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Green Synthesis of Cu–ZnO Nanocomposite Using Pomegranate Peel Mesocarp Extract for Controlling Xanthomonas Campestris Causing Bacterial Blight in Tomato

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ABSTRACT

Zinc oxide/copper (ZnO/Cu) nanoparticles were synthesized using a green synthesis method based on pomegranate pulp peel extract. The purpose of the study is to manufacture an environmentally friendly zinc and copper oxide nanocomposite using plant residues from pomegranate pulp peel extract, and to use it to combat bacterial blight in tomatoes. The synthesized sample was examined to characterize the nanoparticles using UV-Vis spectroscopy, energy-dispersive spectroscopy (EDS), field-emission scanning electron microscopy (FE-SEM), and X-ray diffraction (XRD).The observed peak at 480 nm in the surface plasmon resonance of the zinc oxide nanoparticles indicated the formation of nanoparticles of varying sizes, both large and small. The successful synthesis of the compound was inferred from the shift in the UV-Vis absorption peaks using pH, time, concentration, copper-to-zinc oxide ratio, and temperature. X-ray diffraction (XRD) and phase morphology analysis revealed the crystalline nature of the nanoparticles. The average crystal size of the ZnO nanoparticles synthesized using the extract and ZnO/Cu nanocomposites was 24.16 nm, representing the highest point in the halo. However, this is not considered a sharp "crystalline peak," and the presence of sharp peaks at angles of 25.85, 29.45, and 31.85 is strong evidence of a regular atomic arrangement. Morphological analysis of the synthesized nanoparticles using high-resolution electron microscopy (HR-TEM) revealed an irregular to nearly spherical shape with sizes ranging from 10 to 200 nm.

Introduction

The seeds, juice, and peel are among the most important parts of the pomegranate (Punica granatum L.) that are the subject of intensive research in various fields (Monika p. *et al*.,2024). Among the most important Pomegranate peel contains phenolic compounds are caffeic acid, quercetin, apigenin, granatin A, granatin B, chlorogenic acid, cyanidin, ellagic acid, gallic acid, pelargonidin, punicalagin, and punicalin, all of which are useful in medicine (Eghbali *et al*., 2021). Pomegranate peel extract is attracting increasing attention for its antioxidant, antibacterial, anticancer, anti-inflammatory, and antiulcer properties (Chen *et al*., 2020). Additionally, these compounds have been demonstrated to contribute significantly to nanoparticle production.

Green synthesis is an eco-friendly and cost-effective method for producing nanoparticles. Using this method, high-quality, impurity-free nanoparticles can be obtained (P. et al., 2025).

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Nanomaterials are employed in biomedical and environmental waste management due to their intrinsic qualities. On the other hand, humans may be poisoned by nanoparticles. To solve this issue, experts have created ecologically friendly manufacturing techniques. The production of environmentally safe nanomaterials depends on the use of biological extracts for surface functionalities rather than dangerous chemicals (Biswas, P.,*et al*.,2023).

In scientific research, several types of inorganic metal oxides are commonly used. Among them, zinc oxide (ZnO) is the most popular inorganic metal oxide due to its safety. Zinc oxide nanoparticles (ZnONPs) possess numerous properties (P. *et al.,* 2025).

Biosynthesis is an environmentally friendly method for producing zinc oxide nanoparticles in the laboratory. This is because the biosynthetic pathway relies on the principles of green chemistry, utilizing renewable raw materials and safer solvents, resulting in safer chemicals. The biosynthetic process generally involves adding a zinc salt, such as zinc nitrate or zinc acetate, to a bio-extract prepared from fungi, plants, algae, or bacteria. The solution is then subjected to heat treatment, resulting in zinc oxide nanoparticles. Factors affecting the reaction include temperature, time, pH, and zinc salt concentration (MFaja *et al.,* 2022).

In order to tackle diseases that are becoming more resistant to current antibiotics, new antibiotics have been created by utilizing the development of nanoparticles and metal oxides. (Lithi, I. J., *et al*., 2025) This is because of their special qualities, which include their strong photocatalytic and antibacterial capabilities as well as their high surface area-to-volume ratio. (Babayevska, N. *et al*., 2022) Due to their enhanced ability to generate reactive oxygen species when exposed to light, their regulated release of zinc ions, their direct physical harm to bacterial cells, and their relatively low toxicity to human cells, zinc oxide nanoparticles are more effective antibacterial agents than other metal oxides.(Qu, B.*et al*., 2025)

Zinc oxide has garnered significant attention due to its non-toxicity, low cost, high physical and chemical stability, suitable oxidation-reduction potential, and biocompatibility (Endeshaw *et al.,* 2025). Because of its UV-protective properties, it is used in the cosmetics industry. This material also exhibits high paraconductivity, making it suitable for biomedical applications. It is used in cancer therapies, diabetes treatments, and drug delivery systems. It also possesses excellent antibacterial properties. Furthermore, it is used in the production of rubber and coatings, and in the absorption of arsenic from water (Özcakir, 2023).

