

ECO-FRIENDLY GREEN BIOSYNTHESIS OF COPPER NANOPARTICLES FROM GARLIC (*ALLIUM SATIVUM*) TO COMBAT POST HARVEST FUNGAL PHYTOPHTHORA IN MANGO

MUHAMMAD TAHIR¹, AREEBA BANO² AND SHAZIA IRAM^{3*}

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

²Key Laboratory of Silviculture and Conservation of the Ministry of Education, College of Forestry, Beijing Forestry University, Beijing 100083, China

³Department of Environmental Science, Fatima Jinnah Women University (FJWU), The Mall, 46000, Rawalpindi, Pakistan

*Corresponding author's email: shaziairam@fjwu.edu.pk

Abstract

Lasiodiplodia theobromae is a major fungal pathogen that severely compromises mango fruit quality, leading to rapid postharvest decay and reduced shelf life. Green synthesis offers an eco-friendly and efficient strategy to combat such phytopathogens. This study focuses on a simple, rapid, and cost-effective method for the synthesis of copper nanoparticles (CuNPs) and evaluates their antifungal efficacy against *L. theobromae*. Fungal strain LTTK16-3 identified and its susceptibility to CuNPs were observed. CuNPs were synthesized by using garlic (*Allium sativum*) with copper sulfate as a precursor. CuNPs underwent characterization via a variety of instrumental methods like UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD), and Scanning electron microscope (SEM). The functional group of the NPs and presence of various biomolecules was confirmed by the analysis of FTIR while SEM analysis revealed that the particles are rod-like flakes due to the aggregation of smaller particles. The XRD pattern demonstrated the CuNPs falls in the category of 2.9 nm in size while UV-visible spectroscopy revealed a surface plasmon resonance peak of CuNPs was 432nm under optimal synthesis conditions. The antifungal activity of CuNPs was assessed through testing against fungal strain LTTK16-3. At concentrations of 0.5% extract solution, CuNPs exhibited strong effectiveness for the control of *L. theobromae* fungus of mango phytophthora. The findings of this study support the assertions that CuNPs, are an effective antifungal agent and exhibit significant potential in mitigating fungal infections and proliferation.

Key words: Green synthesis; *Allium sativum*; CuNPs; Mango pathogen; *Lasiodiplodia theobromae*

Introduction

Nanotechnology is a field of research focused on the manipulation and application of materials at the nanoscale (Darwish *et al.*, 2024). Nanotechnology is primarily dedicated for the production of nanoparticles that can be used for human benefits and exhibit a variety of sizes, shapes, chemical compositions, and at least one dimension in the nanoscale range of 1-100 nm (Bouqellah *et al.*, 2019). The size and surface area of nanoparticles can be determined through various well-established techniques. These methods are widely employed to assess the sizes, shapes, and crystallographic structures of nanoparticles. Methods including Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-Ray Diffraction (XRD) offer comprehensive insights into the morphological and structural properties of nanoparticles (Wadhwa *et al.*, 2022).

The biological approach offers a sustainable alternative to traditional chemical and physical methods for nanoparticle synthesis. Additionally, no expensive, risky, or toxic medications are used in this operation. The biological synthesis approach, which has gained considerable attention in modern time, facilitates the formation of metallic nanoparticles with diverse

dimension, morphology, material compositions, and physicochemical characteristics (Khan *et al.*, 2022). Synthesis can be achieved in a single step using biological agents, including bacteria, actinobacteria, yeast, molds, algae, plants, and their by-products. The important key elements consider during the production of nanoparticles are temperature, time and concentrations (Fig. 1). Molecules responsible for the reduction-based synthesis of nanoparticles include alkaloids, proteins, phenolic compounds, amines, enzymes, pigments, and various other bioactive compounds found in plants and microorganisms (Nadaroglu *et al.*, 2017).

Chemical methods are commonly employed for nanoparticle synthesis due to their precision in controlling particle size and shape, as well as their effectiveness. However, these methods can be environmentally harmful and pose risks during handling. This is why researchers have shifted towards greener methods because of their environmentally friendly attributes (Parveen *et al.*, 2016; Shah *et al.*, 2022; Amjad *et al.*, 2021). Green synthesis methods for nanoparticle production exhibit minimal toxicity, and numerous investigations have provided compelling evidence that plants and herbs possess the potential for use in such eco-friendly synthesis processes. Various plant parts, including stems, leaves, roots, and barks, are utilized in green synthesis. These secondary

metabolites are preset in plants, which assume a crucial role as reducing or capping agents in the synthesis of nanoparticles (Naaz *et al.*, 2021). Green methods have been successfully employed to synthesize a diverse range of nanoparticles, including silver, gold, silica, palladium, iron, and zinc oxide and currently attracting significant interest (Padma *et al.*, 2018).

Visual, catalytic, mechanical, and electrical characteristics of copper nanoparticles make them very interesting. Copper nanoparticles are used in bandages, highlighting its antibacterial activity to mitigate or reduce the risk of infection caused by pathogenic bacteria (Pecharromán *et al.*, 2006). The copper nanoparticles (2–5 nm) had a potent antibacterial action and demonstrated the capacity to diminish microbial levels by 99.9% (Sehmi *et al.*, 2015). Although several plant species, including *Aloe vera*, *Magnolia kobus*, *Terminalia arjuna*, and *Bifurcaria bifurcata*, have been successfully employed for the green synthesis of copper nanoparticles (CuNPs), garlic (*Allium sativum*) presents distinct advantages owing to its rich phytochemical composition. Garlic contains abundant organosulfur compounds, such as allicin and alliin, along with phenolics and flavonoids that can serve as natural reducing, stabilizing, and capping agents during nanoparticle synthesis. These bioactive constituents possess strong antioxidant and antimicrobial activities, which may enhance nanoparticle stability and contribute to their biological efficacy (Dayakar *et al.*, 2017; Ghosh *et al.*, 2020).

Allium sativum, a member of the Alliaceae family, is widely cultivated and consumed worldwide as both a culinary ingredient and a medicinal plant (Choudhary *et al.*, 2022). Garlic has long been recognized for its broad spectrum of biological activities, including antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anticarcinogenic, and cardioprotective effects (El-Saber Batiha *et al.*, 2020). These properties are largely attributed to its diverse phytochemical profile, which includes organosulfur compounds, thiosulfates, flavonoids, vitamins, peptides, glycosides, and trace elements (Manpreet *et al.*, 2021). Among these compounds, allicin (diallyl thiosulfate) is considered one of the principal bioactive molecules and has demonstrated potent inhibitory activity against a wide range of Gram-positive and Gram-negative microorganisms (Bhatwalkar *et al.*, 2021). The rich phytochemical composition of garlic makes it a suitable biological source for CuNP synthesis, as these compounds may contribute to copper-ion reduction, nanoparticle stabilization, and antimicrobial activity (Velsankar *et al.*, 2020; Amatya & Joshi, 2020). Based on these properties, garlic-mediated CuNPs warrant evaluation as a potential approach for the control of postharvest fungal pathogens.

L. theobromae, as one of the causative fungal agents associated with mango postharvest disease and also called stem-end rot (SER) (Zhan *et al.*, 2023). *L. theobromae* is the chief causative organism for SER worldwide. This fungus is capable of causing diseases in over 500 different host plants, including citrus, avocado, mango, papaya, banana, and guava, across tropical and subtropical regions globally, resulting in a wide range of disease manifestations (Linh *et al.*, 2021). Applications of many fungicides including prochloraz is still main strategy to control post-harvest mango rot. However, excessive use of

drug led to drug resistant pathogen and environmental pollution (de Souza *et al.*, 2024). The biosynthesis of CuNPs mediated by *Allium sativum* isolated compounds, along with the subsequent assessment of their antifungal activity against *L. theobromae*, remains underexplored. However, the application of garlic mediated CuNPs for the control of *L. theobromae* associated with mango postharvest disease remains poorly explored. Therefore, the present study investigated the synthesis of CuNPs using garlic extract and evaluated their antifungal activity against *L. theobromae*, with potential application in postharvest mango disease management. Given the well-documented antimicrobial and antifungal properties of garlic, this study explores whether garlic-mediated copper nanoparticles can enhance antifungal efficacy for the control of postharvest mango infections caused by *L. theobromae*.

Materials and Methods

Collection of materials: Fresh bulb of *Allium sativum*, a primary component for nanoparticle development, was locally sourced from Rawalpindi and transported to the Mycology laboratory at Fatima Jinnah Women's University. CuSO₄ utilized in the preparation of copper nanoparticles. The deionized water and copper sulphate (CuSO₄) were available in the mycology lab of FJWU.

Preparation of garlic extract: The papery outer layers of the garlic bulbs were carefully peeled away, after which 400 grams of the cleaned cloves were accurately measured using a precision balance for further processing. The cloves were thoroughly washed with distilled water and subsequently dried in an oven set to 55°C for a period of one week. Once dried, the cloves were ground using an electric blender, and 30 grams of the resulting powder were weighed. A paste was then prepared by adding 100 mL of distilled water and blending the mixture in a stirring machine. The aqueous garlic mixture was sonicated using a Sonopuls HD 2200 probe ultrasonicator (Bandelin GmbH and Co. KG, Berlin, Germany) in plastic tubes at a frequency of 20 kHz for eight minutes at 100 W power. A shaking incubator was used to continuously mix the solution at 300 rpm. The samples were centrifuges at 12000x g for 10 minutes to separate impurities. Supernatant were collected in sterilize tube and pellets were discarded. The centrifuged supernatant was further filtered through sterile Whatman filter papers to obtain a purified extract. This extract was then transferred into two 250 mL volumetric flasks, each containing 200 mL of the clarified solution, and stored at room temperature for further use. (Kebede Urge *et al.*, 2023).

