


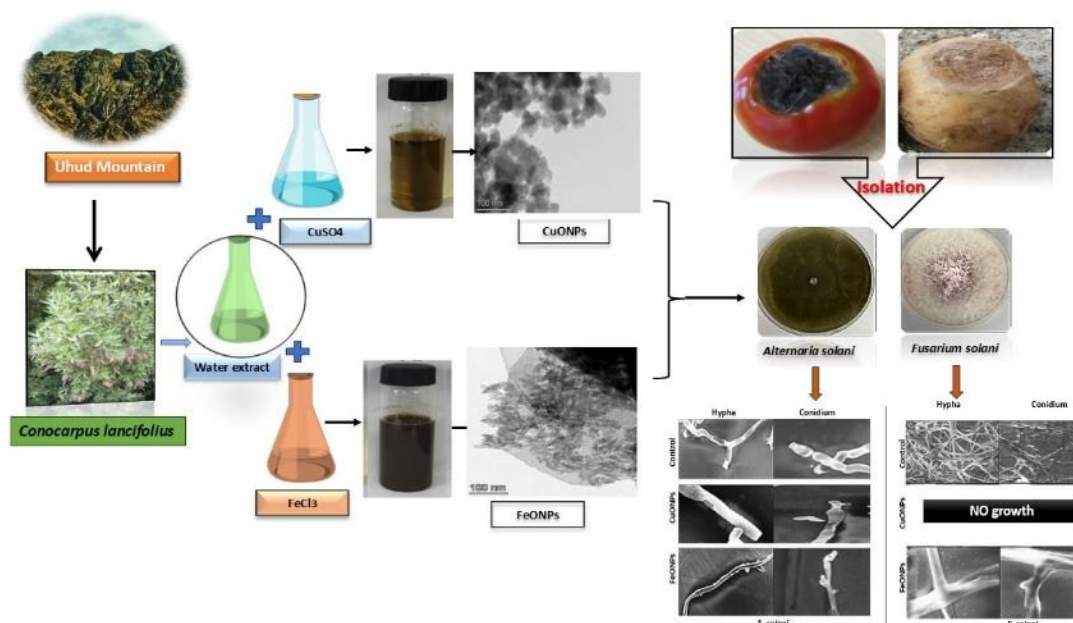
# Green Synthesis of Copper Oxide and Iron Oxide Nanoparticles using *Conocarpus lancifolius* Leaf and their Activity against some Phytopathogenic Fungi

Fayza Kouadri,<sup>a</sup> Reham Fallatah,<sup>b</sup> Safia A. A. Mohammed,<sup>b</sup> and Asmaa M. M. Mawad <sup>b,c,\*</sup>


\*Corresponding author: ammawad@taibahu.edu.sa

DOI: 10.15376/biores.20.4.11041-11055

## GRAPHICAL ABSTRACT



# Green Synthesis of Copper Oxide and Iron Oxide Nanoparticles using *Conocarpus lancifolius* Leaf and their Activity against some Phytopathogenic Fungi

Fayza Kouadri,<sup>a</sup> Reham Fallatah,<sup>b</sup> Safia A. A. Mohammed,<sup>b</sup> and Asmaa M. M. Mawad <sup>b,c,\*</sup>

Iron oxide (FeONP) and copper oxide (CuONP) nanoparticles were synthesized using the leaf extract of *Conocarpus lancifolius*. Their activity against two phytopathogenic fungi, *Alternaria solani* and *Fusarium solani*, was investigated. The colonies' diameter and morphological changes in the fungal hyphae and conidia treated with nanoparticles were examined using scanning electron microscopy (SEM). CuONPs showed oval particles with a wide particle size distribution of ~57 nm in length and ~28 nm in width. FeONPs showed elliptical disks with a wider particle size distribution of 32 to 39 nm in length and 5 to 14 nm in width. Both types of nanoparticles exhibited significant antifungal activity against *A. solani* and *F. solani*. CuONPs inhibited *A. solani* growth by 88.9% to 91.1% in terms of the fungal colony diameter after 12 days of incubation. They completely inhibited the growth of *F. solani*. In contrast, FeONPs reduced the growth of *A. solani* from 68.9% to 73.3%. The SEM images suggest that CuONPs and FeONPs damaged and distorted the fungus structure, consequently limiting and inhibiting fungal growth. Therefore, the green synthesized nanoparticles could be used as antifungal candidates to protect plants against phytopathogenic fungi.

DOI: 10.15376/biores.20.4.11041-11055

**Keywords:** Antifungal activity; *Alternaria solani*; *Fusarium solani*; Nanoparticles

**Contact information:** a: Faculty of Allied Medical Sciences, Applied Science Private University, 11937 Amman, Jordan; b: Department of Biology, College of Science, Taibah University, Madinah, Saudi Arabia; c: Botany and Microbiology Department, Faculty of Science, Assiut University, 71516 Assiut, Egypt; \*Corresponding author: ammawad@taibahu.edu.sa

## INTRODUCTION

Every year, plant diseases cause approximately 10% to 15% of crop loss globally, with phytopathogenic fungi accounting for 70% to 80% of this loss. This leads to an annual economic cost estimated at billions of dollars. Phytopathogenic fungi not only cause crop loss, but they also cause the extinction of some wild plant species (Zhang *et al.* 2024). One of the most prominent plants-pathogenic fungi is *Alternaria solani*, which belongs to the fungal phylum Ascomycota. It causes a devastating crop production loss, especially in potatoes and tomatoes (Schmey *et al.* 2024). *Alternaria solani* causes early blight, which is a plant disease that causes a major crop loss that can reach 78%. The symptoms include dark leaf lesions with rings, stem lesions, and rotting fruits. *A. solani* mycotoxins could also affect human and animal health (Jindo *et al.* 2021). Another aggressive phytopathogenic fungus is *Fusarium* species, a filamentous fungi belonging to the fungal phylum Ascomycota that have been reported to cause plant, animal, and human infections.

About one-third of pathological members belong to *Fusarium solani* species (O'Donnell *et al.* 2020). This species causes plant-damaging diseases in economically significant crops pre- or post-harvesting. It can also cause diseases in animals and humans (Srivastava *et al.* 2018; Pan *et al.* 2023). *F. solani* can cause *Fusarium* wilt, which is a devastating plant disease that causes plant death or stunting, which reduces the crop yield. The symptoms include seed stunting, leaves yellowing, stem browning, leaves and stems wilting, and then dying (Lal *et al.* 2024).

Over the last century, many types of different fungicides have been developed to limit the phytopathogenic fungi's impact on crops. However, today, fungi have become highly resistant to most of the chemical fungicides (Yin *et al.* 2023). This is due to the fungal genome being highly flexible and their rapid reproduction rate (Fisher *et al.* 2018). Conventional fungicides, which are frequently expensive, hazardous to the environment, and dangerous to human health, are losing their effectiveness against phytopathogenic fungi. This led to the rising need for environmentally friendly and sustainable plant disease management strategies. Because of their broad-spectrum antimicrobial capabilities and reduced environmental impact, green-synthesized nanoparticles present a promising substitute. Although a variety of plant extracts have been used in the synthesis of nanoparticles, the phytochemical diversity and potential of this drought-tolerant and medicinally significant plant (*C. lancifolius*) in the biosynthesis of nanoparticles have not been largely unexplored (Yin *et al.* 2023).