Tomatoes (Solanum lycopersicum L.) are among the most important and widely used crops in both households and industries (Khan, Z. and Upadhyaya, H.,2023) (Lengai, G.M. *et.al*. 2021) Tomatoes are utilized in many different ways in the food industry, generating economic benefits for farmers, with tomato production reaching approximately 200 million tons (Ansari et al., 2023). Tomatoes are particularly popular because they add color and flavor to food, as well as being a rich source of vitamin C. (P.K. Mallick ,2021) Tomatoes contain lycopene, which is good for human health because it has antioxidant properties that help neutralize free radicals and stop or fix cell damage (Mendez *et al.,* 2011).

Large quantities of fertilizers and fungicides are used to produce the largest possible amount of tomatoes. Excessive use of these pesticides is harmful to humans and other living organisms. Farmers use 2.5 million tons of pesticides on their crops annually (Ansari *et al.,* 2023).

This work focuses primarily on the environmentally friendly manufacture of a zinc and copper oxide nanocomposite using pomegranate pulp peel extract, and its use in combating bacterial blight in tomatoes.

Materials and Methods

Materials

Pomegranates were purchased fresh from a local market in Babylon, Iraq. After washing them under running water until clean, the outer peel and seeds were removed, leaving only the white pomegranate peel.

We will need Inner white part of pomegranate peel (mesocarp), Zinc nitrate hexahydrate [$Zn(NO_3)_2{\cdot}6H_2O$], Copper sulfate pentahydrate [$CuSO_4{\cdot}5H_2O$] ,NaOH (to adjust pH) ,Distilled water ,Xanthomonas campestris pv. vesicatoria bacteria.

Extraction

To extract the desired compounds, 10 grams of finely chopped white pomegranate peel were weighed and combined with 100 milliliters of deionized water using an electric mixer (TJ LASSCO INDIA). The mixture was heated for two hours at 70–80°C in a magnetic heating device, filtered through filter paper, and then stored at 4°C for later use.

Synthesis of Cu–ZnO Nanocomposite

A 0.01 M solution of zinc nitrate ($Zn(NO_3)_2$) and a 0.01 M solution of copper sulfate ($CuSO_4$) were prepared at a copper to zinc oxide ratio of 1:1 (w/w). White pomegranate peel extract was added gradually with continuous stirring. The pH was measured (and adjusted to 9–10 using sodium hydroxide). The mixture was then heated at 70 °C for 2 hours. The sample was dried at 60 °C, and the precipitate was washed off. The production of copper to zinc oxide nanoparticles was confirmed by observing the change in color of the extract from brown to bluish-green. Finally, a mild calcination process was carried out at 400 °C for 2 hours.

Study of the Optimal Conditions for Synthesis of Copper-Zinc Oxide Nanocomposite

The optimal conditions for Nanocomposite synthesis were analyzed. Tests were conducted under various conditions, including zinc nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] and copper sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$] at concentrations of 0.01,0.02, 0.03 M, pH levels of 4, 6, 8, 10, and 12, temperatures of 60, 70, 80, and 90 °C, storage periods of 1, 2, 4, 6, and 8 days, and ratios of [$Zn(NO_3)_2 \cdot 6H_2O$] to [$CuSO_4 \cdot 5H_2O$] 1:1, 1:2, 1:3, 2:1, and 2:3. The effect of these parameters on the structure of copper and zinc oxide nanocomposites was monitored using ultraviolet and visible spectroscopy.

Characterization of Green Synthesis of Cu–ZnO Nanocomposite

Color Change

A successful color change indicates the formation of copper-zinc oxide nanocomposites when hot aqueous extracts of white pomegranate peel are added to a 0.1 M solution prepared using zinc nitrate ($Zn(NO_3)_2$) and copper sulfate ($CuSO_4$) solution in a 1:1 (w/w) copper-to-zinc oxide ratio. Within a few minutes, the color changes. This is a fundamental test for verifying nanoparticle formation.

UV-Visible Spectrophotometer Analysis

The optical properties of Cu–ZnO nanocomposites were analyzed using UV-Vis spectroscopy to study absorbance and bandgap and this is used to confirm the nano synthesis The highest absorption of Cu–ZnO nanoparticles is illustrated in Figure . The manufacturing process of Cu–ZnO nanocomposites and absorption at 423 nm are shown.

