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Green synthesis of copper bionanoparticles to control the bacterial leaf blight disease of rice

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Copper bionanoparticles were successfully synthesized using leaf aqueous extract of *Datura innoxia* from copper sulphate. Nanoparticles were characterized with the help of UV–Vis spectroscopy, Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-ray Spectroscopy and Fourier Transform Infrared Spectroscopy. FESEM analysis showed that the particles were spherical in shape with size ranging

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from 5 to 15 nm. The antimicrobial activity of copper bionanoparticles revealed that they are effective growth inhibitors against *Xanthomonas oryzae* pv. *oryzae*, the causative organism of bacterial leaf blight of paddy.

Keywords: Bacterial leaf blight, copper bionanoparticles, *Datura innoxia*, green synthesis, rice.

IN recent years, the biocidal properties of copper nanoparticles (CuNPs) are being widely used in treating wounds. They are used through processed bandages with low-cost preparation as well as exceptional physical and chemical properties[1,2]. They have industrial usage such as gas sensors, catalytic processes, high-temperature super conductors, solar cells, and so on[3,4]. Besides, their high surface-to-volume ratio makes them easily reactive to other particles, and increases their antimicrobial efficiency, thus enabling decrease in the microbial concentration by 99.9%. Green synthesis of CuNPs has been achieved by using microorganisms[5] as well as higher plants[6].

Datura innoxia (Solanaceae), the thorn apple native to America, was introduced in Africa, Asia, Australia and Europe[7]. It has whitish pubescent flowers and grows as weed in wastelands[8].

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most severe diseases of rice[9]. It is also one of the oldest known diseases, first noted by farmers in Kyushu Province, Japan around 1884 (ref. 10). It is a vascular disease that produces tannish-grey to white lesions all along the veins in rice. The disease increases with plant growth, peaking in the flowering stage, while symptoms are noted as early as at the tillering stage[11].

For synthesizing copper bionanoparticles, both the precursor and the reducing agents were mixed in a clean beaker in 1 : 1 proportion[12]. For reduction of Cu ions, 5 ml of filtered plant extract was mixed with 5 ml of freshly prepared 1 mM aqueous $CuSO_4$ solution and incubated for 1 h. The change in colour was noted from light yellow to sea green. The colour change indicates reduction into CuNPs.

The reduction of nanoparticles was monitored using the Perkin-Elmer, Lambda 35 double beam UV–visible spectrophotometer against distilled water used as a blank. The UV–visible spectrum was measured in the range 190–1100 nm. The morphology, size and elemental analysis of the prepared CuNPs were examined using Field Emission Scanning Electron Microscopy (FESEM) with EDAX (Carl Zeiss, SUPRA 55 model). Fourier Transform Infrared Spectroscopy (FTIR) spectra were obtained using a spectrophotometer (Spectrum RX 1, Perkin Elmer, Singapore) in the spectral region of 4000–400 cm^{-1}.

The biosynthesized CuNPs of aqueous extract of *D. innoxia* leaves can easily be monitored using UV–visible spectroscopy (Figure 1). Nano-sized particles exhibit unique optical properties. There is an exponential decay Mie scattering profile with decreasing photon energy. In this study the absorption peaks of copper nanoparticles ranged from 236 to 262 nm, which has been confirmed by earlier published works[13]. Nanoparticle formation is strongly indicated by increase in the peak with increase in reaction time and concentration of biological extracts with salt ions. UV–Vis absorption spectrum shows peaks in surface plasmon resonance of nano-sized particles[14,15].

Typical FESEM image shows that the product mainly consists of particle-like copper nano-clusters with panoramic view; the size ranges from 90 to 200 nm and the shape of the particles is spherical. However, further observations using high magnification revealed that these