Recently, metal oxide and reduced metal nanoparticles (NPs) have attracted more attention due to their ultra-small size in addition to their unique chemical and physical traits (Helmy *et al.* 2023). The small size and large surface area-to-volume ratio of nanoparticles have made them very efficient for many applications. They have several pathways to affect microbes, making microbial adaptation and resistance a great challenge (Amaro *et al.* 2021). There are several approaches to synthesize NPs; chemical, physical (top-down), and biological (green) approaches (Szczyglewska *et al.* 2023). Green synthesis or the biogenic approach is more affordable, reliable, eco-friendly, and an effective alternative method to reduce or completely avoid the side effects of industrially produced NPs (Sánchez-López *et al.* 2020; Mukherjee *et al.* 2021). The green synthesis of NPs using plants is faster and produces more stable particles when compared with the mycogenic or bacteriogenic NPs (Ali *et al.* 2020). Many different plants can be used for preparation of metal-based nanoparticles, including *Conocarpus lancifolius*, which is an evergreen plant native to hot arid areas like the Arabian Peninsula, Yemen, Somalia, and exotic to Pakistan and India. *C. lancifolius* can be used in antioxidant production due to the presence of phenolic acids, ascorbic acid, and flavonoids (Rasheed *et al.* 2020). This plant shows high resistance to drought and salinity. These qualities enabled *C. lancifolius* to survive in the elevated temperatures and light intensity of the desert regions of Asia (Redha *et al.* 2021; Saadullah *et al.* 2024).

Copper oxide nanoparticles (CuONPs) are broadly used commercially due to their flexible traits, such as high stability, cost efficiency, and long shelf life. Microbial cells by different mechanisms, such as altering cell membrane permeability, cause oxidative damage to the cell's structure, and disrupt DNA replication (Hasheminya *et al.* 2018). Their antibacterial activity is employed against antibiotic-resistant bacteria, both Gram-positive and Gram-negative. The textile and plastics industries use CuONPs as an effective antifungal agent (Naz *et al.* 2020). The agriculture industry also uses CuONPs; studies have shown that CuONPs increase bean root and shoot growth. CuONPs improve yield and degrade starch, which enhances stress tolerance (Siddiqi and Husen 2020).

Iron oxide nanoparticles (FeONPs) have different medical and bioengineering applications due to their magnetic characteristics and their ability to be synthesized in different shapes and sizes. FeONPs are used in magnetic resonance imaging (MRI), magnetic-field-mediated cancer therapy, tissue engineering, bioimaging, and drug delivery (Predoi *et al.* 2023). The antimicrobial effect of FeONPs has been demonstrated in several studies against both Gram-positive and Gram-negative bacteria. FeONPs kill microbes by damaging their cell walls, binding to key enzymes, inhibiting DNA replication, and forming vacuoles inside cells (Gudkov *et al.* 2021). Plant-assisted synthesis of FeONPs is the best green approach. It is rapid, produces large quantities, and results in more stable particles. A one-step process involving constant stirring and heat can produce FeONPs from plant extract (Saif *et al.* 2016).

The current study enabled a comparative evaluation of the antifungal efficacy of CuO and FeO NPs that were biosynthesized using the same plant extract. Additionally, both nanoparticles selectively target commercially and agriculturally relevant fungal diseases, which makes the results easily adaptable to plant protection strategies. Therefore, the main objective of this study was to synthesize CuO and FeO NPs using *C. lancifolius* leaf extract and to investigate their activity against two significant phytopathogen. To the extent of the authors' knowledge, no research has been done on the synthesis of CuO and FeO nanoparticles using *C. lancifolius* or how they affect the growth and hyphae of phytopathogenic fungi such as *A. solani* and *F. solani*.

## EXPERIMENTAL

### Plant Material

Samples of *C. lancifolius* plant leaves were collected from Uhud Mountain area in Madinah region, Saudi Arabia. The leaves were harvested into sterile bags, labeled, and transported to the laboratory for further processing within 24 h. A plant taxonomist at Taibah University authenticated the collected plants.

#### *Preparation of plant extract*

Freshly collected leaves of the plant were rinsed with distilled water, dried, and chopped into smaller pieces. Plant extract was prepared by mixing the plant material with distilled water in a ratio of 1:20 into an Erlenmeyer flask using 40 g of plant material for 800 mL of distilled water. After 20 min in an 80 °C water bath, the mixture was allowed to cool for 40 min and then filtered using 15 cm-Whatman's No. 1 filter paper (Amer and Awwad 2020). The filtrate was stored at 4 °C for subsequent processing and utilized as a crude source for CuSO<sub>4</sub> and FeCl<sub>3</sub> reduction.

### Green Synthesis of Nanoparticles

CuONPs and FeONPs were prepared by separately adding 500 mL of *C. lancifolius* leaves extract to 100 mL of 0.1 M CuSO<sub>4</sub> and 0.1 M FeCl<sub>3</sub> solutions in a ratio of 5:1 into an Erlenmeyer flask. The mixtures were magnetically stirred on a hot plate at 70 °C for 1 h, then left to cool down. A color change was visually observed, indicating the formation of nanoparticles. CuSO<sub>4</sub> and FeCl<sub>3</sub> solutions without addition of plant extract were used as controls and incubated along with experimental samples. The formed nanoparticles were harvested and transferred into 1.5 mL Eppendorf tubes, purified by three successive centrifugations at 14000 rpm for 5 min, and washed with ethanol and then distilled water.

The nanoparticles were left to dry at room temperature for 48 h, then stored in an amber bottle for subsequent analyses (Amer and Awwad 2020; Kumar *et al.* 2020).

### Characterization of Nanoparticles

A distinct visual color change served as preliminary evidence of formation of nanoparticles (NPs). UV-visible spectroscopy for observing the surface plasmon resonance (SPR) absorbance peak of the synthesized NPs was recorded using a UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) in a wavelength range of 200 to 900 nm at room temperature.

Nanoparticles were subjected to transmission electron microscopy (TEM) for size and morphological analysis. A volume of 5 mL of nanoparticles (NPs) was dispersed in 70% ethanol and ultrasonicated for 10 minutes to ensure uniform dispersion. A drop of the well-dispersed suspension was then placed onto a formvar/carbon-coated copper grid (200 mesh). The formvar film provides a thin, electron-transparent support for the nanoparticles, as electrons cannot pass through the copper or the carbon-coated copper portion of the grid. The grid was left to air-dry overnight at room temperature to allow solvent evaporation and proper adherence of the nanoparticles to the formvar film surface. After complete drying, the grid was mounted on the TEM sample holder and examined using a transmission electron microscope (Thermo Fisher Scientific, Waltham, MA, USA) operated at an accelerating voltage of 200 kV. Images were acquired at different magnifications to evaluate particle size distribution and morphological characteristics.

### Isolation and Identification of Phyto-Pathogenic Fungi

Diseased tomato (*Solanum lycopersicum*) fruits with black spots and potato tubers (*Solanum tuberosum*) with wrinkles and dark lesions on the crust were collected from a local market in Madinah, Saudi Arabia. After surface sterilization with 70% ethyl alcohol, the symptomatic tissues were cut using a scalp and then dipped in 10% bleach for 30 s to ensure that the growing fungi were from the internal tissue. After that, tissues were rinsed with sterilized water and then inoculated on a Potato Dextrose Agar (PDA) medium. The plates were incubated at 25 °C for seven days. The developed colonies were purified on the PDA medium. Then, the fungal isolates were purified and morphologically identified up to the species level *via* microscopic examination (Moubasher 1995; Zemankoa and Lebeda 2001). The fungal phytopathogenicity was confirmed using the four steps of Koch postulates (Popp *et al.* 2019).

