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SYNTHESIS AND EVALUATION OF ANTIMICROBIAL POTENTIAL OF CUO NANOPARTICLES

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ABSTRACT

Low temperature chemical synthesis of copper oxide nanoparticles (CuO) was achieved in aqueous solution using L-Ascorbic acid as a reductant and the cationic surfactant Cetyltrimethyl ammonium bromide (CTAB) as a capping agent to assessed their antimicrobial potential against phytopathogens.CuO nanoparticles shown antibacterial activity against all the tested bacterial cultures *viz*., Escherichia coli (12.87±1.80), *Proteus sp.* (12.5±2.39), *Pseudomonas aeruginosa* (7.3±1.92), *Staphylococcus* (23.87±2.1), *Xanthomonas axonopodis pv. punicae* (21.7± 2.65); similarly antifungal activity against fungal cultures *viz*., *Aspergillus niger* (15.62±1.06), *Alternaria sesami* (22.25±2.31), Colletotrichum lindemunthianum (11.6±1.92), *Fusarium udum (*17.5±2.20*).* The maximum activity of CuO nanoparticles was found against *Streptococcus aureus* (23.875±2.1) while the minimum activity was found against *Pseudomonas aeruginosa* (7.3 ±1.92). Copper sulphate, L-Ascorbic acid did not shown any antimicrobial activity where as CTAB shown relatively less activity as compared to CuO nanopaticles. The synthesized cupper nanoparticles will presumably useful in formulation of various biopesticides and ecologically feasible effective management strategy against harmful pathogenic microorganism.

INTRODUCTION

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm in one dimension. Remarkable growth in this up and coming technology has opened novel fundamental and applied frontiers, including the synthesis of nanoscale materials and exploration or utilization of their exotic physicochemical and optoelectronic properties.

Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug gene delivery, energy science, optoelectronics, catalysis, reorography, single electron transistors, light emitters, nonlinear optical devices, and photoelectron chemical applications (Wang,1991; Colvin, 1994). Research on the synthesis of nano sized material is of great interest because of their unique properties like optoelectronic, magnetic, and mechanical, which differs from bulk (Ingole *et al*., 2010).

Currently, many nanoparticles are synthesized from noble metals such as gold and silver, in spite of their high cost. However, copper is a promising alternative material due to its high conductivity and lower cost. In particular, copper nanoparticles have received considerable attention because of their potential use in nanomaterials, thermal conducting applications, lubrication, nano fluids, and catalysts (Lu *et al*., 2000). In these applications, control of the size, shape, and structure of copper nanoparticles are sensitive to reaction conditions.

In this work, an attempt has been made to analyse formation of CuO nanoparticles. The shape and size of these particles was investigated using particle size analyzer. Further, the antimicrobial potential of CuO nanoparticles was also evaluated.

MATERIALS AND METHODS

Analytical grade precursor salt Copper sulphate ($CuSO_4 \cdot 5H_2O$), L-Ascorbic acid ($C_6H_8O_6$), Cetyltrimethyl ammonium bromide (CTAB), and NaOH were used as received without further purification.

Synthesis of CuO nanoparticles

In a typical experiment, 0.01M $CuSO_4 \cdot 5H_2O$ and 0.11M Ascorbic acid were dissolved in 100 ml of deionised water in a 250 ml beaker flask equipped with hot water bath, a small magnetic stirrer and a thermometer. 0.03M CTAB was introduced into the solution with rapid stirring at room temperature. An aqueous NaOH was employed to adjust pH to 6.5. The mixture was agitated at 85ºC without any inert gas protection. In this process, the white coloured mixture turned brick red, then reddish. CuO nanoparticles were separated by filtration with Watman paper no.5 and filtrate containing nanoparticles collected in separate beaker.

Characterization of CuO Nanoparticles

Absorption spectra of copper nanoparticle colloids were measured using a Hitachi U-2900 UV-VIS spectrophotometer at resolution on 1nm from 200nm to 800nm wavelength range. Size of the CuO nanoparticles were measured by Particle size Analyser.