Preparation of CuSO₄ solution: Copper sulphate was utilized for the preparation of CuNPs. A 100 mL solution of 1 mM copper sulfate was prepared by dissolving 15.96 mg of copper sulfate in 100 mL of distilled water. The mixture was then placed in a water bath at 70°C for 30 minutes to ensure complete dissolution of the salt (Bouqellah *et al.*, 2019).

Biological synthesis of CuNPs: The garlic extract flasks were placed on a magnetic stirrer for 10 minutes. To each

flask, 10 mL of copper sulfate solution was incrementally poured to the extract. The flasks were subsequently closed with aluminum foil and shaken for an additional 10 minutes. The plant extract underwent erosion and chemical degradation, leading to the formation of CuNPs. The flasks were then positioned in a shaker, maintained at 30°C and set to 150 rpm for agitation. After 72 hours, the color shifted from green to brown, signaling the development of nanoparticles. Centrifugation were done at 4000 rpm for 15 minutes to acquire solid material. The supernatant liquid was removed from tubes. The centrifuge tubes were heated at 80°C in hot oven and solid pellets were produced. The pellets were then ground into a fine nano powder with the

use of a mortar and pestle. The resulting CuNPs were then subjected to further characterization to confirm their biomolecular composition, size, shape, and absorbance properties (Murthy *et al.*, 2020a).

Characterization of CuNPs

FTIR spectroscopy analysis: FTIR spectroscopy, employing the KBr pellet method, is a widely recognized technique for identifying the variety of bonds linked with specific functional groups in the sample. FTIR measurements were conducted in the wave number range of 4000–400 cm⁻¹ with a resolution of 4 cm⁻¹.

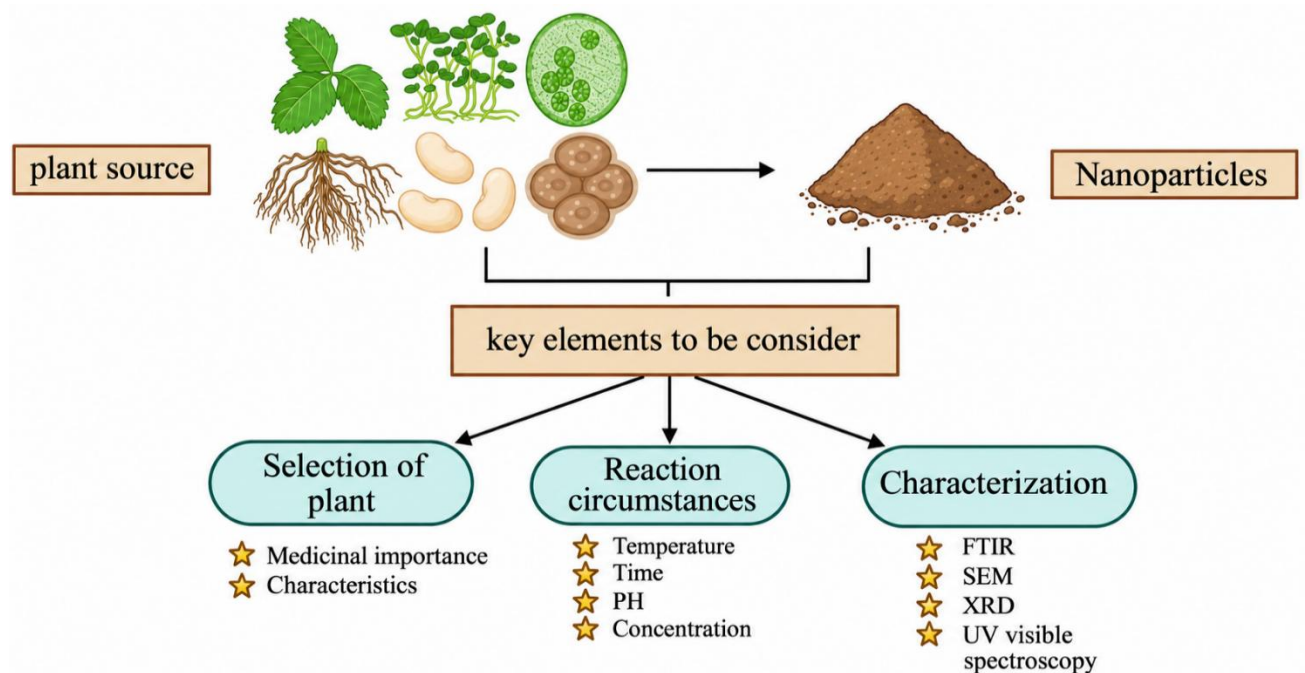


Fig. 1. key elements to be consider in the creation of environmentally friendly nanoparticles.

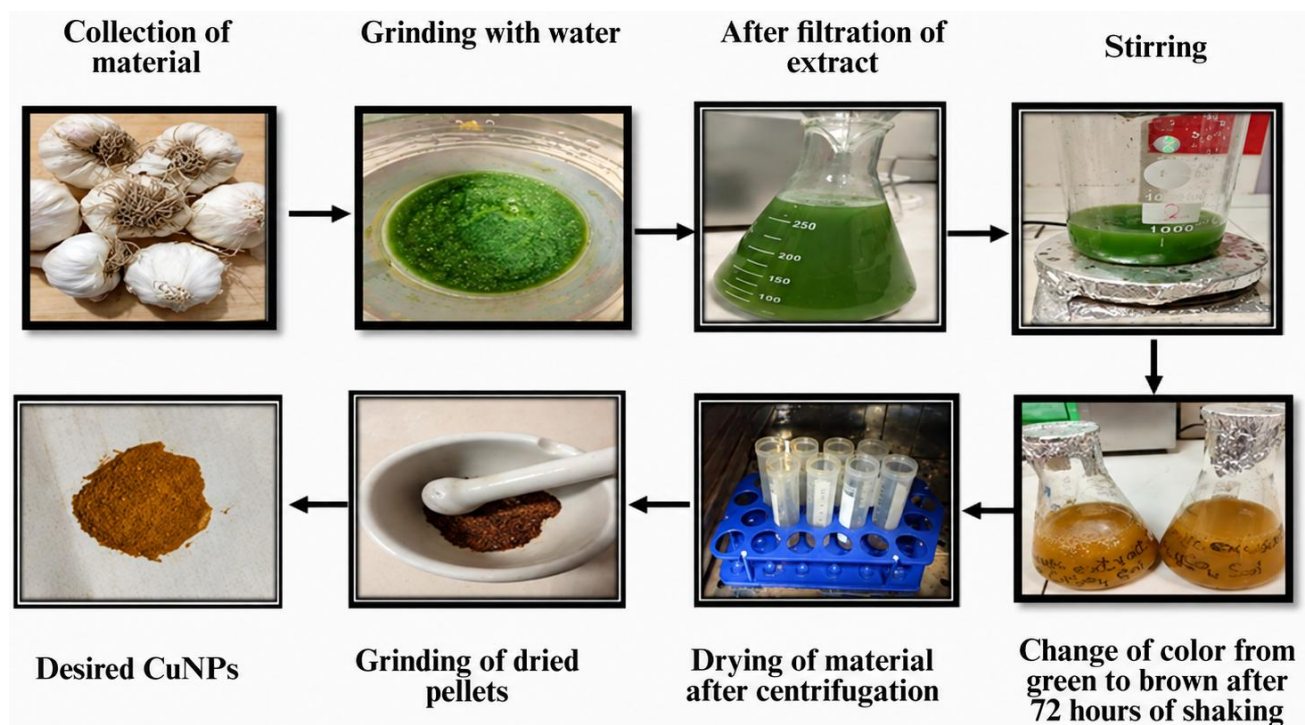


Fig. 2. Final desired CuNPs nanoparticles from garlic (*Allium sativum*) and change of color of extract after shaking 72 hours.

Scanning electron microscopy (SEM): The surface morphology of the biologically produced CuNPs was examined using SEM, which provides information on the NPs' topography and shape. It generates three-dimensional (3D) pictures in order to examine the phase variation. It was done by using Hitachi S-4500 machine (Pavithran *et al.*, 2020).

X-ray diffraction analysis (XRD): X-ray diffraction spectroscopy was carried out to identify the existence of various phases in the synthesized samples. The generator's voltage and current were adjusted to 40KV and 30 mA, respectively. The obtained samples' particle sizes were determined using Scherrer's Debye-equation which is:

$$N\lambda = 2d\sin\theta.$$

where d is the size of CuNPs crystallites and $\sin\theta$ is the full width half maximum (FWHM) of the diffraction peak, X-ray source wavelength used in XRD was 0.154 nm. The diffracted intensities were investigated between the ranges of 10–70° 2 θ angles.

UV-visible spectroscopy analysis: The UV-visible spectroscopy analysis was performed to confirm the absorbance range of Cu NPs. The solution containing CuNPs was characterized using a continuous scan UV-visible 4000 spectrophotometer within a frequency range of 400–700nm at resolution of 1 nm. Double-distilled water served as the blank control and measurement was recorded with time interval of 15 minutes. (Amatya & Joshi, 2020).

Propagation of microorganisms: The pathogenic fungal strains of *L. theobromae* isolated from mango fruit were received from Mycology and Toxicology Laboratory of Fatima Jinnah women University. The strain was cultivated on potato dextrose agar (PDA) plates for a period of seven days. Conidia were collected into sterile water through gently scraping and pathogen inoculum was prepared by adjusting the level up to 1×10^6 conidia mL^{-1} by using a haemocytometer (Oliveira *et al.*, 2015).