FTIR

Using a KBr disk, FTIR analysis was performed in the wavenumber range of 400 to 4000 cm^{-1} to identify the chemical functional groups and the role of the extract formed between the Cu–ZnO nanocomposites.

X-Ray Diffraction (XRD) Studies

To determine the crystalline phase and confirm Cu doping, X-ray diffraction and phase structure analysis were used for copper zinc oxide nanoparticles. Morphological analysis using a Shimadzu XRD-6000 (3K.NOPC) X-ray diffraction spectrometer was also used to determine the crystal structure of the metallic nanoparticles. The Debye-Scherer equation was employed to determine the particle size.

$$Dp=0.9 \lambda / \beta \cos\theta$$

The particle width is represented by Dp in A°, the X-ray wavelength by λ, the Bragg angle by θ, and the peak's full width at half maximum (FWHM) by β.

Scanning Electron Microscopy Analysis (SEM)

Scanning electron microscopy (SEM) analysis is used to study the morphology of the synthesized Cu–ZnO nanocomposites, determining their size and shape. The carefully formed Cu–ZnO nanocomposites were deposited and then dried on a glass slide. The same glass slide was used during the SEM analysis. (expected 20–50 nm)

Energy Dispersive X-Ray Spectroscopy (EDX)

EDX analysis provides information on the type and quantity of elements that may be involved in the formation of nanocomposites. To confirm the presence of Cu and Zn.

Results and Discussion

Characterization of Green Synthesis of Cu–ZnO nanocomposites

Color Change

The successive color changes indicate the formation of a copper-zinc oxide nanocomposite by hot aqueous extracts of white pomegranate peel. These extracts were added to a 0.1 M solution prepared using zinc nitrate ($Zn(NO_3)_2$) and copper sulfate ($CuSO_4$) in a 1:1 (w/w) copper-to-zinc oxide ratio within a few minutes, resulting in a color change from brown to bluish-green (Figure 1). This test is essential for verifying the formation of the copper-zinc oxide nanocomposite.

Bluish-green (turquoise): This is the color of the composite when the copper doping ratio is increased, as it begins to absorb certain wavelengths of visible light and turns brown or dark gray: This occurs if the ratio is very high or if the copper has been converted to copper(II) oxide as a separate phase on the nano-surface.

Figure 1. Color Change

(a) *H*ot aqueous extracts of white pomegranate peel.

(b) The aqueous extract indicates the formation of Cu–ZnO nanocomposites.

UV-Visible 4.1.2.Spectrophotometer Analysis

Pure zinc oxide (ZnO) has strong light absorption in the ultraviolet (UV) region, typically at wavelengths below 400 nm. While copper oxide absorbs light in the visible light region and even near-infrared radiation. The UV-Vis diagram typically shows a redshift at the absorption edge compared to pure zinc oxide. This indicates that the composite begins to absorb longer wavelengths (lower energy) towards the visible light region . Absorption increases in the visible light region (above 400 nm) as showen in Figure 2.

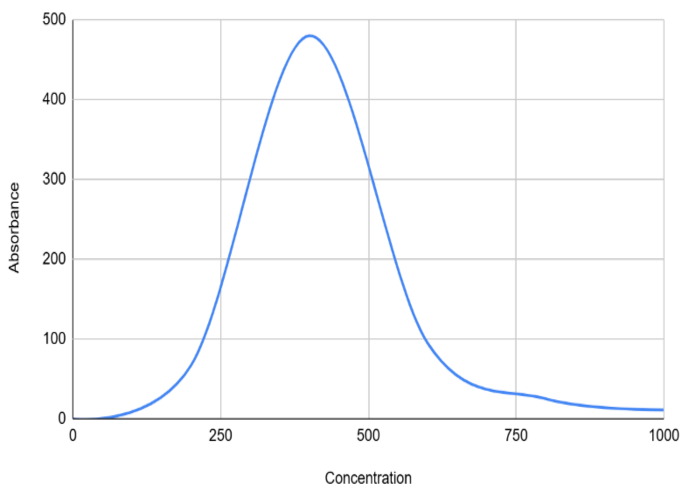


Figure 2. UV-Visible Spectrum Cu–ZnO nanocomposites.

According to Rana H. Adlan *et. al.*, 2022 The presence of ZnO NPs was confirmed by a significant absorption band in the 320–400 nm range, with a maximum of 370 nm. The absorption wavelength of zinc oxide also changes from red to blue. After doping with tin ions, the absorption range extends from approximately 389 nm to approximately 406 nm. The fabricated zinc oxide nanostructures exhibit strong UV emission, emitting green light at approximately 584 nm and other light at approximately 387 nm (Ameen S. *et al.*, 2012).