### Antifungal Activity of Synthesized Nanoparticles

Fresh seven-day-old cultures on the SDA medium of the two pathogenic isolated fungi, *A. solani* and *F. solani*, were used for this test. PDA medium supplemented with CuONPs or FeONPs at a concentration of 5 mmol/L, and a NP-free medium were separately poured into the 9-cm Petri dishes. Plates with PDA medium without any NPs were used as a control. After the medium was solidified, the fungi were inoculated by taking a 6-mm-disk of seven-day-old fungal culture and placing the inoculum at the center of each plate. The plates were then incubated at 25 °C for 12 days. To determine the antifungal activity of the NPs, the diameter of the fungal colony was measured at time intervals of 2, 4, 6, 8, and 12 days.



## Effect of Nanoparticles on the Fungal Hyphae and Conidia

A Scanning electron microscope (SEM) was used for the examination of the morphological change in *A. solani* and *F. solani* treated with CuONPs and FeONPs compared to the control. After the eight-day incubation period, pieces of mycelium were cut from the edge of the fungal culture and directly examined under the SEM.

## Statistical Analysis

All experiments were performed in triplicate. Minitab 21.1.0 was used to statistically analyze the results using ANOVA tests and a Tukey pairwise comparison at significance  $p < 0.05$ . The data was expressed in terms of the mean  $\pm$  SD.

## RESULTS AND DISCUSSION

### Characterization of Copper Oxide and Iron Oxide Nanoparticles

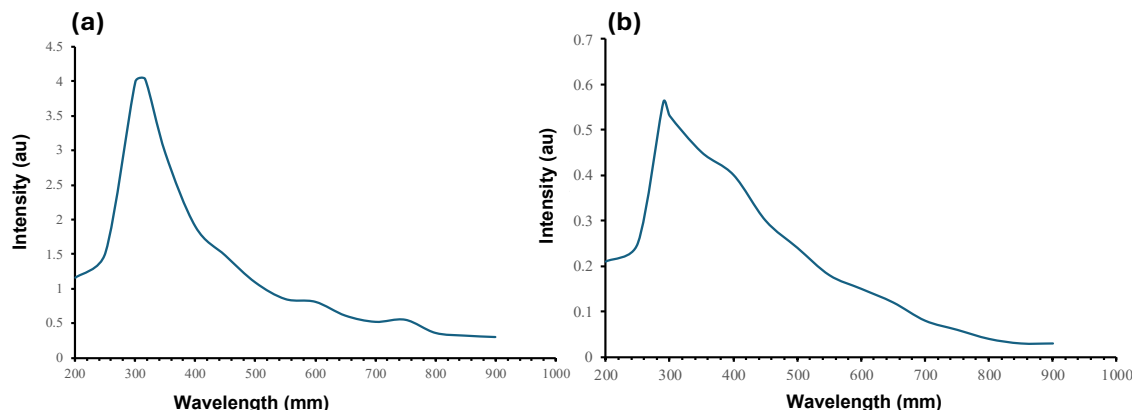
The color changes from light blue to brown with precipitation within 10 min indicated the CuONPs formation. While the immediate color change after adding  $\text{FeCl}_3$  from pale yellow to black indicated the FeONPs formation. In the present study, CuO and FeO NPs were successfully prepared using *C. lancifolius* leaf extract. The color change of the reaction solution with precipitation indicates the formation of nanoparticles (Vishveshvar *et al.* 2018; Amer and Awwad 2020).

A possible mechanism of nanoparticle formation involved the bioreduction of metal ions and subsequent oxidation to form metal oxide nanoparticles by phytochemicals present in *C. lancifolius* extract. Alkaloids, phenols, flavonoids, and saponins are examples of phytochemicals found in plant extracts that act as both reducing and stabilizing agents, leading to the observed color change during synthesis. These biomolecules facilitate the initial reduction of  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  ions to lower oxidation states, which are subsequently oxidized to form stable copper and iron oxides ( $\text{CuO}$  and  $\text{Fe}_2\text{O}_3$ ) (Mobarak *et al.* 2025). This differs from the mechanism reported by Benassai *et al.* (2021), who described the complete reduction of metal ions to their metallic ( $\text{Cu}^0$  and  $\text{Fe}^0$ ) form. In that study, to obtain the metallic nanoparticles, the amounts of  $\text{CuSO}_4$  and  $\text{FeCl}_3$  precursor solutions to plant extract were kept at a ratio of 1:5.

Small concentrations and ratios have been shown to be effective in obtaining small-sized nanoparticles in this study and several other studies (Bouafia *et al.* 2021; Mani *et al.* 2021). The types of phytochemical compounds included in plant extracts, as well as the volume of extract used, influence the synthesis of nanoparticles and have a significant impact on their morphological characteristics and biological activities, according to Mohamed *et al.* (2023).

Different plants can be used for the green synthesis of metal nanoparticles, including *C. lancifolius*, which was used by Raheema and Shoker (2020) in the fabrication of silver nanoparticles. This study is unique because of the use of *C. lancifolius* leaf extract to green-synthesize CuONPs and FeONPs. This preparation was achieved using an easy, economic, and eco-friendly method.

The UV-Vis spectra of CuONPs and FeONPs are presented in Fig. 1. The absorbance peaks of both CuONPs and FeONPs indicated that CuONPs and FeONPs were both successfully synthesized.



**Fig. 1.** UV-Vis absorption spectra of CuONPs (a) and FeONPs (b)

In the UV-Vis spectrophotometer, CuONPs and FeONPs showed characteristic maximum absorption peaks at 316 nm and 290 nm, respectively (Fig 1a and 1b. FeONP's characteristic wavelengths range between 300 nm and 325 nm (Prakash *et al.* 2022). FeONPs characteristic wavelength ranges between 250 nm and 300 nm (Saranya *et al.* 2017). Researchers have previously reported similar shape and size of nanoparticles for both CuONPs (Yugandhar *et al.* 2018; Shammout and Awwad 2021) and FeONPs (Ahmad *et al.* 2020).