Application of CuNPs on mango for the control of postharvest disease: The pathogens identified from mango fruit were isolated and stored in slant bottles. To rejuvenate the pathogens, a small sample of the culture was transferred from the slants and plated onto pre-prepared PDA petri plates. The petri plates were subsequently tightly closed with parafilm and incubated at approximately 28°C for periods of 7 days to facilitate the growth of fungal colonies.

Preparation of CuNPs solution and infection of mango fruit: A copper nanoparticles solution was prepared by dissolving 0.2 and 0.5 grams of CuNPs powder in 100 ml of distilled water. The mixture was then shaken vigorously until the powder was fully mixed-up in the water (Jagana *et al.*, 2017).

For the application, mango fruit were chosen. Fruits were washed with distilled water after being cleaned with 70% ethanol. With the use of a sterile needle, a little cube of a fungus colony was removed from a petri plate and

injected into the incision. 8 mm cork borer were used to inflict 4 mm wounds on mangoes. Fifteen mangoes were used in the experiment, five of them received 10 ml spray of 0.2% solution of CuNPs and infected with fungal spore, five fruits were sprayed with 0.5% solution of CuNPs and infected with fungal spore and five mango fruits received solely fungal pathogen treatment without spray of CuNPs solution. All fruits were placed in laminar flow until fruit become fully dry. For infection, 100 μL of fungal spore with density of 1×10^5 spores per milliliter were dropped on the boring place in fruits. Fruits were wrapped in parafilm to prevent contamination of the wound and left at room temperature in a germinator for 6 days to analyze the growth of fungus and measure the lesions' length and width (Jagana *et al.*, 2017). The size of the lesions was calculated longitudinally and vertically to see the effect of CuNPs on the inhibition of fungal disease.

Statistical analysis

All experiments were performed with three independent biological replicates per treatment combination ($n = 3$). Data were analyzed using two-way analysis of variance (ANOVA) with Day (2 levels: 2nd and 6th day), CuNPs concentration (3 levels: 0%, 0.2%, 0.5%), and their interaction as fixed factors. Normality of residuals was assessed using the Shapiro–Wilk test, and homogeneity of variances was verified using Levene's test. Following a significant interaction, post-hoc pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test. Statistical significance was set at $\alpha = 0.05$. All analyses were performed using (version 4.4.2; R Core Team, Vienna, Austria)

Results and Discussion

Synthesis of CuNPs: In the study, CuSO_4 was employed as the precursor for synthesizing the biogenic CuNPs, meanwhile, the plant extract functioned as a reducing, capping, and stabilizing agent. After the addition of CuSO_4 salt, the change in color was noted which indicate the generation of nanoparticles (Mali *et al.*, 2020). In general, it was discovered that the color evolution of the reaction solution at 30°C temperatures may be used to predict the evolution of particle size. The plant extract's color changed from green to brown at a reaction temperature of 30°C for shaking 72 hours at 150 rpm. Depending on the reaction temperature, the solution containing the synthesized copper nanoparticles changed color to dark brown (Fig. 2) as the reaction time was extended which support the findings of another researcher (Saikia *et al.*, 2023). For particles created at 30°C, a brown solution was noted. The extract color shifted from green to a light brownish hue when placed in the shaker and visually demonstrating the creation of copper nanoparticles (Amatya & Joshi, 2020). The reaction mixture was then centrifuged for the collection and purification of the nanoparticles, followed by heating to obtain CuNPs powder. The formation of CuNPs was likely facilitated by phytochemicals present in the garlic extract, which may have acted as reducing, capping, and stabilizing agents, consistent with previous reports (Jagana *et al.*, 2017).

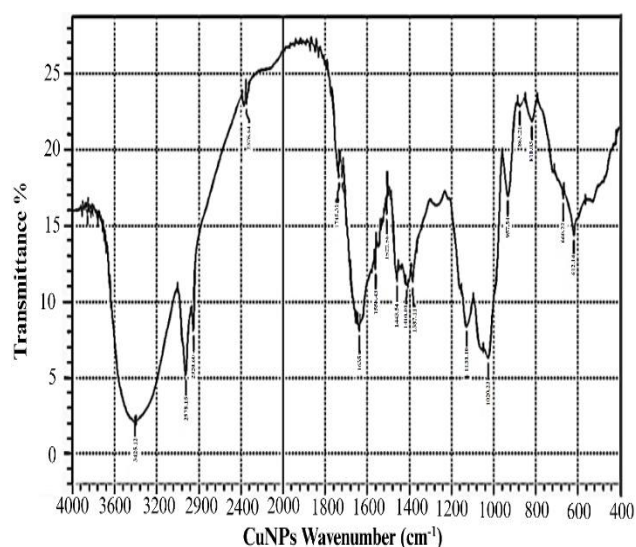


Fig. 3. FTIR analysis of CuNPs prepared from garlic (*Allium sativum*) extract.

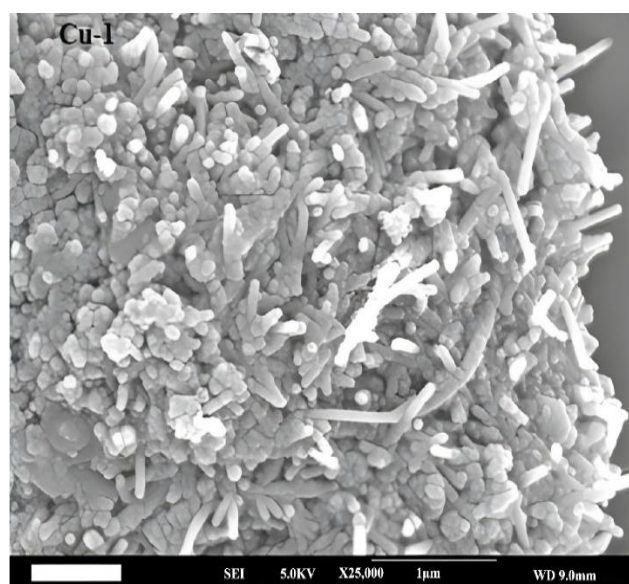


Fig. 4. SEM of CuNPs prepared from garlic (*Allium sativum*) extract.

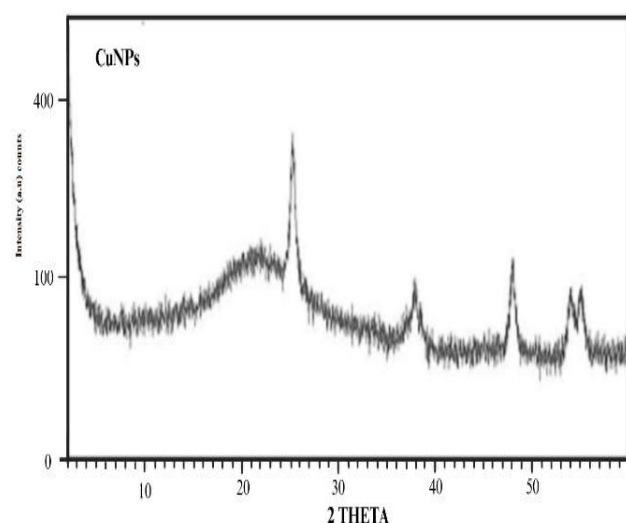


Fig. 5. XRD patterns of Cu NPs prepared from garlic (*Allium sativum*) extract.

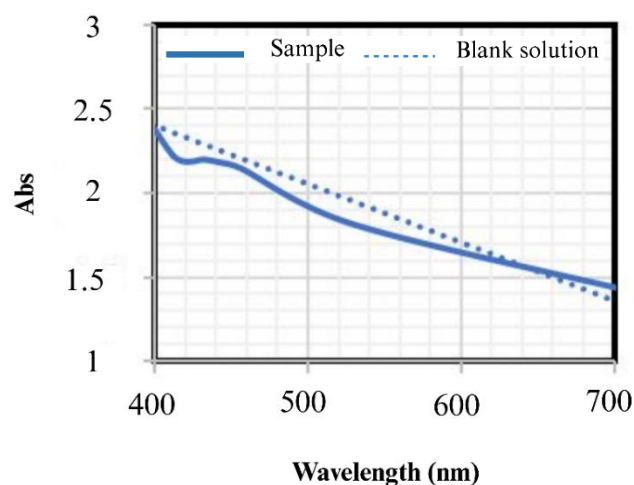


Fig. 6. UV-visible graph of CuNPs prepared from garlic (*Allium sativum*) extract.

FTIR (Fourier Transform Infrared Spectroscopy): The FTIR spectrum of biosynthesized CuNPs (Fig. 3) confirms the involvement of phytochemical constituents of *Allium sativum* in nanoparticle formation, consistent with green synthesis approaches reported in previous studies (Amer & Awwad, 2020). A broad absorption band at around 3404 cm^{-1} corresponds to O–H and N–H stretching vibrations of phenolic and amine groups, which may be associated with hydroxyl and amine containing phytochemicals and proteins present in the plant extract (Amatya & Joshi, 2020). The peaks observed at 2368 and 2345 cm^{-1} may be related to atmospheric CO_2 interference, as previously reported in FTIR analysis of plant-mediated nanoparticles (Murthy *et al.*, 2020b). The bands at 1718 cm^{-1} and 1637 cm^{-1} are attributed to C=O stretching of carbonyl compounds and N–H bending of amide groups, suggesting the presence of carbonyl- and amide-containing compounds that may contribute to interactions with the nanoparticle surface, consistent with previous biosynthetic nanoparticle studies (Elhabal *et al.*, 2022).