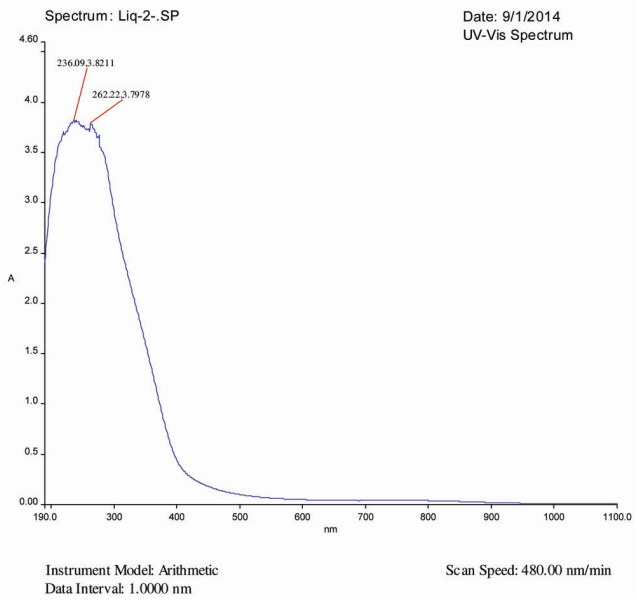


Figure 1. Ultraviolet–visible spectrum of bionanoparticles.

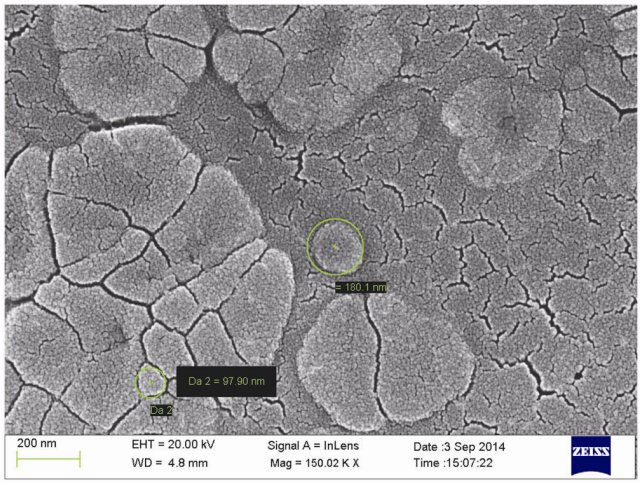
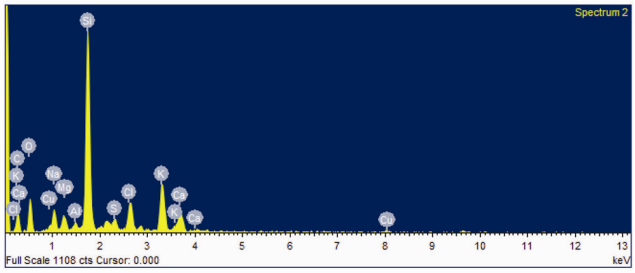


Figure 2. Field emission scanning electron microscopic image of bionanoparticles.



Element	Weight (%)	Atomic (%)
C	6.20	11.39
O	33.18	45.76
Na	4.25	4.08
Mg	2.17	1.97
Al	0.70	0.57
Si	27.25	21.41
S	1.76	1.21
Cl	6.11	3.80
K	11.06	6.24
Ca	5.00	2.75
Cu	2.31	0.80
Total	100.00	

Figure 3. Energy-Dispersive X-ray Spectroscopic image of bionanoparticles.

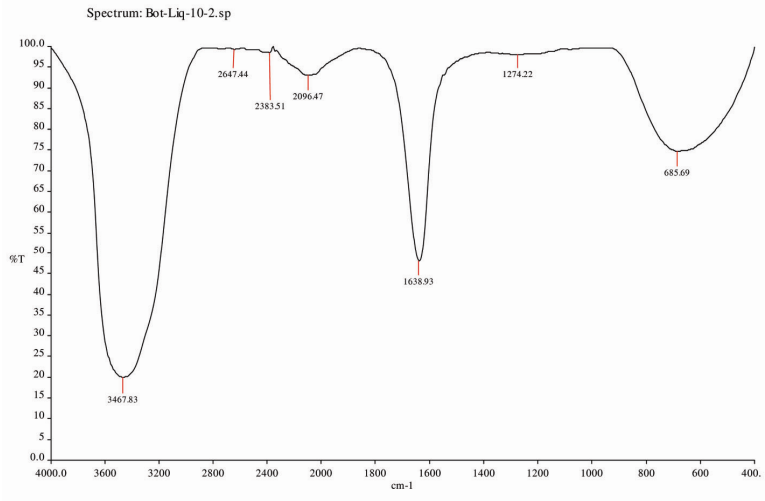


Figure 4. Fourier Transform Infrared Spectrum of bionanoparticles.