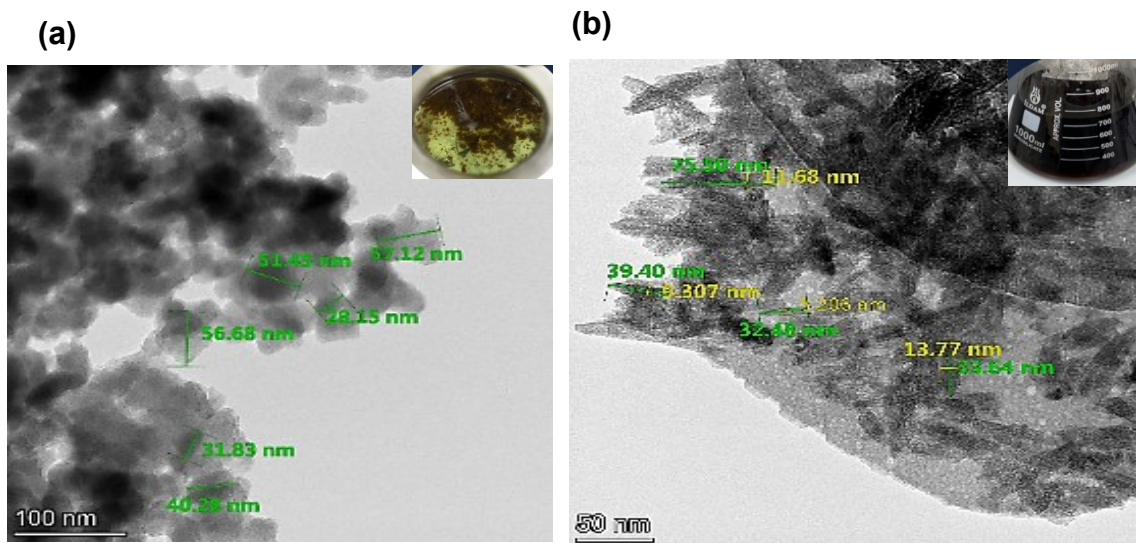
### TEM Analysis of Copper Oxide and Iron Oxide Nanoparticles

The morphology and particle size of nanoparticles were analyzed using TEM instrument, after dispersing samples in ethanol and 45 min of ultrasonication. In the study by Mali *et al.* (2019), CuONPs were green synthesized using leaf extract of *Enicostemma axillare*. The average particle size was 30 nm. The size of synthesized CuONPs in this study (oval particles with a size distribution of ~28.15 to 57.12 nm) differed from the nanoparticles isolated by Naika *et al.* (2015) (Fig. 2a). Their particles were spherical in shape, and the size was found to be in the range of 5 to 10 nm.

Many studies have reported a wide range of sizes with different shapes, *i.e.*, CuONPs produced using *Punica granatum* peel extract and Indian medicinal plant *Tabernaemontana divaricate* leaf extract had an average size of 40 nm and  $48 \pm 4$  nm, respectively (Sivaraj *et al.* 2014; Ghidan *et al.* 2016). Narayanan *et al.* (2022) isolated spherical CuONPs with sizes ranging from 61 to 69 nm using *Thespesia populnea* aqueous bark extract. *Carica papaya* leaf extract was used to make larger copper oxide nanoparticles. These particles had a rod shape and were about 140 nm in size (Sankar *et al.* 2014). This variation in nanoparticle size and form can be attributed to the copper salt utilized in the synthesis of copper nanoparticles.

It has been reported that the copper salt significantly affects the morphology of copper nanoparticles; for example, when copper sulfate was used, the shape was spherical, and when copper acetate was used, the form was rod-shaped. Additionally, increasing the salt concentration results in increased NPs size (Sankar *et al.* 2014).

FeONPs showed elliptical disks with a wider particle size distribution (vertical radius 32.5 to 39.4 nm in length and 5.2 to 13.8 nm in width) and horizontal radius (Fig. 2b).



**Fig. 2.** TEM micrograph of CuONPs (a) and FeONPs (b)

A few papers have discussed the green synthesis of FeONPs using plant extracts. For instance, Patra and Baek (2017) described the synthesis of magnetite iron oxide using aqueous extracts of food processing waste, with an estimated crystallite size of 48.9 nm.

### Antifungal Activity of Nanoparticles

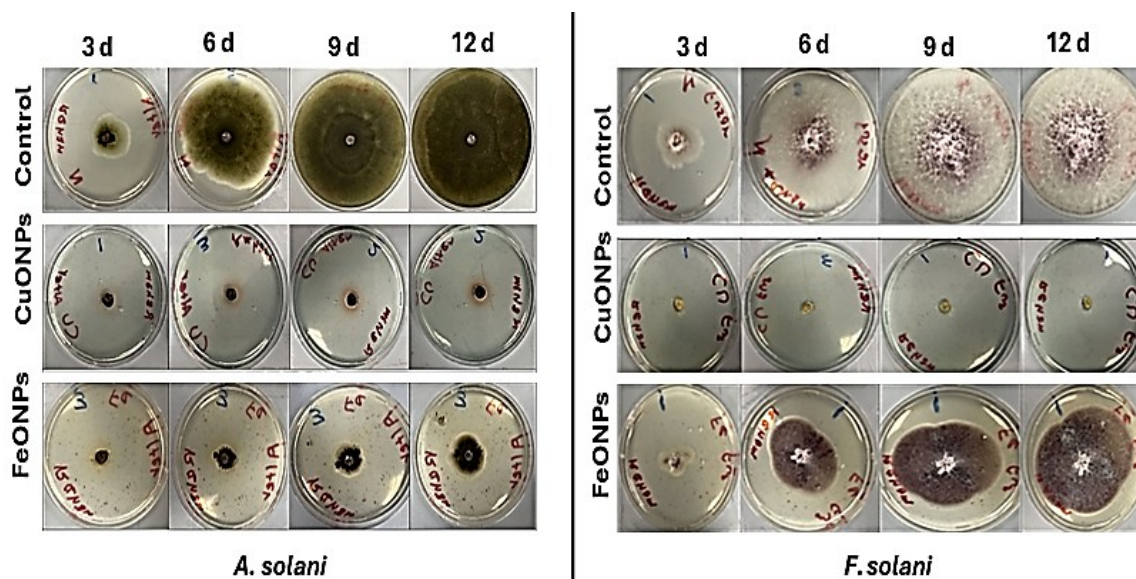
Both CuONPs and FeONPs showed significant ( $p < 0.05$ ) antifungal activity against *A. solani* and *F. solani* (Table 1; Fig. 3). CuONPs limited *A. solani* growth by 88.9% to 91.1% in terms of fungal colony growth diameter after incubation for 12 days and completely inhibited *F. solani* growth. Whereas FeONPs limited *A. solani* growth by 68.9% to 73.3% and *F. solani* growth by 23.3% to 26.7% in terms of fungal colony growth diameter after incubation for 12 days. CuONPs were significantly ( $p < 0.05$ ) more effective against both *A. solani* and *F. solani* than FeONPs (Table 1).

Growth started to occur after four days for *A. solani* using CuONPs and six days using FeONPs compared to the growth of the control, which started after two days of incubation. The growth rate was slower than in the control, leading to a larger diameter in the control after 10 days. By contrast, the growth of *F. solani* was completely inhibited by CuONPs and slowed by FeONPs. It was also noticed after FeONPs treatments that the *F. solani* colony's color changed from white to violet. In addition, FeONPs treatments visibly affected the hyphae's density in the colonies (Fig.3).

Phytopathogenic fungi causes highly significant economic losses to important commercial crops (O'Brien 2017; Poveda *et al.* 2020; Singh and Singh 2018). They are controlled using fungicides, but the emergence of fungicide-resistant fungi caused failure of the phytopathogenic fungi's control (Ishii and Holloman 2015; De Miccolis Angelini *et al.* 2015). Numerous studies have shown that CuONPs strongly hinder the mycelium growth of *A. solani* (El-Batal *et al.* 2020) and *F. solani* (Mohamed *et al.* 2023). This study added to those findings. In contrast to the current study, it has been reported that FeONPs have no inhibitory effect against *A. solani* (Vera-Reyes *et al.* 2019). This could be due to the much larger size of FeONPs than the size reported in this study, in addition to the higher agglomeration rate of FeONPs reported in their study.