The peak at 1560 cm^{-1} corresponds to aromatic C=C stretching of flavonoids and phenolic compounds, which are known to act as strong reducing and chelating agents in nanoparticle synthesis (Pasiczna-Patkowska *et al.*, 2025). The band at 1128 cm^{-1} is assigned to C–N stretching vibrations of aliphatic amines, further supporting the presence of nitrogen-containing biomolecules involved in capping.

The absorption band at 669 cm^{-1} is attributed to Cu–O or metal–biomolecule interactions, confirming successful formation and stabilization of NPs, as similarly reported in plant-mediated copper nanoparticle synthesis (Iravani, 2011). Peaks in the regions $3600\text{--}3200\text{ cm}^{-1}$, $1600\text{--}1800\text{ cm}^{-1}$, and $\sim 2400\text{ cm}^{-1}$ correspond to O–H, N–H, and C=O stretching vibrations, respectively (Amatya & Joshi, 2020). Overall, the FTIR spectrum confirms that biomolecules such as saponins, tannins, flavonoids, alkaloids, and polyphenols present in garlic extract act as reducing and capping agents during CuNP synthesis (Asemani & Anarjan, 2019).

SEM (Scanning electron microscopy): The SEM micrographs of the biosynthesized CuNPs (Fig. 4) reveal the surface morphology and structural characteristics of the nanoparticles. The analysis indicates that the particles

predominantly exhibit rod-like and flake-shaped morphologies. This variation in shape may be attributed to the anisotropic growth of nanoparticles and the presence of bioactive compounds from *Allium sativum* extract acting as capping and stabilizing agents during synthesis (Iravani, 2011; Jeetkar *et al.*, 2022).

Some degree of aggregation and agglomeration was observed in the SEM micrographs, a feature commonly reported in plant-mediated nanoparticle synthesis and often attributed to the high surface energy of nanoparticles (Mittal *et al.*, 2013). At higher magnification (100,000 $\times$), variation in particle size was evident, which may reflect non-uniform nucleation and growth during green synthesis. The particles appeared relatively compact with limited porosity, which is typical of copper nanoparticles synthesized using biological extracts (Choudhary *et al.*, 2022).

These observations are consistent with reports suggesting that phytochemicals from biological extracts may act as capping agents during nanoparticle synthesis. The observed morphological diversity, including irregular and crystalline-like structures, is in agreement with previous studies on biosynthesized metal nanoparticles (Murthy *et al.*, 2020a; Amatya & Joshi, 2020). Overall, the SEM results support the successful formation of CuNPs with morphological features commonly reported for green synthesis routes.

XRD (X-ray diffraction): The XRD pattern of biosynthesized CuNPs is shown in (Fig. 5). Phase identification of the synthesized sample was carried out using X'Pert High Score software. The obtained samples' particle sizes were determined using Scherrer's Debye-equation, which is $n\lambda = 2d\sin\theta$.

The average crystallite size of the synthesized CuNPs was found to be approximately 2.9 nm, indicating the formation of nanoscale particles. This is consistent with previous reports on biosynthesized metallic nanoparticles. The average crystallite size of the synthesized CuNPs was approximately 2.9 nm, confirming their nanoscale crystalline dimensions. This value is comparable to crystallite sizes reported for biologically synthesized metal nanoparticles in previous studies (Oraibi *et al.*, 2023). The observed broadening of the diffraction peaks is consistent with the small crystallite size of the synthesized CuNPs. The limited crystallite growth may be influenced by phytochemicals, including phenolic, flavonoid, and proteinaceous compounds present in *Allium sativum*, which have been reported to act as capping or stabilizing agents during plant-mediated nanoparticle synthesis (Mittal *et al.*, 2013; Iravani, 2011).

Distinct diffraction peaks were observed at 2θ values of 25°, 38°, and 48.8°, confirming the crystalline nature of the synthesized material. The peak at approximately 38° is attributed to the (111) plane of face-centered cubic (FCC) copper, which is a characteristic reflection of metallic CuNPs. The peaks at 38° and 48.8° correspond to the (111) and (200) planes of FCC copper, respectively, as reported in previous studies. Similar diffraction patterns have been widely reported in plant-mediated synthesis of copper and other metallic nanoparticles, confirming the formation of stable crystalline phases (Thakar *et al.*, 2022; Joseph *et al.*, 2016).

The weak peak observed around 25° may be attributed to residual organic compounds from the *Allium sativum* extract acting as capping agents on the nanoparticle

surface. Similar observations have been reported in green synthesis of metal nanoparticles, where phytochemicals contribute to surface stabilization and slight background diffraction signals (Murthy *et al.*, 2020a; Amatya & Joshi, 2020). The XRD analysis confirms the successful formation of crystalline CuNPs with nanoscale crystallite size. These angles correspond to characteristic peaks observed in the XRD pattern of the analyzed material.

Scherrer's Debye equation is given by $n\lambda = 2d\sin\theta$ where:

- d represents the crystallite size of CuNPs,
- λ is the wavelength of the X-ray source (0.154 nm) used in XRD,
- $\sin\theta$ is the sine of the diffraction angle, and the term for the full width at half maximum (FWHM) of the diffraction peak.

UV-Visible Spectroscopy: The UV–Visible absorption spectrum of the biosynthesized CuNPs was recorded in the range of 400–700 nm (Fig. 6). A distinct absorption band was observed at 432 nm, supporting the formation of copper-based nanoparticles. This optical response may be associated with surface plasmon-related absorption arising from collective oscillation of conduction electrons at the nanoparticle surface (Kelly *et al.*, 2003).

The presence of a single and well-defined absorption peak at 432 nm suggests the formation of relatively stable CuNPs with controlled size distribution. The slight broadening of the peak may indicate moderate polydispersity and possible aggregation, which is commonly observed in plant-mediated nanoparticle synthesis due to biomolecule capping effects (Iravani, 2011).

The observed SPR peak is consistent with previous reports on biosynthesized copper nanoparticles, where absorption bands typically appear in the range of 400–450 nm depending on particle size, shape, and surrounding dielectric environment (Murthy *et al.*, 2020a; Saikia *et al.*, 2023). A comparative study reported an SPR peak at approximately 403 nm, which is slightly lower than the present study. This shift can be attributed to variations in particle size, aggregation state, and differences in phytochemical composition of the plant extract used for synthesis (Amatya & Joshi, 2020; Mittal *et al.*, 2013).

The shift observed at 432 nm in the present study may indicate relatively larger particle size or increased interparticle interaction due to mild agglomeration during synthesis and drying processes. Such shifts in SPR position are commonly reported in green-synthesized copper nanoparticles and are influenced by reaction time, temperature, and reducing agent concentration (Kelly *et al.*, 2003).

Antifungal activity of copper nanoparticles on mango fruit:

The resistance of synthesized CuNPs from *Allium sativum* was assessed on the basis of fungus growth. In order to analyze the growth and control of the fungus on mango fruit, two different concentrations of CuNPs (0.2% and 0.5%) were applied on mango fruit. After the application, the observation was made for 6 days. The results demonstrated that the copper nanoparticles exhibited effective antifungal effect. According to observation both concentrations produced various effects. The zone of inhibition and lesion of fungus were measured in length and width wise using a meter scale. Fig. 7 shows the results of spreading of lesion on mango with experimental and control sample, which were observed in 2nd and 6th days of inoculation of fungus spores.

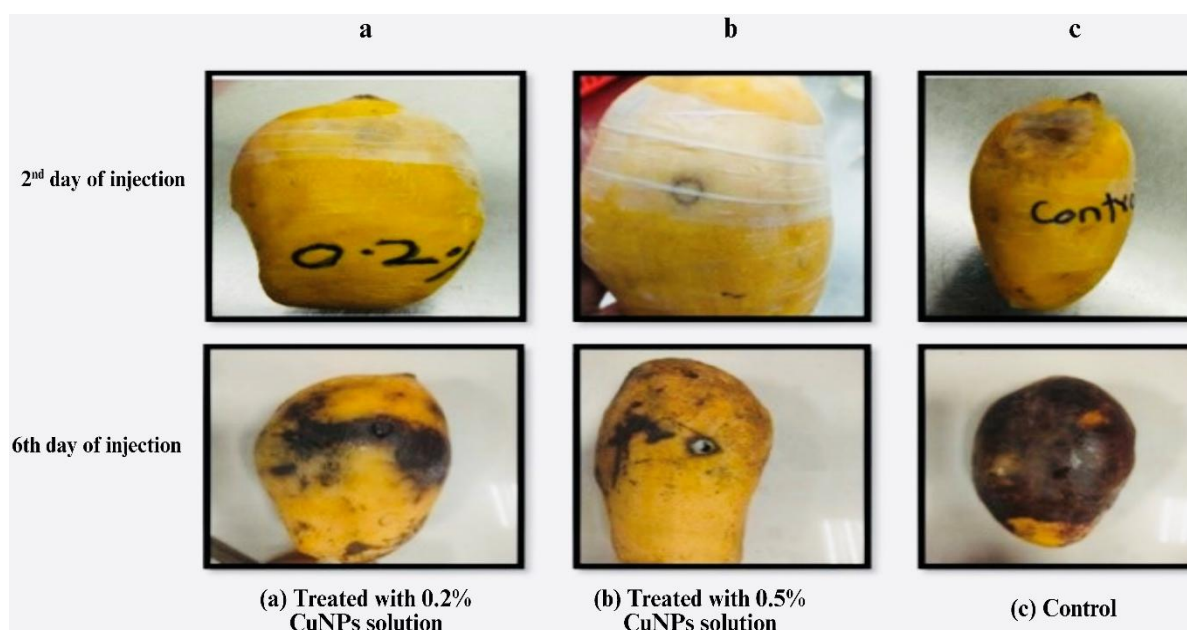


Fig. 7. Observation of spreading of fungus on Mango on 2nd and 6th day with experimental (CuNPs + Fungus spores) and control sample. Control was injected only with fungal spore without treated with Cu-NPs solution.