Antimicrobial activity of CuO nanoparticles

The antimicrobial activity of the nanoparticles was tested against a five bacterial pathogens *viz.*, *Proteus vulgaris, Xanthomonas axonopodis pv. punicae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and four fungal pathogens *viz.*, *Alternaria sesami, Colletotrichum lindemuthianum, Fusarium udum, Aspergillus niger*.

Antimicrobial activity was evaluated by well diffusion method. For antibacterial activity 100 μl bacterial culture and fungal spore suspension were spread on nutrient and potato dextrose agar media respectively. 15 μl CuO nanoparticle solution was filled in wells. Similarly, 15μl copper sulphate, L-Ascorbic acid and CTAB solutions were also added in wells to measure the control activity. Zone of inhibition was observed after 24 hours in bacterial and 3-4 days in fungal cultures.

RESULTS AND DISCUSSION

Synthesis of CuO nanoparticles

Low temperature chemical synthesis of copper oxide nanoparticles was achieved in aqueous solution using ascorbic acid as a reductant and the cationic surfactant Cetyltrimethyl ammonium bromide (CTAB) as a capping agent. The color of reaction mixture was changed from white to brick red. This indicates synthesis of CuO nanoparticles (Figure 1).

Characterization of CuO nanoparticles

The UV-VIS absorption spectra of nanoparticle showed a sharp and narrow absorption peak at 293 nm (574-200nm), which can be attributed to absorption by nanosize copper oxide particle, confirming the presence of nanoparticles copper (Figure 2), and are in good agreement with other studies reporting characteristics of Cu oxide nanoparticles (Bicer and Sisman, 2010). The absence of a characteristic absorption band for copper oxide at 800 nm was also noteworthy.

Size of the CuO nanoparticles was found to be 46 nm and total concentration is 2.92 particles/frame (0.11E8 particles/ml) (Figure 3). Ethiraj *et al.* (2012) reported a successful synthesis of copper oxide nano wires with an average diameter of 90 nm and lengths of several micrometers by using a simple and inexpensive wet chemical method whereas smaller size ~3-5nm was reported by Nahhal *et al.* (2012), Ritu *et al.* (2013), Shaffiey *et al.* (2014).

Table 1: Antimicrobial activity of CuO nanoparticles.

Sr. No. Bacteria	Name of the microorganism	Diameter of zone of inhi bition (mm)
1.	*Escherichia coli*	12.87±1.80
2.	*Proteus vulgaris*	12.5±2.39
3.	*Pseudomonas aeruginosa*	7.3± 1.92
4.	*Staphylococcus aureus*	23.875 ± 2.1
5.	*Xanthomonas axonopodis pv. punicae*	21.7± 2.65
Fungi		
1.	*Aspergillus niger*	15.62±1.06
2.	*Alternaria sesame*	22.25± 2.31
3.	*Colletotrichum lindemuthianum*	11.6±1.92
4.	*Fusarium udum*	17.5±2.20
Control		
1.	CTAB	3.75±0.88
2.	L-Ascorbic acid	—
3.	$CuSO_4 \cdot 5H_2O$	—

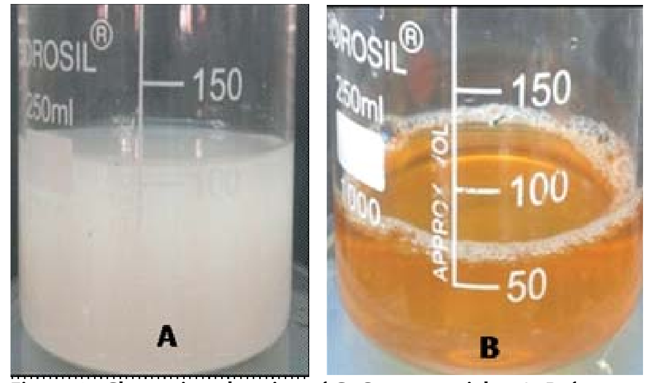

Figure 1: Change in coloration of CuO nanoparticles: A: Before and B: After reaction respectively.