The observed reduction in the growth of *A. solani* in the range from 68.9% to 73.3% upon treatment with FeONPs can be attributed to the nanoparticles' antifungal properties, which are likely to act through multiple mechanisms. FeONPs are known to generate reactive oxygen species (ROS), which can disrupt cellular structures and damage vital biomolecules in fungal cells, including lipids, proteins, and DNA. This oxidative stress compromises cell membrane integrity and impairs metabolic functions, ultimately inhibiting fungal growth. Additionally, FeONPs may interact directly with the fungal cell wall and membrane, leading to structural damage, increased permeability, and leakage of cellular contents. These effects contribute to the reduction in mycelial growth (Gudkov *et al.* 2021). In agreement with this study, Muhammad *et al.* (2019) also showed that FeONPs had a positive inhibitory effect against the growth of *F. solani*. Koka *et al.* (2019) reported a positive inhibitory effect of FeONPs against *A. alternata*. Moreover, CuONPs in this study completely inhibited the growth of *F. solani* while limiting the growth of *A. solani* by 88.9% to 91.1%. This difference in inhibitory activity may result from how each fungus protects itself from CuO NPs. Another reason for this variation is the possibility that there are different mechanisms of CuONPs against the two different fungus species.



**Fig. 3.** Biological Activity of green synthesized CuONPs and FeONPs against *A. solani* and *F. solani* along 12 days of incubation at 30 °C

**Table 1.** Antifungal Activity of Green Synthesized Nanoparticles on *A. solani* and *F. solani*

Fungus	Fungal Diameter (mm) Mean $\pm$ SD					
	2 Days	4 Days	6 Days	8 Days	10 Days	12 Days
<i>A. solani</i> + CuONPs	0	8.3 $\pm$ 0.5	8.3 $\pm$ 0.5	8.6 $\pm$ 1.1	8.6 $\pm$ 1.1	9 $\pm$ 1.0
<i>A. solani</i> + FeONPs	0	0	1.4 $\pm$ 2.5	16.3 $\pm$ 0.5	19.6 $\pm$ 2.0	26.6 $\pm$ 2.3
<i>A. solani</i> control	17.3 $\pm$ 1.5	42.6 $\pm$ 8.3	75.6 $\pm$ 5.1	86.6 $\pm$ 5.7	90 $\pm$ 0.0	90 $\pm$ 0.0
<i>F. solani</i> + CuONPs	0	0	0	0	0	0
<i>F. solani</i> + FeONPs	15.3 $\pm$ 0.5	31 $\pm$ 5.2	49 $\pm$ 1	53.6 $\pm$ 2.3	60.3 $\pm$ 2.8	67 $\pm$ 1.7
<i>F. solani</i> control	19.6 $\pm$ 1.5	44.6 $\pm$ 3	70.3 $\pm$ 1.5	83.3 $\pm$ 5.7	90 $\pm$ 0	90 $\pm$ 0

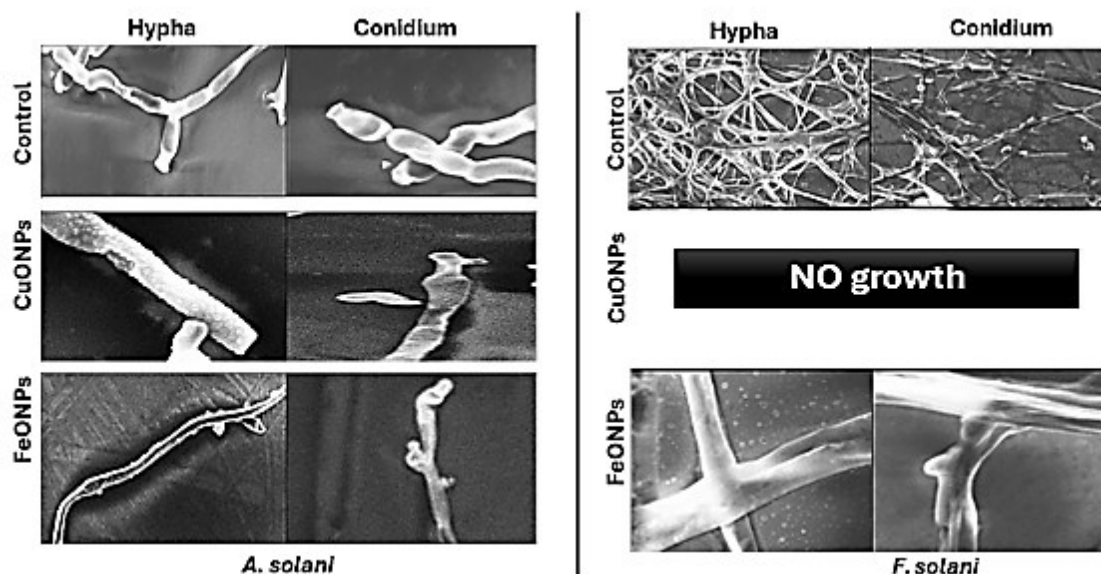
The values represent the mean  $\pm$  SD of triplicate experiments (n = 3, p < 0.05)

### Effect of Nanoparticles on the Fungal Hyphae and Conidia

An SEM analysis was performed to investigate the effect of CuONPs and FeONPs on *A. solani* and *F. solani*. The *A. solani* hyphae in the control group had a smooth surface with an intact wall and a regular-shaped apical conidium (ovoid) (Fig. 4). After the treatment, CuONPs *A. solani* hyphae showed pores and cavities on the surface and a deformed apical conidium. After the treatment of *A. solani* with FeONPs, the hyphae lost their smoothness, with damaged walls with many fractures and a deformed apical conidium. The *F. solani* hyphae in the control group exhibited a smooth, cylinder-shaped, and uniform structure (Fig. 4). In contrast, the FeONPs-treated hyphae exhibited a bulge and ruptured surface. These suggest that both CuONPs and FeONPs damaged and distorted the fungal structure, consequently limiting and inhibiting the fungal growth.

SEM analysis showed that both CuONPs and FeONPs caused mycelium structural deformation and surface wounds to the fungus. That explains the visible effect of FeONPs on the hypha density. The cytotoxicity of ZnO and CuO nanoparticles (NPs) on fungal cells was primarily attributed to their strong antifungal and oxidative properties, which disrupt essential cellular functions (El Sayed and El-Sayed 2020). Both types of NPs may be adhered to fungal cell walls and penetrate through the membrane, where they release  $Zn^{2+}$  or  $Cu^{2+}$  ions and generate reactive oxygen species (ROS); these steps can lead to oxidative stress, lipid peroxidation, and membrane damage (Hasheminya et al. 2018). This results in cytoplasmic leakage, enzyme inactivation, and disruption of mitochondrial activity, ultimately causing cell death (Pariona et al. 2019; El Sayed and El-Sayed 2020). ZnO NPs can also interfere with spore germination, hyphal elongation, and cell wall synthesis, while CuO NPs, due to their higher redox activity, often exhibited stronger antifungal toxicity by inducing greater ROS levels and damaging nucleic acids and proteins (Siddiqi and Husen 2020). The degree of cytotoxicity varies depending on nanoparticle concentration, size, surface charge, and fungal species (Gudkov et al. 2021).