Table 1. The percentage inhibition of fungal growth relative to untreated control.

Day	Concentration %	Lesion length (cm)	% inhibition	Lesion width (cm)	% Inhibition
2 nd	0 (control)	0.71 ± 0.05	—	0.32 ± 0.02	—
2 nd	0.2	0.32 ± 0.03	54.9% ***	0.27 ± 0.04	15.6% ns
2 nd	0.5	0.19 ± 0.04	73.2% ***	0.12 ± 0.03	62.5% ***
6 th	0 (control)	4.40 ± 0.10	—	2.92 ± 0.16	—
6 th	0.2	2.10 ± 0.20	52.3% ***	2.10 ± 0.20	28.1% ***
6 th	0.5	1.13 ± 0.12	74.3% ***	1.13 ± 0.12	61.3% ***

Significance levels: * $p < 0.001$; ns = Not significant ($p = 0.352$) based on Tukey HSD post-hoc test

Note: Values are mean ± SD ($n = 3$). Percentage inhibition calculated relative to untreated control

The fungicidal potential of the biosynthesized CuNPs was assessed by monitoring fungal growth in both length and width dimensions under different treatments (Truong *et al.*, 2023). On the second day of incubation, the fungal colony exhibited a growth of 0.65 cm in length in the presence of 0.2% CuNPs, whereas no visible lesion expansion was observed in the treatment containing 0.5% CuNPs. By the sixth day, the 0.5% CuNP treatment showed superior antifungal efficacy, restricting lesion development to only 1.0 cm compared with 4.3 cm in the untreated control, indicating a concentration-dependent inhibitory effect on *L. theobromae* (Table 1)

One of the primary mechanisms through which CuNPs inhibit fungal growth is the generation of reactive oxygen species (ROS). CuNPs undergo oxidation in the conditions involving water and oxygen, releasing copper ions (Cu^{2+}). These ions catalyze Fenton-like reactions, triggering the formation ROS, such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot\text{OH}$) (Gaba *et al.*, 2022).

According to the (Fig. 8a), the boxplot represents the effect of different concentrations of CuNPs on controlling fungal lesions, as measured by lesion length (in cm). The boxplot of control sample indicating a high variation in lesion lengths. The median (horizontal line within the box) suggests that most lesion lengths are significantly higher compared to the treated groups. While the lesion length with 0.2% CuNPs solution reduced as compared to 0.50%

CuNPs solution. This suggests that increasing the Cu NP concentration to 0.5% leads to better fungal control than 0.2% (Jagana *et al.*, 2017).

Boxplot (Fig. 8b) illustrates the time-dependent progression of *L. theobromae* infection on mango fruit. By the second day of incubation, fungal growth was minimal and only small, localized fungal colonies were observed, indicating the early stage of infection. However, by the sixth day, the untreated control fruits exhibited extensive mycelial growth and severe tissue deterioration, demonstrating rapid disease progression under favorable conditions. In contrast, fruits treated with CuNPs showed markedly reduced lesion development, indicating effective suppression of fungal growth and delayed disease advancement.

The observed antifungal activity may be associated with the nanoscale dimensions and high surface-area-to-volume ratio of the CuNPs, which may promote closer interactions with fungal cell surfaces and thereby contribute to their inhibitory effects (Ren *et al.*, 2009). The prolonged reduction in disease severity throughout the storage period highlights the potential of biosynthesized CuNPs as an eco-friendly strategy for postharvest disease management in mangoes, with the added benefits of reducing fruit spoilage, extending shelf life, and lowering dependence on conventional synthetic fungicides (Mostafa *et al.*, 2026).

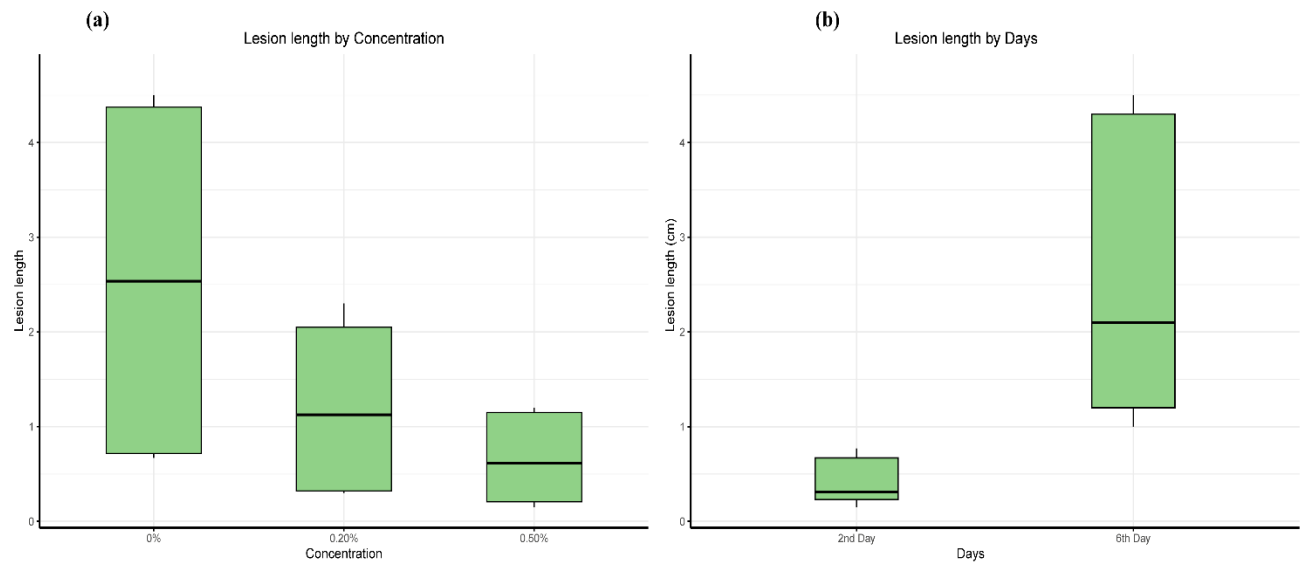


Fig. 8. Boxplot shows the spreading of lesion on mango in length wise on experimental and control sample (a) with concentrations (b) with days.

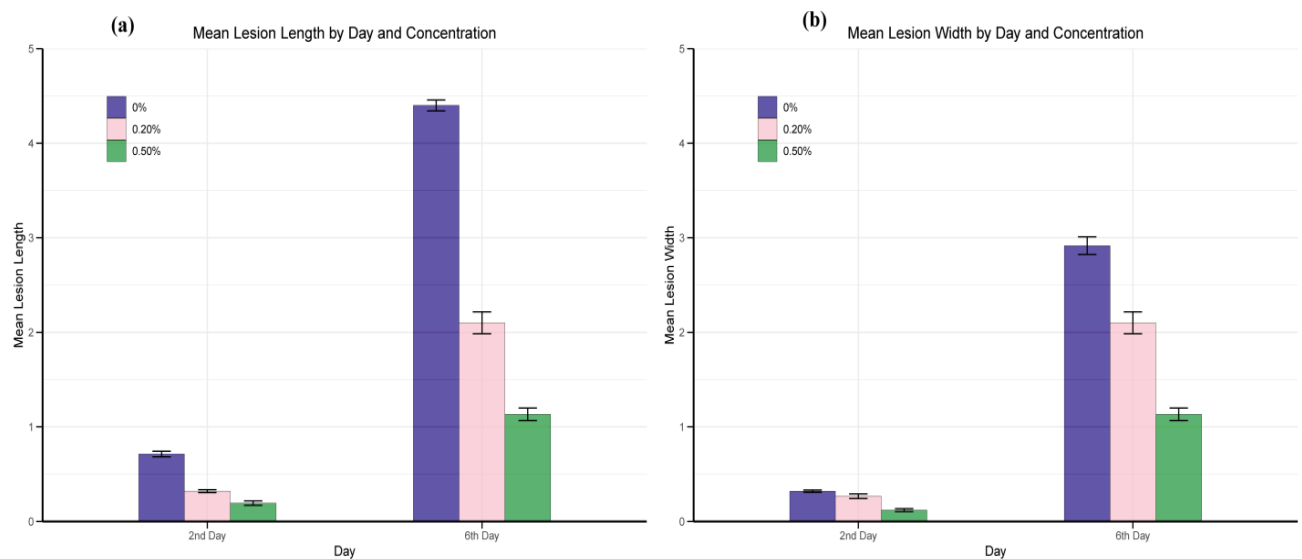


Fig. 9. Spreading of lesion on mango by day and concentration, lesion length (a) and lesion width (b).