Effect of Cu–ZnO Concentration

A low concentration of the material may be the reason for the weak peak. If the concentration is very low, the material may not absorb enough light, resulting in a weak peak. The type of solvent used can affect the absorption spectrum, as the solvent may react with the substance or absorb itself in the same spectral region.

different concentration of [$Zn(NO_3)_2{\cdot}6H_2O$] and [$CuSO_4{\cdot}5H_2O$] at concentrations of 0.01,0.02, 0.03 M as showen in Figure 3.

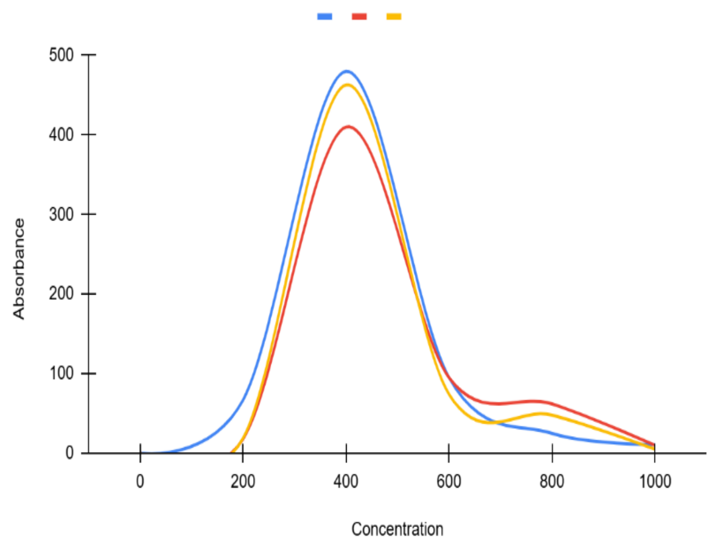


Figure 3. UV Spectrum of Synthesis of Cu–ZnO nanocomposites at different concentration of [$Zn(NO_3)_2{\cdot}6H_2O$] and [$CuSO_4{\cdot}5H_2O$] at concentrations of 0.01,0.02, 0.03 M

Effect of Temperature

The temperature was increased from 60-90 degrees Celsius Increasing the temperature increases absorption, accelerates the reaction, and then begins to decrease due to loss of stability. The curve was shifted to higher wavelengths as showen in Figure 4, and the curve became distorted with increasing temperature. It was observed that as the temperature increased from 60 to 130 degrees Celsius in 10-degree Celsius increments, the current increased while the voltage remained constant. This is because the higher the temperature, the more thermal energy electrons need to transition from the valence band to the conduction band, thus increasing the current (Dirar Mubarak et al., 2018). Abd El-Wahab H. *et al.*, 2024 study concentrates on the synthesis of zinc oxide and copper oxide nanoparticles through eco-friendly methods and plant extracts. The study found that the films with these synthetic oxides worked very well, showing no signs of damage or color change even when the temperature reached 500°C.

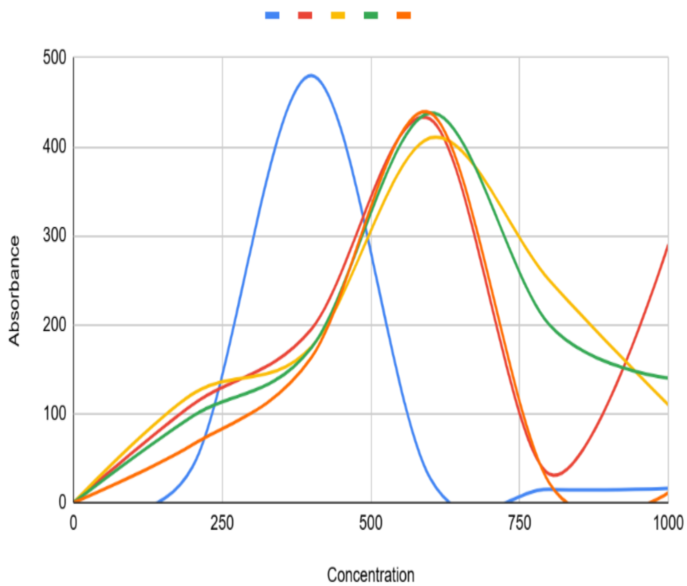


Figure 4. UV Spectrum of Synthesis of Cu–ZnO nanocomposites showing the effect of temperature.

Effect of pH

We observe as showen in Figure 5 that the blue curve reaches its highest absorption value (near 480), while the red curve shows the lowest absorption at its peak. This indicates that the substance is at its most stable or "photoactive" concentration at the pH of the blue curve. 4, 6, 8, 10, and 12, Slight shift: There is a slight shift at the peaks of the curves along the concentration axis, suggesting that absorption efficiency varies with the acidity or alkalinity of the medium. Deviation from the law due to pH change.