Other studies have reported similar effects of metallic nanoparticles against several fungi (Pariona et al. 2019; El Sayed and El-Sayed 2020).



**Fig. 4.** SEM images showing the effect green synthesized CuONPs and FeONPs on the hyphae and conidia of *A. solani* and *F. solani*

## CONCLUSIONS

1. This study applied a fast and eco-friendly CuONPs and FeONPs that were successfully synthesized using *C. lancifolius* leaf extract. To the best of the authors' knowledge, no earlier research has considered the synthesis of CuO and FeO nanoparticles from *C. lancifolius*, nor their impact on the growth and appearance of phytopathogenic fungi.
2. These green-synthesized nanoparticles showed extraordinary inhibitory effects against the phytopathogenic fungi *A. solani* and *F. solani*. CuONPs and FeONPs can significantly improve crop yield by protecting plants from phytopathogenic fungi.
3. The application of these particles as agricultural fungicides could be an efficient alternative to regular chemical fungicides. However, further studies are needed to investigate the possibility and safety level of using different concentrations of CuONPs and FeONPs against fungi, along with the evaluation of the efficiency of these nanoparticles in fields.

## ACKNOWLEDGMENTS

The authors would like to thank Ms Mai Aldeeb; a Microbiology lab technician, for her helping in plant preparation and fungal isolation.

## REFERENCES CITED

- Ahmad, W., Khan, A. U., Shams, S., Qin, L., Yuan, Q., Ahmad, A., Wei, Y., Khan, Z. U. H., Ullah, S., and Rahman, A. U. (2020). "Eco-benign approach to synthesize spherical iron oxide nanoparticles: A new insight in photocatalytic and biomedical applications," *J. Photochem. Photobiol.- B* 205, article 111821. DOI: 10.1016/j.jphotobiol.2020.111821
- Ali, M. A., Ahmed, T., Wu, W., Hossain, A., Hafeez, R., Islam Masum, M. M., Wang, Y., An, Q., Sun, G., and Li, B. (2020). "Advancements in plant and microbe-based synthesis of metallic nanoparticles and their antimicrobial activity against plant pathogens," *Nanomaterials (Basel)* 10(6), article 1146. DOI: 10.3390/nano10061146
- Amaro, F., Morón, Á., Díaz, S., Martín-González, A., and Gutiérrez, J. C. (2021). "Metallic nanoparticles-friends or foes in the battle against antibiotic-resistant bacteria?," *Microorganisms* 9(2), article 364. DOI: 10.3390/microorganisms9020364
- Amer, M., and Awwad, A. (2020). "Green synthesis of copper nanoparticles by Citrus limon fruits extract, characterization and antibacterial activity," *Chemistry International* 7(1), 1-8. DOI: 10.5281/zenodo.4017993
- Benassai, E., Del Bubba, M., Ancillotti, C., Colzi, I., Gonnelli, C., Calisi, N., Salvatici, M. C., Casalone, E., and Ristori, S. (2021). "Green and cost-effective synthesis of copper nanoparticles by extracts of non-edible and waste plant materials from *Vaccinium* species: Characterization and antimicrobial activity," *Mat. Sci. Eng.- C* 119, Article 111453. DOI: 10.1016/j.msec.2020.111453
- Bouafia, A., Laouini, S. E., Khelef, A., Tedjani, M. L., and Guemari, F. (2021). "Effect of ferric chloride concentration on the type of magnetite (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles biosynthesized by aqueous leaves extract of *Artemisia* and assessment of their

- antioxidant activities,” *Journal of Cluster Science* 32(4), 1033-1041. DOI: 10.1007/s10876-020-01868-7
- De Miccolis Angelini, R. M., Pollastro, S., and Faretra, F. (2015). “Genetics of fungicide resistance,” *Fungicide Resistance in Plant Pathogens: Principles and a Guide to Practical Management*, Springer, Tokyo, Japan, pp. 13-34. DOI: 10.1007/978-4-431-55642-8\_2
- El Sayed, M. T., and El-Sayed, A. S. A. (2020). “Bioremediation and tolerance of zinc ions using *Fusarium solani*,” *Heliyon* 6(9), article e05048. DOI: 10.1016/j.heliyon.2020.e05048 PMID:33024860
- El-Batal, A. I., El-Sayyad, G. S., Mosallam, F. M., and Fathy R. M. (2020). “*Penicillium chrysogenum*-mediated mycogenic synthesis of copper oxide nanoparticles using gamma rays for *in vitro* antimicrobial activity against some plant pathogens,” *Journal of Cluster Science* 31(1), 79-90. DOI: 10.1007/s10876-019-01619-3
- Fisher, M. C., Hawkins, N. J., Sanglard, D., and Gurr, S. J. (2018). “Worldwide emergence of resistance to antifungal drugs challenges human health and food security,” *Science* 360(6390), 739-742. DOI: 10.1126/science.aap7999 PMID:29773744
- Ghidan, A. Y., Al-Antary T. M., and Awwad, A. M. (2016). “Green synthesis of copper oxide nanoparticles using *Punica granatum* peels extract: Effect on green peach Aphid,” *Environmental Nanotechnology, Monitoring & Management* 6, 95-98. DOI: 10.1016/j.enmm.2016.08.002
- Gudkov, S. V., Burmistrov, D. E., Serov, D. A., Rebezov, M. B., Semenova, A. A., and Lisitsyn, A. B. (2021). “Do iron oxide nanoparticles have significant antibacterial properties?,” *Antibiotics* (Basel) 10(7), article 884. DOI: 10.3390/antibiotics10070884
- Hasheminya, S.-M., Rezaei Mokarram, R., Ghanbarzadeh, B., Hamishekar, H., and Kafil, H. S. (2018). “Physicochemical, mechanical, optical, microstructural and antimicrobial properties of novel kefiran-carboxymethyl cellulose biocomposite films as influenced by copper oxide nanoparticles (CuONPs),” *Food Packaging and Shelf Life* 17, 196-204. DOI: 10.1016/j.fpsl.2018.07.003
- Helmy, Y. A., Taha-Abdelaziz, K., Hawwas, H. A. E.-H., Ghosh, S., AlKafaas, S. S., Moawad, M. M. M., Saied, E. M., Kassem, I. I., and Mawad, A. M. M. (2023). “Antimicrobial resistance and recent alternatives to antibiotics for the control of bacterial pathogens with an emphasis on foodborne pathogens,” *Antibiotics* 12(2), article 274. DOI: 10.3390/antibiotics12020274
- Ishii, H., and Holloman, D. (2015). *Fungicide Resistance in Plant Pathogens*, Springer, Tokyo, Japan. DOI: 10.1007/978-4-431-55642-8
- Jindo, K., Evenhuis, A., Kempenaar, C., Pombo Sudré, C., Zhan, X., Goitom Teklu M., and Kessel, G. (2021). “Review: Holistic pest management against early blight disease towards sustainable agriculture,” *Pest Management Science* 77(9), 3871-3880. DOI: 10.1002/ps.6320
- Khatami, M., Varma, R. S., Heydari, M., Peydayesh, M., Sedighi, A., Agha Askari, H., Rohani, M., Baniasadi, M., Arkia, S., and Seyedi, F. (2019). “Copper oxide nanoparticles greener synthesis using tea and its antifungal efficiency on *Fusarium solani*,” *Geomicrobiology Journal* 36(9), 777-781. DOI: 10.1080/01490451.2019.1621963
- Koka, J., Wani, A., and Bhat, M. (2019). “Evaluation of antifungal activity of Magnesium oxide (MgO) and Iron oxide (FeO) nanoparticles on rot causing fungi,”