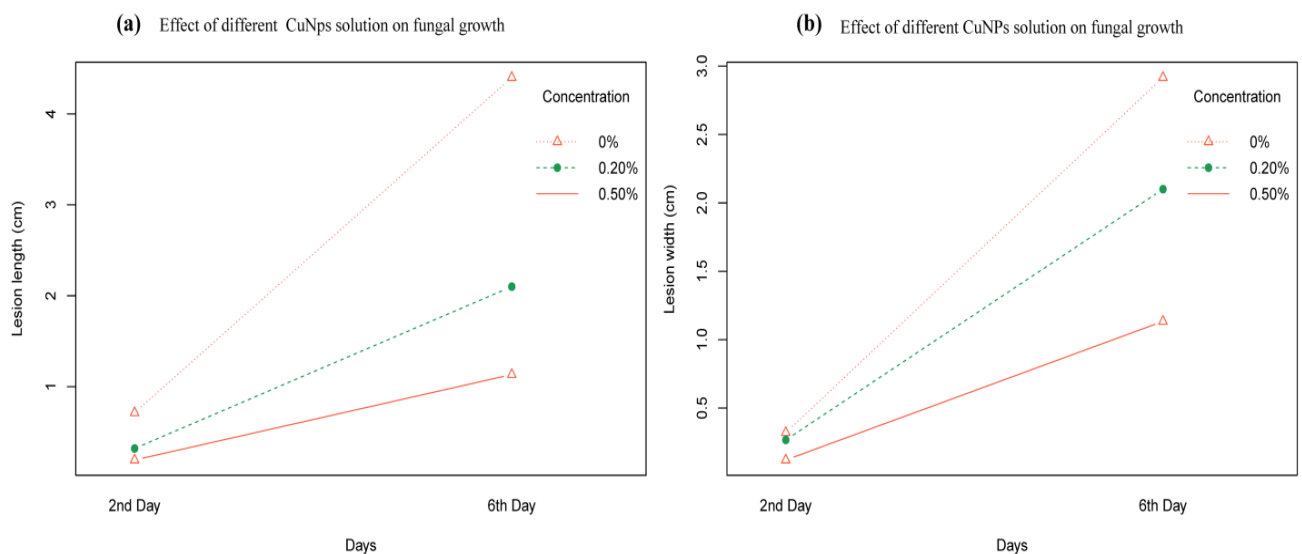


Fig. 10. Change in lesion length (a) and width (b) over time with varying CuNP concentrations.

Bar graph (Fig. 9a) illustrates the mean lesion lengths and width with day and concentration of fungal infections at two time points (2nd and 6th day) under three treatment conditions: 0% CuNPs (control), 0.2% CuNPs, and 0.5% CuNPs. The results highlight the dose-dependent efficacy of CuNPs in controlling fungal lesions. Untreated control group (0% CuNPs) exhibited the highest lesion growth, increasing sharply from approximately 1 cm on the 2nd day to over 4 cm by length on the 6th day. In contrast, the 0.2% CuNPs treatment moderately suppressed fungal growth, with lesion lengths increasing from less than 1 cm on the 2nd day to around 2.5 cm on the 6th day. The 0.5% CuNPs treatment was the most effective, maintaining the smallest lesion lengths, increasing only slightly from below 1 cm on the 2nd day to approximately 1.5 cm on the 6th day. Control group (Fig. 9b) exhibited the highest lesion growth by width, sharp increased from 2nd day to 6th day and approximately maximum 2.8 cm on 6th day. In contrast, with 0.2% CuNPs treatment moderately suppress the fungal growth, with lesion width increasing from less than 0.5cm on 2nd day and 2cm on 6th day. The 0.5% copper solution was more effective, maintaining the smallest width, increasing only less than 0.5cm on 2nd day and 1cm on 6th day. These results demonstrate a dose-dependent and time-dependent suppression of fungal growth, with 0.5% CuNPs providing the greatest control.

The line graph (Fig. 10) illustrates the effect of different concentrations of CuNPs on fungal lesion growth over a 6-day incubation period. Lesion length increased with time in all treatments; however, the extent of lesion expansion varied markedly among CuNP concentrations. According to (Fig. 10a) on the 2nd day, the control treatment (0% CuNPs) showed the largest lesion length (approximately 0.8 cm), whereas fruits treated with 0.20% and 0.5% CuNPs exhibited smaller lesions of approximately 0.4 cm and 0.3 cm, respectively. By the 6th day, lesion length in the control increased substantially to about 4.3 cm. In contrast, lesion lengths in fruits treated with 0.2% and 0.5% CuNPs were approximately 2.1 cm and 1.2 cm, respectively. The effect of different concentrations of CuNPs on fungal lesion width is shown in (Fig. 10b). On the 2nd day, lesion widths were approximately 0.35 cm, 0.30 cm, and 0.15 cm for the control (0%), 0.2%, and 0.5% CuNPs treatments, respectively. By the 6th day, the lesion width in the control increased sharply to about 2.95 cm, whereas the 0.2% CuNP treatment limited lesion expansion to approximately 2.10 cm. The smallest lesion width (1.15 cm) was observed in fruits treated with 0.5% CuNPs.

The variation in fungicidal efficacy among two samples is influenced by several factors. The toxicity of copper nanoparticles is determined by a combination of factors, including concentration, exposure duration, humidity, and temperature (Dorjee *et al.*, 2023). Copper is known to affect microorganisms through multiple mechanisms, such as disrupting membrane integrity, inducing lipid peroxidation, modifying proteins, and degrading nucleic acids, which collectively result in microbial cell death. In this study, an increase in the concentration of copper nanoparticles corresponded with enhanced antifungal effectiveness, eventually achieving complete inhibition of the fungal growth (Truong *et al.*, 2023). The fig. 7 shows that the more the Cu NPs concentration increases, the more the diameter (length and width) of fungal lesion on fruit decreases (Fatima *et al.*, 2024).

The present study demonstrated that garlic-mediated CuNPs effectively inhibited the growth of *L. theobromae*, a major causal agent of mango dieback and postharvest decay. A concentration-dependent response was observed, where 0.5% CuNPs exhibited greater antifungal activity than 0.2% CuNPs, indicating that increasing nanoparticle concentration enhanced fungal growth inhibition. Similar concentration-dependent antifungal effects of plant-mediated nanoparticles have been reported in the previous study (Chalandar *et al.*, 2017). The high efficacy observed in this study suggests that biosynthesized CuNPs have considerable potential for controlling postharvest fungal diseases in mango.

The antifungal properties of garlic are largely attributed to sulfur-containing bioactive compounds such as allicin and ajoene. These compounds have been reported to suppress spore germination and mycelial growth in several fruit-infecting fungi, including *Botrytis cinerea* and *Penicillium expansum* (Sarfraz *et al.*, 2020; Daniel *et al.*, 2015). Therefore, garlic phytochemicals may contribute not only to the reduction and stabilization of CuNPs during synthesis but also to the overall antifungal activity of the nanoparticle formulation. Similar findings have been reported for natural antifungal compounds such as thymol, which showed strong inhibitory effects against postharvest pathogens including *L. theobromae* isolated from avocado and mango (Perumal *et al.*, 2016; Suwanamornlert *et al.*, 2018).

The enhanced antifungal activity of CuNPs can be attributed to both their nanoscale properties and their unique mode of action. Similar to Ag, TiO₂, and ZnO nanoparticles, CuNPs can alter fungal membrane morphology, increase membrane permeability, disrupt nutrient transport, and cause deformation of fungal hyphae (Linh *et al.*, 2021; Li *et al.*, 2022). In addition, CuNPs undergo surface oxidation and gradually release Cu²⁺ ions, which promote the generation of reactive oxygen species (ROS) including superoxide radicals (O₂^{•-}), hydrogen peroxide (H₂O₂), and hydroxyl radicals (•OH) (Lemire *et al.*, 2013). Excessive ROS accumulation induces oxidative stress, resulting in lipid peroxidation, protein denaturation, enzyme inactivation, mitochondrial dysfunction, and DNA damage, ultimately leading to fungal cell death. Furthermore, released Cu²⁺ ions may interact with cellular proteins and membrane components, potentially contributing to membrane disruption, leakage of intracellular contents, and interference with essential metabolic processes (Ren *et al.*, 2009; Rai *et al.*, 2018). These mechanisms have been proposed for copper-based nanoparticles and may contribute to the antifungal activity against *L. theobromae* observed in the present study.

Compared with conventional fungicides such as prochloraz, CuNPs have been reported to act through multiple mechanisms, including ROS generation, copper-ion release, membrane damage, and interference with cellular metabolism. Such multiple modes of action may provide advantages for antifungal applications; however, their potential effects on resistance development require further investigation (Crisan *et al.*, 2021).

Although garlic-mediated CuNPs showed antifungal activity against *L. theobromae* in the present study, their practical application requires further evaluation. No obvious visual symptoms of fruit injury were observed at the tested concentrations; however, nanoparticle residues, fruit-quality attributes, consumer safety, and environmental fate were not systematically assessed and therefore remain to be investigated.

A limitation of this study is the absence of a garlic-extract-only control, which prevents clear distinction between the antifungal effects of garlic phytochemicals and those of the CuNPs. Previous research has shown that nano-modification of garlic extract can enhance its antifungal activity compared with garlic extract alone (Zhao *et al.*, 2022). However, because such a comparison was not included in the present study, future studies should incorporate a garlic-extract-only treatment to determine the relative contribution of garlic phytochemicals and CuNPs to the observed antifungal activity.

Although copper is an essential micronutrient, excessive exposure to copper can cause toxic effects in biological systems (Kah *et al.*, 2021). The biological responses to nanoparticles are strongly influenced by concentration, particle size, surface properties, and exposure duration. Therefore, comprehensive studies are required to evaluate copper residue levels, nanoparticle persistence on fruit surfaces, potential impacts on fruit quality attributes, and dietary exposure risks before field or commercial application.