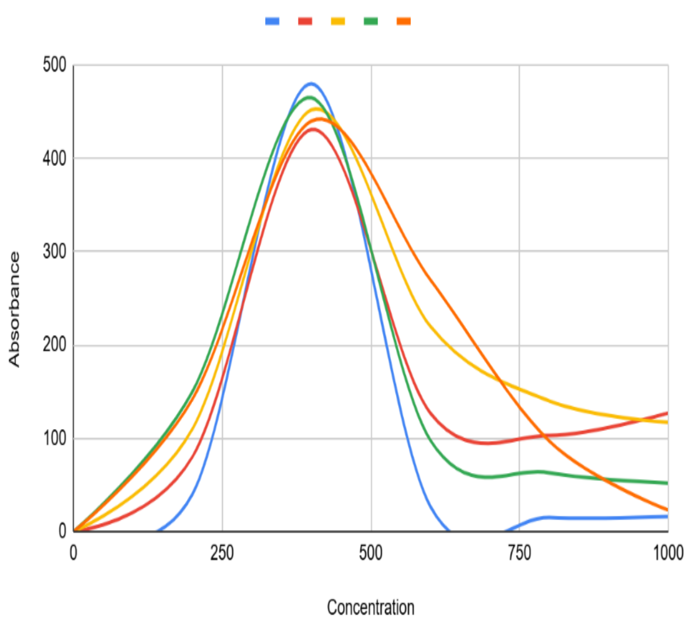


Figure 5. UV Spectrum of Synthesis of Cu–ZnO nanocomposites showing the effect of pH

Concentration Ratio of Metal and Solanum Melongena L. Extract

In the UV-Vis absorption spectrum of Cu–ZnO nanocomposites, the following [$Zn(NO_3)_2 \cdot 6H_2O$] to [$CuSO_4 \cdot 5H_2O$] concentration ratios were used: 1:1, 1:2, 1:3, 2:1, and 2:3. We observed as shown in Figure 6 that the intensity of the absorption peak increased with increasing copper oxide concentration, as CuO has a higher photoacid absorption capacity in the visible region compared to ZnO. Therefore, the highest peak was observed at the 1:3 ratio, while the lowest was at the 2:1 ratio. This is consistent with the study he conducted (Salas Jesús Plácido Medina *et al.*, 2023).

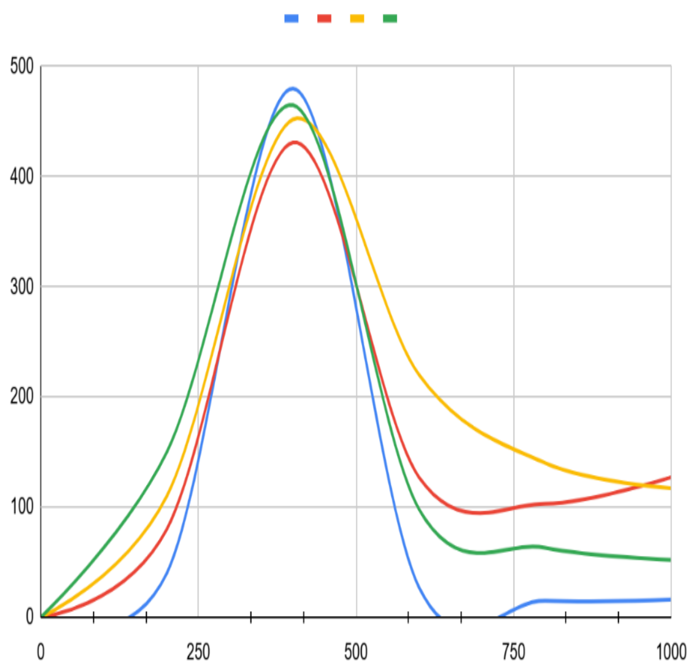


Figure 6. The effect of Concentration Ratio of Metal

Effect of the Time of Storage

The study demonstrated the biosynthesis of copper-zinc oxide nanocomposites over time during packaging, indicating that the UV-Vis and visible spectra of the copper-zinc oxide nanocomposites prepared using the green synthesis method remained stable at different reaction times: 1, 2, 3, 6, and 8 days. During this period, the solution was unaffected, with a peak center at 480 nm and increasing absorbance. The optimal storage period for the bio-prepared Cu–ZnO nanocomposite using pomegranate peel extract is typically six days when stored at 4°C in opaque containers, due to the completion of the reduction and biostabilization processes. UV-Vis peak intensity reaches its highest value ,Low particle agglomeration , Highest colloidal stability and Optimal antibacterial activity.