Figure 5. Antibacterial activity of *Datura innoxia* and its copper nanoparticles. ***a***, *D. innoxia* leaf extract; ***b***, Plantomycin chemical solution; ***c***, Synthesized copper nanoparticles solution.

copper nano-clusters are assembled by smaller nanoparticles, which exhibit non-uniformity, and the average diameter is about 5–15 nm (Figure 2). The particle size was calculated using the software Image J (IJ). The Energy Dispersive X-ray Spectroscopy (EDX) analysis revealed that pure copper (2.31%) was present in the solution (Figure 3). The synthesized samples were transferred to the FESEM chamber for microstructural analysis without disturbing the actual nature of the products. The FESEM images confirm that the nanoparticles have grown with well-defined morphology[16].

FTIR analysis was used to identify biomolecules that are responsible for capping and stabilization of CuNPs with *D. innoxia* leaf extract. A strong peak at 3434 cm^{-1} can be attributed to hydrogen-bonded O–H groups of alcohols and phenols, and also to the presence of amines N–H of amide (Figure 4). This peak shifted to 3467 cm^{-1} in the synthesized CuNPs. The peak at 2256 cm^{-1} can be attributed N=C=O groups of isocyanates and Si–H silane stretch mode. The bands at 1638 cm^{-1} in *D. innoxia* leaf extract could be attributed to C–H stretch mode in aldehydes. The bands at 1461 and 1374 cm^{-1} are assigned to C–H deformation mode in alkanes. The bands at 1038 cm^{-1} are assigned to C–O stretch mode in ethers, while those at 687 cm^{-1} are assigned to C–Cl stretch mode in halogens. The bands at 2096 cm^{-1} can be attributed to diazo ketones and those 2383 cm^{-1} to P–H-phosphine in the synthesized CuNPs. The bands at

Table 1. Growth inhibitory activity of *Datura innoxia* and its copper nanoparticles

Types of extract	Diameter of inhibition zone (mm)
Plant extract	17
Copper nanoparticles	24
Plantomycin	19

1274 cm^{-1} are assigned to C–H bend in plane bending bonds, and those at 685 cm^{-1} are assigned to C–H-strong vinyl mode in alkenes. This clearly indicates that the coordination of carboxylic acids in the protein of *D. innoxia* leaf extract with CuNPs plays a major role in dispersing, stabilizing and capping CuNPs. FTIR spectrum of CuNPs suggests that they are surrounded by different organic molecules such as alcohols, aldehydes and carboxylic acid[17].

Antibacterial activity of the synthesized CuNPs was studied using well-diffusion method. The inhibitory effect of nanoparticles on bacterial growth was reflected by the zone of inhibition formed and comparison with known antibiotics (Table 1). Biosynthesized CuNPs showed clear zone of inhibition against *X. oryzae* pv. *oryzae*. Zone of inhibition was 24 mm for CuNP solution, 17 mm for *D. innoxia* leaf aqueous solution and 19 mm for plantomycin solution (antibiotic). Antibacterial activity of nanoparticles was assessed based on the zone of inhibition around the well (Figure 5). The results establish that the synthesized CuNPs have significant antibacterial action due to the greater affinity of copper ions to profusely present amines and carboxyl groups on the cell surface[18]. The extremely large surface area of CuNPs provides efficient antibacterial property by binding with the DNA molecules that leads to deformation of the helical structure by cross-linking of the nucleic acid strands[19]. Copper ions inside the bacterial cells also cause disruption of other biochemical processes[20].

The present work reports an eco-friendly, clean, non-toxic and simple process for synthesis of copper bionanoparticles using *D. innoxia* leaf aqueous extract. The synthesized CuNPs are highly stable and have been observed to possess antibacterial activity against rice pathogen *X. oryzae* pv. *oryzae* isolates from bacterial leaf blight diseased rice plant.

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