- Journal of Drug Delivery & Therapeutics* 9(2), 173-178. DOI: 10.22270/jddt.v9i2-s.2479
- Kumar, B., Smita, K., Galeas, S., Sharma, V., Guerrero, V. H., Debut A., and Cumbal, L. (2020). "Characterization and application of biosynthesized iron oxide nanoparticles using *Citrus paradisi* peel: A sustainable approach," *Inorganic Chemistry Communications* 119, article 108116. DOI: 10.1016/j.inoche.2020.108116
- Lal, D., Dev, D., Kumari, S., Pandey, S., Aparna, N., Sharma, S., Nandni, R., Jha, K., and Singh, A. (2024). "*Fusarium* wilt pandemic: current understanding and molecular perspectives," *Functional & Integrative Genomics* 24(2), article 41. DOI: 10.1007/s10142-024-01319-w
- Mali, S. C., Raj, S., and Trivedi, R. (2019). "Biosynthesis of copper oxide nanoparticles using *Enicostemma axillare* (Lam.) leaf extract," *Biochemistry and Biophysics Reports* 20, article 100699. DOI: 10.1016/j.bbrep.2019.100699
- Mani, V. M., Kalaivani, S., Sabarathinam, S., Vasuki, M., Soundari, A. J. P. G., Ayyappa Das, M. P., Elfakhany, A., and Pugazhendhi, A. (2021). "Copper oxide nanoparticles synthesized from an endophytic fungus *Aspergillus terreus*: Bioactivity and anti-cancer evaluations," *Environmental Research* 201, article 111502. DOI: 10.1016/j.envres.2021.111502
- Mobarak, M. B., Sikder, M. F., Muntaha, K. S., Islam, S., Rabbi, S. F., and Chowdhury, F. (2025). "Plant extract-mediated green-synthesized CuO nanoparticles for environmental and microbial remediation: A review covering basic understandings to mechanistic study," *Nanoscale Advances* 7(9), 2418-2445.
- Mohamed, A., Atta, R. R., Kotp, A. A., Abo El-Ela, F. I., Abd El-Raheem, H., Farghali, A., Alkhalifah, D. H. M., Hozzein, W. N., and Mahmoud, R. (2023). "Green synthesis and characterization of iron oxide nanoparticles for the removal of heavy metals ( $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$ ) from aqueous solutions with antimicrobial investigation," *Scientific Reports* 13(1), article 7227. DOI: 10.1038/s41598-023-31704-7
- Moubasher (1995). "Soil fungi are an active partner of our ecosystem. Their biodiversity and activities should be appreciated," *Qatar University Science Journal* 15(1), 239-247.
- Muhammad, W., Khan, M. A., Nazir, M., Siddiquah, A., Mushtaq, S., Hashmi, S. S., and Abbasi, B. H. (2019). "*Papaver somniferum* L. mediated novel bioinspired lead oxide ( $\text{PbO}$ ) and iron oxide ( $\text{Fe}_2\text{O}_3$ ) nanoparticles: *In-vitro* biological applications, biocompatibility and their potential towards HepG2 cell line," *Materials Science & Engineering C: Materials for Biological Applications* 103, article 109740. DOI: 10.1016/j.msec.2019.109740
- Mukherjee, A., Sarkar, D., and Sasmal, S. (2021), "A review of green synthesis of metal nanoparticles using algae," *Frontiers in Microbiology* 12, article 693899. DOI: 10.3389/fmicb.2021.693899
- Naika, H. R., Lingaraju, K., Manjunath, K., Kumar, D., Nagaraju, G., Suresh, D., and Nagabhushana, H. (2015). "Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity," *Journal of Taibah University Science* 9(1), 7-12. DOI: 10.1016/j.jtusci.2014.04.006
- Narayanan, M., Hussain, F. A. J., Srinivasan, B., Sambantham, M. T., Al-Keridis, L. A., and Al-mekhlafi, F. A. (2022). "Green synthesizes and characterization of copper-oxide nanoparticles by *Thespesia populnea* against skin-infection causing microbes," *Journal of King Saud University - Science* 34(3), article 101885. DOI: 10.1016/j.jksus.2022.101885



- Naz, S., Gul, A., and Zia, M. (2020). "Toxicity of copper oxide nanoparticles: A review study," *IET Nanobiotechnology* 14(1), 1-13. DOI: 10.1049/iet-nbt.2019.0176
- O'Brien, P. A. (2017). "Biological control of plant diseases," *Australian Plant Pathology* 46, 293-304. DOI: 10.1007/s13313-017-0481-4
- O'Donnell, K., Al-Hatmi, A. M., Aoki, T., Brankovics, B., Cano-Lira, J. F., Coleman, J. J., De Hoog, G. S., Di Pietro, A., Frandsen, R. J., and Geiser, D. M. (2020). "No to *Neocosmospora*: Phylogenomic and practical reasons for continued inclusion of the *Fusarium solani* species complex in the genus *Fusarium*," *MSphere* 5(5), 00810-00820. DOI: 10.1128/mSphere.00810-00820
- Pan, C., Yang, K., Erhunmwunsee, F., Li, Y.-X., Liu, M., Pan, S., Yang, D., Lu, G., Ma, D., and Tian, J. (2023). "Inhibitory effect of cinnamaldehyde on *Fusarium solani* and its application in postharvest preservation of sweet potato," *Food Chemistry* 408, article 135213. DOI: 10.1016/j.foodchem.2022.135213
- Pariona, N., Mtz-Enriquez, A. I., Sánchez-Rangel, D., Carrión, G., Paraguay-Delgado, F., and Rosas-Saito, G. (2019). "Green-synthesized copper nanoparticles as a potential antifungal against plant pathogens," *RSC Advances* 9(33), 18835-18843. DOI: 10.1039/C9RA03110C PMID:35516870
- Patra, J. K., and Baek, K. H. (2017). "Green biosynthesis of magnetic iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles using the aqueous extracts of food processing wastes under photo-catalyzed condition and investigation of their antimicrobial and antioxidant activity," *Journal of Photochemistry and Photobiology B: Biology* 173, 291-300. DOI: 10.1016/j.jphotobiol.2017.05.045
- Popp, C., Grunewaldt-Stöcker, G., and Maiss, E. (2019). "A soil-free method for assessing pathogenicity of fungal isolates from apple roots," *Journal of Plant Diseases and Protection* 126, 329-341. DOI: 10.1007/s41348-019-00236-6
- Poveda, J., Eugui, D., and Velasco, P. (2020). "Natural control of plant pathogens through glucosinolates: An effective strategy against fungi and oomycetes," *Phytochemistry Reviews* 19(4), 1045-1059. DOI: 10.1007/s11101-020-09699-0
- Prakash, J., Shekhar, H., Yadav, S. R., Sonkar, P. K., and Kumar, N. (2022). "Synthesis and characterization of plant derived copper oxide nanoparticles and their application towards oxygen reduction reaction," *ChemistrySelect* 7(1), article e202103594. DOI: 10.1002/slct.202103594
- Predoi, S. A., Iconaru, S. L., and Predoi, D. (2023). "In vitro and in vivo biological assays of dextran coated iron oxide aqueous magnetic fluids," *Pharmaceutics* 15(1), article 177. DOI: 10.3390/pharmaceutics15010177
- Raheema, R. H., and Shoker, R. M. (2020). "Phytochemicals screening and antibacterial activity of silver nanoparticles, phenols and alkaloids extracts of *Conocarpus lancifolius*," *EurAsian Journal of BioSciences* 14(2), 4829-4835.
- Rasheed, F., Zafar, Z., Waseem, Z. A., Rafay, M., Abdullah, M., Salam, M. M. A., Mohsin, M., and Khan, W. R. (2020). "Phytoaccumulation of Zn, Pb, and Cd in *Conocarpus lancifolius* irrigated with wastewater: Does physiological response influence heavy metal uptake?," *International Journal of Phytoremediation* 22(3), 287-294. DOI: 10.1080/15226514.2019.1658711
- Redha, A., Al-Hasan, R., and Afzal, M. (2021). "Synergistic and concentration-dependent toxicity of multiple heavy metals compared with single heavy metals in *Conocarpus lancifolius*," *Environmental Science and Pollution Research* 28(18), 23258-23272. DOI: 10.1007/s11356-020-12271-0