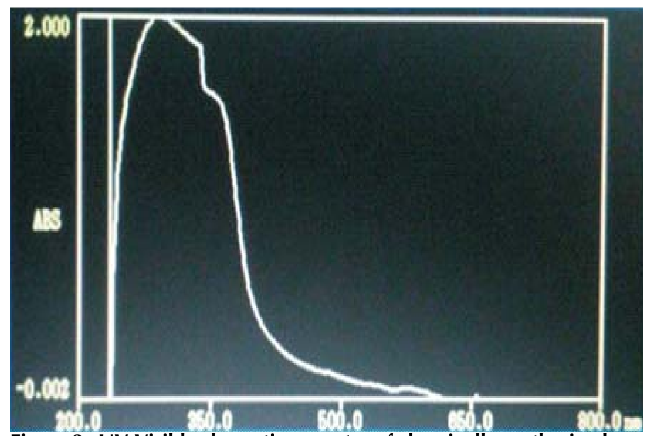

Figure 2: UV-Visible absorption spectra of chemically synthesized CuO nanoparticles.

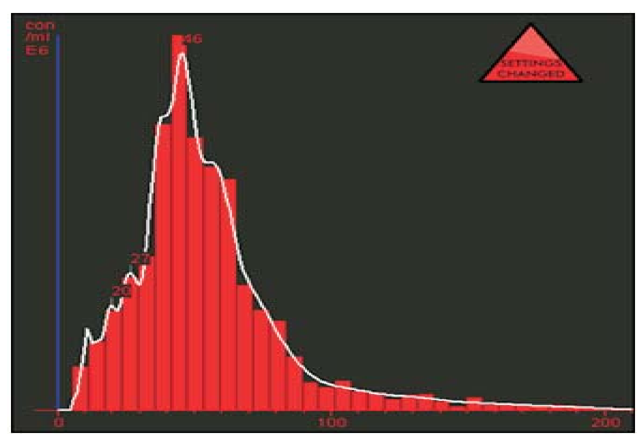

Figure 3: Particle size spectra of CuO nanoparticles.

Antimicrobial activity of copper oxide (CuO) nanoparticles

CuO nanoparticles possess potent antimicrobial activity and in comparative study it was found significant as compared to control. CuO nanoparticles shown antibacterial activity against

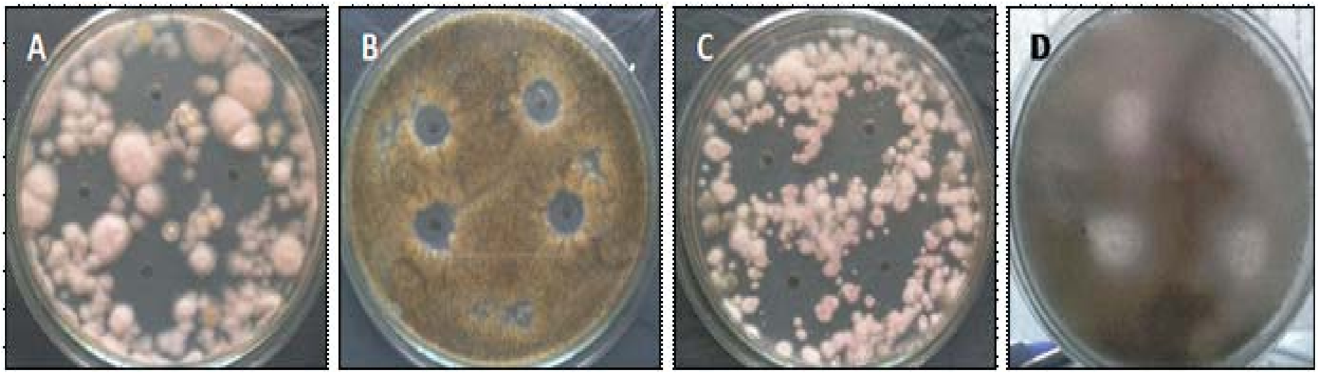

Figure 5: Antifungal activity of copper oxide nanoparticles against A-*Alternaria sesami*, B-*Colletotrichum lindemuthianum*, C-*Fusarium udum*, D-*Aspergillus niger*.