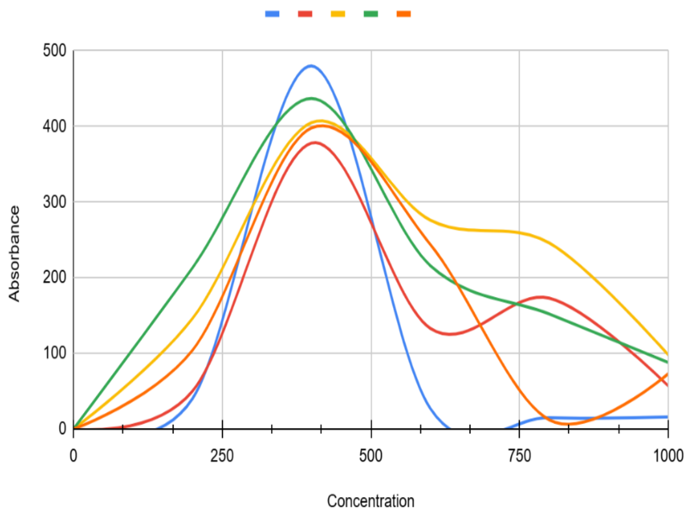


Figure 7. The effect of Time of Storage

Fourier Transforms Infrared (FTIR) Spectroscopy Analysis

A very strong and sharp band appears at the end of the spectrum (below 500 cm⁻¹), representing ZnO, this band is due to the vibration of the Zn-O bond. In the second sample (shown in purple), we observe a slight change in the shape or intensity of this band, confirming the intrusion of copper (Cu) into the crystal lattice and the formation of Cu-O bonds. Hydroxyl groups (O-H) exhibit their broad band at (3400–3600 cm^{-1}): these represent the stretch vibration of the O-H bonds. Their presence indicates water molecules adsorbed onto the sample surface or surface-bound hydroxyl groups. Those appearing at (approximately 1200 cm^{-1}) represent the bending vibration of the hydroxyl group. O-C-O and C=O appear in the regions between 1400 and 1600 cm^{-1}, indicating the presence of carbonyl or carboxyl groups. A small peak near 2900 cm^{-1} represents carbon-hydrogen bonds. A peak appears at approximately 800 cm^{-1}, which may indicate a change in the surface chemical structure after the addition of copper.

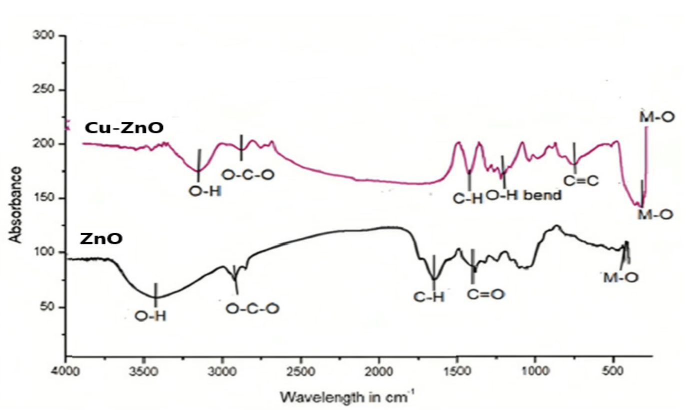


Figure 8.Sample A ZnO (Amorphous): Showed a broad hump, indicating a disordered atomic structure. Sample B Cu–ZnO (Crystalline): Demonstrated sharp, well-defined peaks, confirming a transition to a stable crystalline phase (Hexagonal Wurtzite structure).

Scanning Electron Microscopic (SEM)

Scanning electron microscopy (FE-SEM) provides valuable information about the size and shape of nanoparticles and is an effective method for studying the morphology of materials. Figure 1 shows Cu–ZnO Nanocomposite made from Pomegranate Peel Mesocarp Extract . Nanoparticles are solids with a heterogeneous nature in terms of both size and shape. The particles appear as rock fragments or broken crystals with sharp, irregular edges. This shape often indicates materials resulting from grinding or mechanical crushing processes. There is considerable variation in particle sizes (multi-dispersion), with very large particles surrounded by finer, smaller particles. Size the large particles are approximately 150–200 micrometers long. Medium-sized particles range from 30 to 70 micrometers in length. Ultrafine particles (fine dust) are less than 10 micrometers in size.