- Román, L. E., Gomez, E. D., Solís, J. L., and Gómez, M. M. (2020). "Antibacterial cotton fabric functionalized with copper oxide nanoparticles," *Molecules* 25(24), article 5802. DOI: 10.3390/molecules25245802
- Saadullah, M., Fakhar-e-Alam, M., Atif, M., Asif, M., Irshad, K., and Ali, Z. (2024). "Biological and *in silico* investigation of isolated novel bioactive compound from *Conocarpus lancifolius*," *Journal of King Saud University Science* 36(4), article 103121. DOI: 10.1016/j.jksus.2024.103121
- Saif, S., Tahir, A., and Chen, Y. (2016). "Green synthesis of iron nanoparticles and their environmental applications and implications," *Nanomaterials* (Basel) 6(11), article 209. DOI: 10.3390/nano6110209
- Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A. L., Galindo, R., Cano, A., Espina, M., Ettcheto, M., Camins, A., *et al.* (2020). "Metal-based nanoparticles as antimicrobial agents: An overview," *Nanomaterials* (Basel) 10(2), article 292. DOI: 10.3390/nano10020292
- Sankar, R., Manikandan, P., Malarvizhi, V., Fathima, T., Shivashangari, K. S., and Ravikumar, V. (2014). "Green synthesis of colloidal copper oxide nanoparticles using *Carica papaya* and its application in photocatalytic dye degradation," *Spectrochim Acta A Mol. Biomol. Spectrosc.* 121, 746-750. DOI: 10.1016/j.saa.2013.12.020
- Saranya, S., Vijayarani, K., and Pavithra, S. (2017). "Green synthesis of iron nanoparticles using aqueous extract of *Musa ornata* flower sheath against pathogenic bacteria," *Indian Journal of Pharmaceutical Sciences* 79(5), article 1000280. DOI: 10.4172/pharmaceutical-sciences.1000280
- Schmey, T., Tominello-Ramirez, C. S., Brune, C., and Stam, R. (2024). "*Alternaria* diseases on potato and tomato," *Molecular Plant Pathology* 25(3), article e13435. DOI: 10.1111/mpp.13435
- Shammout, M., and Awwad, A. (2021). "A novel route for the synthesis of copper oxide nanoparticles using *Bougainvillea* plant flowers extract and antifungal activity evaluation," *Chemistry International* 7(1), 71-78.
- Siddiqi, K. S., and Husen, A. (2020). "Current status of plant metabolite-based fabrication of copper/copper oxide nanoparticles and their applications: A review," *Biomaterials Research* 24, article 11. DOI: 10.1186/s40824-020-00188-1
- Singh, A., and Singh, I. K. (2018). *Molecular Aspects of Plant-Pathogen Interaction*, Vol. 10, Springer, Singapore, pp. 978-981. DOI: 10.1007/978-981-10-7371-7
- Sivaraj, R., Rahman, P. K., Rajiv, P., Salam, H. A., and Venckatesh, R. (2014). "Biogenic copper oxide nanoparticles synthesis using *Tabernaemontana divaricate* leaf extract and its antibacterial activity against urinary tract pathogen," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 133, 178-181. DOI: 10.1016/j.saa.2014.05.048
- Srivastava, S., Kadooka, C., and Uchida, J. Y. (2018). "*Fusarium* species as pathogen on orchids," *Microbiology Research* 207, 188-195. DOI: 10.1016/j.micres.2017.12.002
- Szczyglewska, P., Feliczak-Guzik, A., and Nowak, I. (2023). "Nanotechnology-general aspects: A chemical reduction approach to the synthesis of nanoparticles," *Molecules* 28(13), article 4932. DOI: 10.3390/molecules28134932
- Vera-Reyes, I., Esparza-Arredondo, I. J. E., Lira-Saldivar, R. H., Granados-Echegoyen, C. A., Alvarez-Roman, R., Vásquez-López, A., Santos-Villarreal G. D., and Díaz-Barriga Castro, E. (2019). "*In vitro* antimicrobial effect of metallic nanoparticles on phytopathogenic strains of crop plants." *Journal of Phytopathology* 167(7-8), 461-469.

- Vishveshvar, K., Aravind Krishnan, M., Haribabu, K., and Vishnuprasad, S. (2018). "Green synthesis of copper oxide nanoparticles using *Ixiro coccinea* plant leaves and its characterization," *BioNanoScience* 8, 554-558. DOI: 10.1007/s12668-018-0508-5
- Yin, Y., Miao, J., Shao, W., Liu, X., Zhao, Y., and Ma, Z. (2023). "Fungicide resistance: Progress in understanding mechanism, monitoring, and management," *Phytopathology* 113(4), 707-718. DOI: 10.1094/PHYTO-10-22-0370-KD
- Yugandhar, P., Vasavi, T., Jayavardhana Rao, Y., Uma Maheswari Devi, P., Narasimha, G., and Savithramma, N. (2018). "Cost effective, green synthesis of copper oxide nanoparticles using fruit extract of *Syzygium alternifolium* (Wt.) Walp., characterization and evaluation of antiviral activity," *Journal of Cluster Science* 29, 743-755. DOI: 10.1007/s10876-018-1395-1
- Zemankoa, M., and Lebeda, A. (2001). "*Fusarium* species, their taxonomy, variability and significance," *Plant Protection Science* 37(1), 25-42.
- Zhang, Z., Zhai, C., Li, Y., Stephenson, S. L., and Liu, P. (2024). "Slime molds (Myxomycetes) causing a "disease" in crop plants and cultivated mushrooms," *Frontiers in Plant Science* 15, article 1411231. DOI: 10.3389/fpls.2024.1411231

Article submitted: May 24, 2025; Peer review completed: August 9, 2025; Revised version received: October 17, 2025; Accepted: October 18, 2025; Published: October 31, 2025.

DOI: 10.15376/biores.20.4.11041-11055