The present findings are supported by previous studies reporting the antimicrobial activity of biosynthesized CuNPs (Jagana *et al.*, 2017; Iliger *et al.*, 2021), as well as the

antifungal potential of garlic-derived bioactive compounds against phytopathogenic fungi (Satyal *et al.*, 2017). These reports collectively reinforce the role of plant-mediated synthesis in developing effective antifungal nanomaterials.

Overall, the results indicate that green-synthesized CuNPs exhibit strong antifungal activity against the postharvest mango pathogen, even at relatively low concentrations. These findings support their potential for postharvest disease management; however, further studies on toxicological safety, fruit quality, residue levels, and practical efficacy are required before field or commercial application.

ANOVA and post-hoc results: Two-way ANOVA revealed that Day, CuNPs concentration, and their interaction significantly affected both lesion length and lesion width (all $p < 0.001$; Table 2). Tukey HSD post-hoc comparisons (Table 3) showed that for lesion length, all treatment pairs differed significantly on both days ($p < 0.01$ for all comparisons). For lesion width, all comparisons were significant ($p < 0.001$) except for 0.2% vs. control on day 2, which was not significant ($p = 0.352$). These results confirm that CuNPs concentration and exposure time independently and synergistically inhibit *L. theobromae* growth.

Table 2. Two-way ANOVA results for lesion length and width of *L. theobromae* treated with biosynthesized CuNPs.

Source	DF	F-value	p-value	F-value	p-value
		Lesion length		Lesion width	
Day	1	1802.9	< 0.001 ***	1084.02	< 0.001 ***
Concentration	2	500.5	< 0.001 ***	108.48	< 0.001 ***
Day X Concentration	2	261.0	< 0.001 ***	68.82	< 0.001 ***
Error	12				

Significance levels: * $p < 0.001$. Note: $n = 3$ per treatment combination

Table 3. Tukey HSD post-hoc comparisons for lesion length and width.

Comparison	Day	M.D (cm)	p-value	M.D (cm)	p-value
		Lesion length		Lesion width	
0.2% vs 0%	2 nd	-0.39	< 0.001 ***	-0.05	0.352 ns
0.5% vs 0%	2 nd	-0.55	< 0.001 ***	-0.20	< 0.001 ***
0.5% vs 0.2%	2 nd	-0.16	0.008 **	-0.15	< 0.001 ***
0.2% vs 0%	6 th	-2.20	< 0.001 ***	-0.91	< 0.001 ***
0.5% vs 0%	6 th	-3.23	< 0.001 ***	-1.86	< 0.001 ***
0.5% vs 0.2%	6 th	-1.03	< 0.001 ***	-0.95	< 0.001 ***

*Significance levels: *** $p < 0.001$; $p < 0.01$; ns = Not significant ($p > 0.05$). Only one comparison (0.2% vs. control on day 2 for lesion width) was non-significant

Conclusion

????

In conclusion, this study demonstrates a sustainable, room-temperature biosynthesis of copper nanoparticles (CuNPs) using *Allium sativum* (garlic) extract. The synthesized CuNPs showed concentration-dependent antifungal activity against *L. theobromae*, with 0.5% CuNPs exhibiting significantly higher inhibition than 0.2%. The antifungal mechanism is attributed to Cu^{2+} ion release and ROS-induced oxidative stress, leading to fungal cell damage. These findings highlight green-synthesized CuNPs as a promising alternative for postharvest mango disease management. However, further studies on residue safety, phytotoxicity, and large-scale application are needed before commercial use.

Author's Contributions: Tahir M. performed data analysis and wrote the manuscript. Bano A. conducted the

experiments. Iram S. supervised the study, reviewed the manuscript, and provided guidance. All authors approved the final version.

Declaration of Interests: The authors have no conflict of interest to declare as there are no financial relationships, professional affiliations, or personal interests that could influence the research or the interpretation of its results.

Data Availability: Data will be made available on a fair request to the corresponding author.

References

Amatya, S.P. and L.P. Joshi. 2020. Bio-synthesis of copper nanoparticles (CuNPs) using garlic extract to investigate antibacterial activity. *Bibechana.*, 17: 13-19.

- Amer, M. and A. Awwad. 2020. Green synthesis of copper nanoparticles by Citrus limon fruits extract, characterization and antibacterial activity.
- Amjad, R., B. Mubeen, S.S. Ali, S.S. Imam, S. Alshehri, M.M. Ghoneim, S.I. Alzarea, R. Rasool, I. Ullah and M.S. Nadeem. 2021. Green synthesis and characterization of copper nanoparticles using *Fortunella margarita* leaves. *Polym.*, 13: 4364.
- Asemani, M. and N. Anarjan. 2019. Green synthesis of copper oxide nanoparticles using *Juglans regia* leaf extract and assessment of their physico-chemical and biological properties. *Green Processing. Synth.*, 8: 557-567.
- Bhatwalkar, S.B., R. Mondal, S.B.N. Krishna, J.K. Adam, P. Govender and R. Anupam. 2021. Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). *Front. Microbiol.*, 12: 613077.
- Bouqellah, N.A., M.M. Mohamed and Y. Ibrahim. 2019. Synthesis of eco-friendly silver nanoparticles using *Allium* sp. and their antimicrobial potential on selected vaginal bacteria. *Saudi J. Biol. Sci.*, 26: 1789-1794.
- Chalandar, H.E., H.R. Ghorbani, H. Attar and S.A. Alavi. 2017. Antifungal effect of copper and copper oxide nanoparticles against *Penicillium* on orange fruit. *Biosci. Biotechnol. Res. Asia.*, 14: 279-284.
- Choudhary, S., M.U. Noor, M.S. Hussain, M. Mishra and S. Tyagi. 2022. Pharmacological properties and phytoconstituents of garlic (*Allium sativum* L.): A review. *Biol. Sci.*, 2: 338-346.
- Crisan, M.C., M. Teodora and M. Lucian. 2021. Copper nanoparticles: Synthesis and characterization, physiology, toxicity and antimicrobial applications. *Appl. Sci.*, 12: 141.
- Daniel, C.K., C.L. Lennox and F.A. Vries. 2015. *In vitro* effects of garlic extracts on pathogenic fungi *Botrytis cinerea*, *Penicillium expansum* and *Neofabraea alba*. *S. Afr. J. Sci.*, 111: 1-8.
- Darwish, M.A., W. Abd-Elaziem, A. Elsheikh and A.A. Zayed. 2024. Advancements in nanomaterials for nanosensors: a comprehensive review. *Nanoscale Adv.*, 6: 4015-4046.
- Dayakar, T., K.V. Rao, K. Bikshalu, V. Rajendar and S.-H. Park. 2017. Novel synthesis and characterization of pristine Cu nanoparticles for the non-enzymatic glucose biosensor. *J. Mater. Sci. Mater. Med.*, 28: 109.
- De Souza, J.F.F., K.R. Da Silva França, M.M. De Medeiros Ferro, I.P. Assunção, G.S. De Andrade Lima, A.R.G. De Farias, F. De Alcântara Neto and M.P. De Melo. 2024. *Lasioidiplodia theobromae* and *Lasioidiplodia brasiliense* causing dieback and rot fruit of jackfruit tree in Brazil. *Crop Prot.*, 106763.
- Dorjee, L., R. Gogoi, D. Kamil, R. Kumar, T.K. Mondal, S. Pattanayak and B. Gurung. 2023. Essential oil-grafted copper nanoparticles as a potential next-generation fungicide for holistic disease management in maize. *Front. Microbiol.*, 14: 1204512.
- El-Saber Batiha, G., A. Magdy Beshbishy, L. G. Wasef, Y.H. Elewa, A. A. Al-Sagan, M.E. Abd El-Hack, A.E. Taha, Y. M. Abd-Elhakim and H. Prasad Devkota. 2020. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. *Nutrients.*, 12: 872.
- Elhabal, S.F., H.M. Elwy, S. Hassanin, A.A. El-Rashedy, A.A. Hamza and M.A. Khasawneh. 2022. Biosynthesis and characterization of gold and copper nanoparticles from *Salvadora persica* fruit extracts and their biological properties. *Int. J. Nanomed.*, 17: 6095.
- Fatima, Q., A. Shoaib, N. Gull, S. Khurshid and U. Fatima. 2024. Chitosan-mediated copper nanohybrid attenuates the virulence of a necrotrophic fungal pathogen *Macrophomina phaseolina*. *Sci. Rep.*, 14: 23193.
- Gaba, S., A.K. Rai, A. Varma, R. Prasad and A. Goel. 2022. Biocontrol potential of mycogenic copper oxide nanoparticles against *Alternaria brassicae*. *Fron. Chem.*, 10: 966396.
- Ghosh, M.K., K. Jain, S. Khan, K. Das and T.K. Ghorai. 2020. New dual-functional and reusable bimetallic Y₂ZnO₄ nanocatalyst for organic transformation under microwave/green conditions. *ACS omega.*, 5: 4973-4981.
- Iligier, K.S., T.A. Sofi, N.A. Bhat, F.A. Ahanger, J.C. Sekhar, A.Z. Elhendi, A.A. Al-Huqail and F. Khan. 2021. Copper nanoparticles: Green synthesis and managing fruit rot disease of chilli caused by *Colletotrichum capsici*. *Saudi J. Biol. Sci.*, 28: 1477-1486.
- Iravani, S. 2011. Green synthesis of metal nanoparticles using plants. *Green Chem.*, 13: 2638-2650.
- Jagana, D., Y.R. Hegde and R. Lella. 2017. Green nanoparticles: A novel approach for the management of banana anthracnose caused by *Colletotrichum musae*. *Int. J. Curr. Microbiol. Appl. Sci.*, 6: 1749-56.
- Jeetkar, T., S. Khataokar, A. Indurkar, A. Pandit and M. Nimbalkar. 2022. A review on plant-mediated synthesis of metallic nanoparticles and their applications. *Adv. Nat. Sci: Nanosci. Nanotechnol.*, 13: 033004.
- Joseph, A.T., P. Prakash and S. Narvi. 2016. Phytofabrication and characterization of copper nanoparticles using *Allium sativum* and its antibacterial activity. *Int. J. Sci. Eng Technol.*, 4: 463-472.
- Kah, M., L.J. Johnston, R.S. Kookana, W. Bruce, A. Haase, V. Ritz, J. Dinglasan, S. Doak, H. Garelick and V. Gubala. 2021. Comprehensive framework for human health risk assessment of nanopesticides. *Nat. Nanotechnol.*, 16: 955-964.
- Kebede Urge, S., S. Tiruneh Dibaba and A. Belay Gemta. 2023. Green synthesis method of ZnO nanoparticles using extracts of *Zingiber officinale* and garlic bulb (*Allium sativum*) and their synergetic effect for antibacterial activities. *J. Nanomater.*, 2023: 1-9.
- Kelly, K.L., E. Coronado, L.L. Zhao and G.C. Schatz 2003. The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment. ACS Publications.
- Khan, N., S. Ali, S. Latif and A. Mehmood. 2022. Biological synthesis of nanoparticles and their applications in sustainable agriculture production. *Nat. Sci.*, 14: 226-234.
- Lemire, J.A., J.J. Harrison and R.J. Turner. 2013. Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat. Rev. Microbiol.*, 11: 371-384.
- Li, L., H. Pan, L. Deng, G. Qian, Z. Wang, W. Li and C. Zhong. 2022. The antifungal activity and mechanism of silver nanoparticles against four pathogens causing kiwifruit post-harvest rot. *Front. Microbiol.*, 13: 988633.
- Linh, N.V.M., P.T.H. Thảo, N.T.H. Liên, Đ.T.H. Vân, N.V. Hiếu, N.T. Vân and N.H. Châu. 2021. Khả năng ức chế nấm *Lasioidiplodia theobromae* gây bệnh thối cuống trên xoài của nano bạc và đồng. *Tạp chí Công nghệ Sinh học.* 4: 735-740.
- Mali, S.C., A. Dhaka, C.K. Githala and R. Trivedi. 2020. Green synthesis of copper nanoparticles using *Celastrus paniculatus* Willd. leaf extract and their photocatalytic and antifungal properties. *Biotech. Rep.*, 27: e00518.
- Manpreet, K., G. Priyanshi and G. Reena. 2021. Phytochemical analysis, antioxidant and antimicrobial activity assessment of *Allium sativum* (garlic) extract. *Res. J. Biotechnol.*, Vol. 16: 3.
- Mittal, A.K., Y. Chisti and U.C. Banerjee. 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnol. Adv.*, 31: 346-356.
- Mostafa, M., H. Ferdus, S.K. Biswas, M.A. Al Sabbir and M.M. Hossain 2026. Plant-mediated synthesis of nanoparticles for sustainable management of plant diseases. *Emerg. Nanotechnol. Agroecosyst. Manag.*, Springer.
- Murthy, H., T. Desalegn, M. Kassa, B. Abebe and T. Assefa. 2020a. Synthesis of green copper nanoparticles using medicinal plant *Hagenia abyssinica* (Brace) JF. Gmel. leaf extract: Antimicrobial properties. *J. Nanomater.*, 2020.