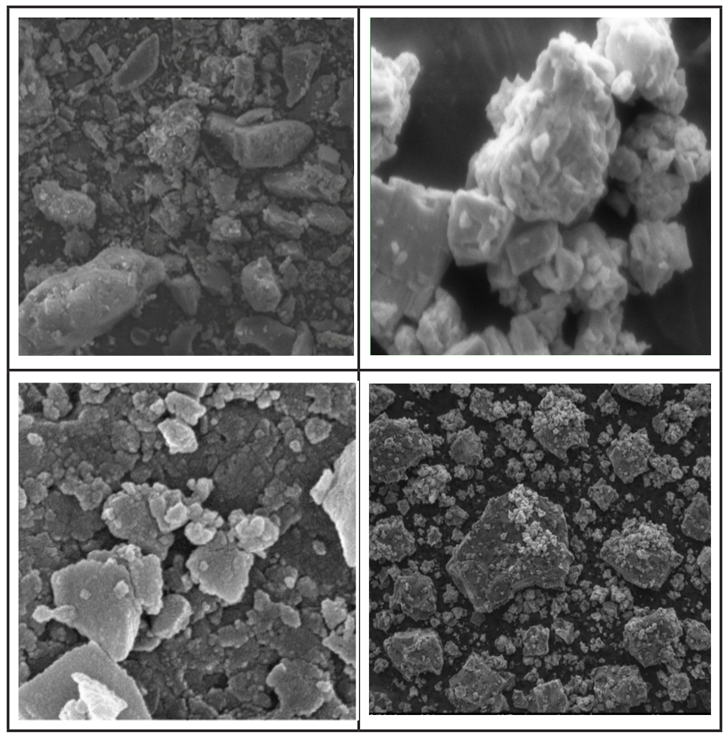


Figure 9. SEM image of Cu–ZnO Nanocomposite

Similar findings may be seen in the work of Rana H. Adlan *et. al.* 2022 Nanoparticles appear as clumped, quasopaceous particles with a rough surface. Their clumped shape emphasizes their surface morphology. The average size of nanoparticles is 38.30 nanometers. The figure shows that the powder contains numerous tightly packed nanoparticles, forming a clump-like structure. Copper oxide (CuO) consists of numerous nanoflakes densely packed at the endpoints of hexagonal zinc oxide (ZnO) rods. Zinc oxide particles are typically hexagonal in shape.One type of structure called a nanoflake is copper oxide (CuO) (Rula M Allaf and Louisa J , 2014)The scanning electron microscopy (SEM) image also shows that the ZnO-CuO nanocomposite we obtained has a homogeneous shape and size with very high clumping. Therefore, these particles did not form large clumps. (Bandekar S. *et al*.,2020) Not all zinc oxide nanoparticles are uniform in size and shape, or they clump together significantly. These particles typically range in size from 150 to 200 nanometers. When tin ions are added to zinc oxide nanostructures, their shape changes dramatically, becoming spindle-like. Each zinc oxide-tin spindle is approximately 350 ± 50 nanometers long. (Ameen S. *et al*.,2012).

X- Ray Diffraction Studies (XRD)

Sample (A): A shapeless material. The curve appears as a wide "halo" with an angular apex 2θ = 24.16 °. After the crystallization process, we see sharp, narrow peaks (acute peaks) at certain angles, such as 23.65° , 25.85°, and 29.45° . The diagram B shows that the atoms are arranged in a regular way, which is different from the shape A. The sharp peaks indicate that the nanocomposite we obtained has a high degree of crystallinity. X-ray diffraction analysis shows that the zinc oxide-copper oxide molecules have the highest peaks at 2θ angles of 53.3° and 83.9°.(Bandekar S. *et al*.,2020). Abd El-Wahab H. *et al*., 2024 focuses on the synthesis of zinc oxide and copper oxide nanoparticles using environmentally friendly techniques and plant extracts. The results showed that the synthesized nanoparticles ranged in size from 10 to 40 nanometers.

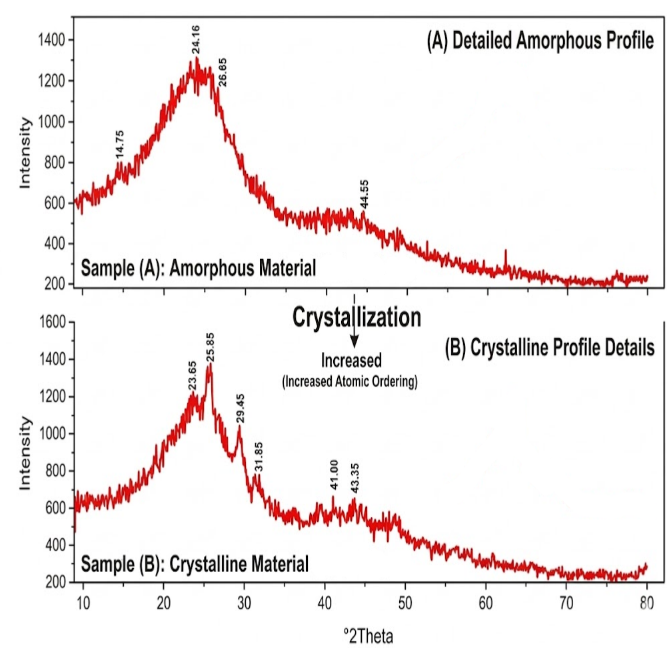


Figure 10. XRD pattern of Cu–ZnO Nanocomposite

Energy Dispersive X-Ray Spectroscopy (EDX)