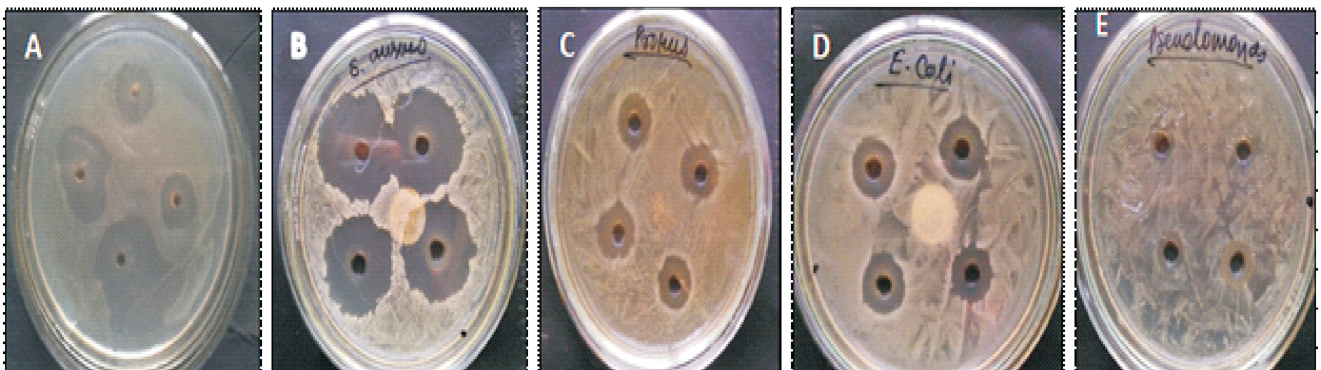

Figure 4: Antibacterial activity of copper oxide nanoparticles against A-*Xanthomonas axonopodis pv. punicae*, B- *Staphylococcus aureus*, C- *Proteus* sp., D- *Escherichia coli*, E- *Pseudomonas aeruginosa*.

all the tested bacterial cultures *viz*., *Escherichia coli* (12.87 ± 1.80), *Proteus vulgaris* (12.5 ± 2.39), *Pseudomonas aeruginosa* (7.3 ± 1.92), *Staphylococcus aureus* (23.875 ± 2.1), *Xanthomonas axonopodis pv. punicae* (21.7 ± 2.65) (Figure 4), similarly antifungal activity against fungal cultures *viz*., *Aspergillus niger* (15.62 ± 1.06), *Alternaria sesami* (22.25 ± 2.31), *Colletotrichum lindemuthianum* (11.6 ± 1.92), *Fusarium udum* (17.5 ± 2.20) (Figure 5). These results of CuO nanoparticles synthesis and their antimicrobial activity are in accordance with the other scientists (Vojislav *et al*., 2010; Nahhal *et al*., 2012; Abboud and Saffaj, 2013; Shaffiey *et al*., 2014; Maqusood Ahamed *et al*., 2014).

The maximum antimicrobial activity of CuO nanoparticles was found against *Staphylococcus aureus* (23.875 ± 2.1) while the minimum activity was found against *Pseudomonas aeruginosa* (7.3 ± 1.92) (Table no. 1). Control activity of copper sulphate, L-Ascorbic acid and CTAB was also tested. Copper sulphate, L-Ascorbic acid did not shown antimicrobial activity where as CTAB shown relatively less activity as compared to CuO nanoparticles. The potent antibacterial activity of CuO nanoparticles was found to be due to ROS-generation by the nanoparticles attached to the bacterial cells, which in turn provoked an enhancement of the intracellular oxidative stress (Applerot *et al*., 2012).

The synthesized cupper nanoparticles in this study will presumably useful in formulation of various biopesticides and ecological feasible effective management strategy against harmful pathogenic microorganism.

These nanoparticles can be used for formulations of agriculturally important insecticides or more effectively controlling human as well as phytopathogens. Further study in this respect may help to synthesize effective formulations than day today used.

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