- Murthy, H.A., T. Desalegn, M. Kassa, B. Abebe and T. Assefa. 2020b. Synthesis of green copper nanoparticles using medicinal plant *Hagenia abyssinica* (Brace) JF. Gmel. leaf extract: Antimicrobial properties. *J. Nanomater.*, 2020: 3924081.
- Naaz, R., V.U. Siddiqui, S.U. Qadir and W.A. Siddiqi. 2021. Green synthesis of silver nanoparticles using *Syngonium podophyllum* leaf extract and its antibacterial activity. *Mater. Today: Proc.*, 46: 2352-2358.
- Nadaroglu, H., A.A. Güngör and İ. Selvi. 2017. Synthesis of nanoparticles by green synthesis method. *International Journal of Innovative Research and Reviews.*, 1: 6-9.
- Oliveira, D.G.P., G. Pauli, G.M. Mascarim and I. Delalibera. 2015. A protocol for determination of conidial viability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. *J. Microbiol. Methods.*, 119: 44-52.
- Oraibi, A., A.A. Rashad and M.H. Ahmed. 2023. Carum carvi mediated green synthesis of copper nanoparticles and its effect on *Solanum lycopersicum* seedlings. *J. Arid. Agric.*, 9: 9-15.
- Padma, P.N., S.T. Banu and S.C. Kumari. 2018. Studies on green synthesis of copper nanoparticles using *Punica granatum*. *Annu. Res. Rev. Biol.*, 23: 1-10.
- Parveen, K., V. Banse and L. Ledwani. 2016. Green synthesis of nanoparticles: Their advantages and disadvantages. AIP conference proceedings, 2016. AIP Publishing.
- Pasieczna-Patkowska, S., M. Cichy and J. Flieger. 2025. Application of Fourier transform infrared (FTIR) spectroscopy in characterization of green synthesized nanoparticles. *Molecules.*, 30: 684.
- Pecharrmán, C., A. Esteban-Cubillo, I. Montero, J.S. Moya, E. Aguilar, J. Santarén and A. Alvarez. 2006. Monodisperse and corrosion-resistant metallic nanoparticles embedded into sepiolite particles for optical and magnetic applications. *J. Am. Ceram. Soc.*, 89: 3043-3049.
- Perumal, A.B., P.S. Sellamuthu, R.B. Nambiar and E.R. Sadiku. 2016. Antifungal activity of five different essential oils in vapour phase for the control of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* *In vitro* and on mango. *Int. J. Food Sci. Technol.*, 51: 411-418.
- Rai, M., A.P. Ingle, R. Pandit, P. Paralikar, S. Shende, I. Gupta, J.K. Biswas and S.S. Da Silva. 2018. Copper and copper nanoparticles: Role in management of insect-pests and pathogenic microbes. *Nanotechnol. Rev.*, 7: 303-315.
- Ren, G., D. Hu, E.W. Cheng, M.A. Vargas-Reus, P. Reip and R.P. Allaker. 2009. Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int. J. Antimicrob. Agents.*, 33: 587-590.
- Saikia, S., B.K. Saud, P. Dutta, S. Baruah and A.K. Gogoi. 2023. Green synthesis and characterization of copper nanoparticles using different plant sources. *Int. J. Plant Soil Sci.*, 35: 37-45.
- Sarfraz, M., M.J. Nasim, C. Jacob and M.C. Gruhlke. 2020. Efficacy of allicin against plant pathogenic fungi and unveiling the underlying mode of action employing yeast based chemogenetic profiling approach. *Appl. Sci.*, 10: 2563.
- Satyral, P., J.D. Craft, N.S. Dosoky and W.N. Setzer. 2017. The chemical compositions of the volatile oils of garlic (*Allium sativum*) and wild garlic (*Allium vineale*). *Foods.*, 6: 63.
- Shah, I.H., M. Ashraf, I.A. Sabir, M.A. Manzoor, M.S. Malik, S. Gulzar, F. Ashraf, J. Iqbal, Q. Niu and Y. Zhang. 2022. Green synthesis and characterization of copper oxide nanoparticles using *Calotropis procera* leaf extract and their different biological potentials. *J. Mol. Struct.*, 1259: 132696.
- Suwanamornlert, P., S. Sangchote, W. Chinsirikul, A. Sane and V. Chonhenchob. 2018. Antifungal activity of plant-derived compounds and their synergism against major postharvest pathogens of longan fruit *In vitro*. *I. J. Food Microbiol.*, 271: 8-14.
- Thakar, M.A., S.S. Jha, K. Phasinam, R. Manne, Y. Qureshi and V.H. Babu. 2022. X ray diffraction (XRD) analysis and evaluation of antioxidant activity of copper oxide nanoparticles synthesized from leaf extract of *Cissus vitiginea*. *Mater. Today: Proc.*, 51: 319-324.
- Truong, H.T., L.C.T. Nguyen and L.Q. Le. 2023. Synthesis and antifungal activity of copper nanoparticles against *Fusarium oxysporum* pathogen of plants. *Mater. Res. Express.*, 10: 065001.
- Velsankar, K., R.M.A. Kumar, R. Preethi, V. Muthulakshmi and S. Sudhahar. 2020. Green synthesis of CuO nanoparticles via *Allium sativum* extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities. *J. Environ. Chem. Eng.*, 8: 104123.
- Wadhwa, P., S. Sharma, S. Sahu, A. Sharma and D. Kumar. 2022. A review of nanoparticles characterization techniques. *Curr. Nanomater.*, 7: 202-214.
- Zhan, X., R.A.A. Khan, J. Zhang, J. Chen, Y. Yin, Z. Tang, R. Wang, B. Lu and T. Liu. 2023. Control of postharvest stem-end rot on mango by antifungal metabolites of *Trichoderma pinnatum* LS029-3. *Sci. Hortic.*, 310: 111696.
- Zhao, X., L. Wang and Y. Wang. 2022. The preparation and mechanism of garlic-templated fluorescent nanoparticles for degradation of *Coriolus versicolor* via one-pot. *Ind. Crops Prod.*, 186: 115281.