EDX in Figure discovered the highest proportion of Cu–ZnO Nanocomposite is clearly visible as the highest peak in the diagram (this indicates that zinc is the primary element in this material). Oxygen appears at a lower energy level; its presence alongside zinc often suggests that the material is zinc oxide (ZnO). Copper appears as small peaks; its presence in trace amounts may indicate impurities or doping. The expected percentage of equivalent mass for ZnO (Zn 54.4%, O 44.2%) (Pakzad K. et al., 2019). CuO (Cu 45.2%, O 53.88%) (Hasnidawani J. et al., 2016) or ZnO/CuO (Cu 16.26%, Zn 39.16%, and O 44.58%).(Alzidi A. and Nasir E.,2025). While the percentage of oxygen was 47.91 and copper 4.56, copper was 41.96 (Gebretsadik, A. et al., 2026)

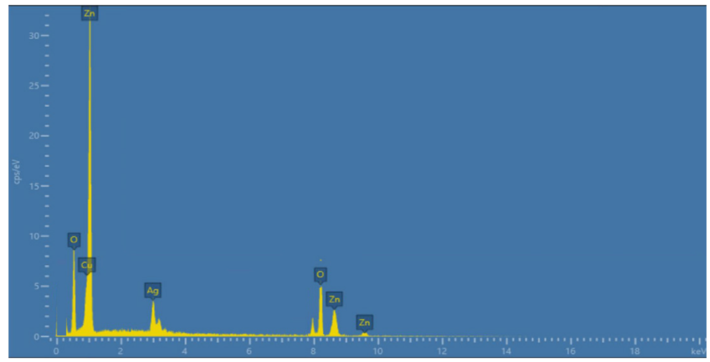
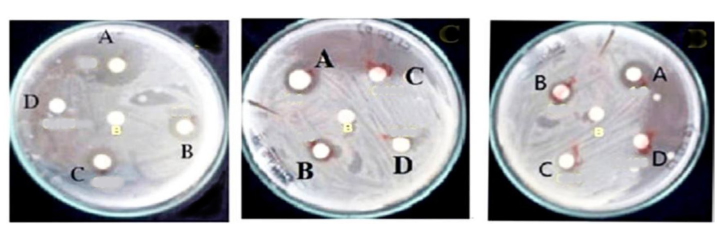


Figure 11. EDAX spectrum of Cu–ZnO Nanocomposite

Effect of Nanocomposites on Bacterial Blight Disease in Tomato Plants.

This compound stimulates the plant to produce active oxygen, which leads to the breakdown of the fungal cell membrane. Copper inhibits the fungus's vital processes, while zinc enhances the plant's immunity and activates protective enzymes. Due to its small size, the compound can penetrate plant tissues and protect them from within and without. Its effect begins by slowing the growth of the fungal mycelium.

This compound is a key means of stopping infection, but it may not be sufficient to eradicate severe infections. A noticeable reduction in the area of spots on the leaves is observed. The plant begins to exhibit an immune response by increasing chlorophyll production.The higher the number of nanoparticles, the greater the likelihood of them adhering to the walls of the fungal spores. At a concentration of 0.03M, the compound completely coats the leaves. The fungus experiences toxic shock as a result of releasing more copper and zinc ions, preventing it from multiplying. At this level, the disease severity index decreases significantly. Thanks to zinc's role in protein synthesis, new leaves appear darker green and stronger. This compound also protects the fruit from blight, reducing crop losses.



Conclusion

In this study, a hot aqueous extract of the inner white peel of pomegranate, a plant byproduct, was used in green synthesis. This method allows for the production of nanoparticles at a low cost and without harmful residues. Plant biosynthesis is the most common type of green synthesis. The nanoparticle synthesis process was optimized by changing several factors, including pH, temperature, reaction time, and the concentration of the plant extract. All these factors affect the size and shape of the nanoparticles. Some settings can be optimized to increase productivity and take advantage of the effectiveness of the copper-zinc oxide complex. FTIR, SEM, XRD, and EDX techniques were used to characterize the optimized nanoparticles. FTIR analysis showed that the plant extract contains different functional groups, and that these groups contributed to the formation of the nanoparticles. Scanning electron microscopy (SEM) images showed round and rod-shaped nanoparticles. XRD analysis confirmed their monoclinic structure and crystalline properties. EDX also determined and analyzed the elemental composition of the synthesized nanoparticles.

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