduced *M. oryzae* colony formation in vitro, an effect attributed to surface-area-dependent hydrogen peroxide generation together with Zn^{2+} ion release.

These convergent lines of evidence indicate that the Citrus sinensis peel-mediated ZnO NPs synthesized in this study possess the physicochemical profile associated with antifungal activity against *M. oryzae*, making them a reasonable candidate for direct antifungal bioassays. Agglomeration and undetermined Zn^{2+} release kinetics remain the two most likely determinants of whether this potential is realized at a given application concentration. While this study did not directly test the nanoparticles against *M. oryzae* in a wet-lab setting, the convergence of phytochemical, optical, vibrational, and elemental evidence provides a strong physical and chemical basis for their potential as a green, sustainable, and cost-effective biocontrol candidate — offering a viable direction toward reducing reliance on synthetic

4. Conclusion

The study concluded that the green-synthesized zinc oxide nanoparticles (ZnO NPs) derived from *Citrus sinensis* peel extract exhibited physicochemical characteristics that make them a promising candidate against *Magnaporthe oryzae*. The presence of various phytochemicals, particularly tannins and flavonoids, supported the extract's role in the green synthesis and stabilization of the nanoparticles. UV-Vis, FTIR, and SEM-EDS analyses consistently confirmed the formation and characteristic properties of ZnO NPs, including near-UV absorption, Zn–O bonding, quasi-spherical nanoscale morphology, and zinc-dominant composition. These properties correspond to characteristics associated with antifungal activity reported in previous studies, particularly through mechanisms involving ROS generation and Zn^{2+} release.

However, since the nanoparticles were not directly tested against *M. oryzae* in this study, their actual antifungal effectiveness remains to be validated through laboratory bioassays. Overall, the findings provide a strong basis for further investigation of *C. sinensis* peel-derived ZnO NPs as a green, sustainable, and cost-effective potential biocontrol agent for rice blast disease.

5. Recommendations

Based on the findings of the study, the following recommendations are proposed to validate, optimize, and further assess the potential of sweet-orange-peel-derived ZnO NPs as a biocontrol agent against *M. oryzae*: Conduct in vitro antifungal bioassays to validate the antifungal potential inferred from SEM, UV-Vis, and FTIR analyses. Determine the Minimum Inhibitory Concentration (MIC) to establish the exact application rate of the ZnO NPs. Employ Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) to obtain a statistically representative particle-size distribution of the nanocrystals. Optimize the green synthesis protocol, particularly the calcination temperature and pH levels, to reduce nanoparticle agglomeration and produce more uniform, well-dispersed ZnO NPs. Quantify the phytochemical content of Citrus sinensis peel extract to better correlate specific bioactive compounds with nanoparticle formation and antifungal potential. Evaluate the ecological safety and non-target toxicity of the ZnO NPs before application and further testing. Lastly, extend antifungal testing to greenhouse and field trials on rice cultivars to assess the practical efficacy, stability, and cost-effectiveness of the ZnO NPs for rice blast management.

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