

Synthesis, Characterization and *in vitro* Antifungal Action of Gum Ghatti Capped Copper Oxide Nanoparticles

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Abstract: Copper oxide nanoparticles gained significance in agriculture for their antifungal action on various fungal plant pathogens. In this context, gum ghatti capped copper oxide nanoparticles (CuO NP) were generated by optimizing metal precursor conc. Synthesized NP were analyzed with different instrumental techniques such as UV-vis spectroscopy (UV-vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering, and transmission electron microscopy. NP showed characteristic absorption at 280 nm in UV-vis spectra and indicated monoclinic crystal structure. FTIR spectra confirmed the capping of NP by functional groups of the gum. Produced NP were spherical and showed a mean particle size of 4.5 ± 1 nm. The *in vitro* antifungal action of the gum-capped NP (25-100 $\mu\text{g/mL}$) was determined using resazurin broth assay against mycelia and sclerotia of *Rhizoctonia solani*, a soil and seed-borne fungal plant pathogen. Minimum inhibitory concentration (MIC) values ranged from 50-75 and 75-100 $\mu\text{g/mL}$, respectively, for mycelia and sclerotia of *R. solani* strains. NP inhibited the mycelial growth and sclerotial germination by 41.4-59% and 46.8-69.3% at respective MIC values. Therefore, the present report paves the way for the production of CuO NP-based fungicidal formulations towards the control and management of different fungal diseases in a broad range of crops.

Keywords: antifungal; copper oxide nanoparticles; gum ghatti; plant disease; plant pathogen; *Rhizoctonia solani*

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1. Introduction

The different copper nanomaterials, such as copper oxide nanoparticles (CuO NP) are widely employed in agriculture to control and manage various plant diseases caused by fungal pathogens [1]. It is mainly due to their facile synthetic methodologies, controllable size and morphologies, versatile applications, and cheaper cost than their counterparts, silver nanoparticles [2-7]. The CuO NP are used as a fungicide against an array of phytopathogenic fungi infecting a broad range of host crops including, cereals, vegetables, fruits, trees, etc. in comparison with other macro copper formulations including, Bordeaux and Burgundy mixtures; and copper sulfate due to the slow copper ion release [8]. Also, the copper nanomaterials gained wide acceptance as antifungal agents due to higher solubility and bioavailability; and enhanced activity at lower copper dosage [1,9-12].

In literature, various colloidal thermal synthesis processes were adapted for the synthesis of CuO NP at alkaline pH using different plant extracts such as tree gum [13], leaf

[14], flower [15], bacteria [16], fungi [17,18], etc. In this scenario, the CuO NP were synthesized using the proteinaceous, edible, exudate tree gum, gum ghatti utilizing its renewability, biodegradability, nontoxicity, and low-cost nature [19]. The gum ghatti was extensively employed for the synthesis of silver [20], gold [21], palladium [22], platinum [23], iron oxide [24] and titanium dioxide [25] nanoparticles.

The synthesized ghatti gum capped CuO NP were analyzed with UV-vis spectroscopy (UV-vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and transmission electron microscopy (TEM). Further, the *in vitro* antifungal action of the CuO NP at variable concentrations was evaluated against the mycelia and sclerotia of various strains of *Rhizoctonia solani* using the resazurin broth assay. The fungus *R. solani* is a soil and seed-borne fungal plant pathogen responsible for sheath blight disease in rice [26].

2. Materials and Methods

2.1. Materials.

Gum ghatti (Girijan Co-operative Corporation Ltd., Hyderabad, India), sodium hydroxide, copper chloride dihydrate (SD Fine, Mumbai, India), potato dextrose agar (PDA), potato dextrose broth (PDB), resazurin (HiMedia, Mumbai, India), propiconazole (Sigma-Aldrich, Bengaluru, India) were used during this work. The gum, salt, and other needed solutions were made with ultra-pure water. Autoclave sterilized plasticware, glassware and media were utilized throughout the experimentation.

2.2. Synthesis of copper oxide nanoparticles (CuO NP).

As reported earlier, 0.5% of gum aqueous solution was prepared [23]. The CuO NP were prepared by autoclaving the 0.5% gum solution at pH 12 by varying the copper chloride concentration (1-10 mM) at 121°C and 15 psi for 30 min with slight modifications [13,23].

2.3 Characterization of CuO NP

The UV-visible (UV-vis) absorption spectra of the produced CuO NP solutions were measured from 250-800 nm against the autoclaved gum blank (Analytik Jena AG, Specord 200 Plus, Jena, Germany). The X-ray diffraction (XRD) pattern of the glass slide deposited NP was acquired (Rigaku, Ultima IV diffractometer, Tokyo, Japan). The infrared (IR) spectra of the lyophilized gum and NP were measured (Bruker Optics, TENSOR 27, Ettlingen, Germany). The hydrodynamic radius of NP and zeta potential of the synthesized NP solutions nanoparticles were recorded (Malvern, Zetasizer Nano ZS90, Malvern, UK). The transmission electron microscope (TEM) images of the NP solution were obtained (FEI, Tecnai 20 G2 S-Twin, Eindhoven, Netherlands) [23].

2.4. Antifungal activity of synthesized CuO NP.

The *in vitro* antifungal action of the synthesized CuO NP was studied against the mycelia and sclerotia of *Rhizoctonia solani* strains (TS-06, TS-10, TS-14, TS-20, TS-22, TS-24) with resazurin assay [26]. The potato dextrose broth (PDB) tubes (2 mL) were augmented with varying concentrations of CuO NP (50-100 µg/mL), and the tubes were inoculated with 2 mm mycelial agar plug from 3 days old actively growing fungus on potato dextrose agar (PDA).

The negative and positive and control PDB tubes were kept with 0.5% autoclaved gum and propiconazole (100 $\mu\text{g}/\text{mL}$), respectively. After 24 h of growth at 27°C, 50 μl of resazurin dye (200 $\mu\text{g}/\text{mL}$) was added into the tubes and further incubated. After a total incubation of 48 h, the tubes were noted for hyphal growth and color change. The fungal growth inhibition was revealed from blue, purple, faint yellow, or yellow color, and live fungi were signified from the pink color of tubes. The minimum inhibitory concentration (MIC) values were obtained based on fungal growth inhibition at tested NP concentrations. The absorption of the tubes was measured at 500 and 600 nm, and the mycelial growth inhibition (%) and sclerotial germination inhibition (%) were calculated [27]. A similar method was adapted for sclerotia (2-3 mg fresh weight) collected from PDA-grown fungus for 7 days. The assay was repeated three times.

3. Results and Discussion

3.1. Synthesis and characterization of CuO NP.

The CuO NP were synthesized by autoclaving the 0.5% gum solution supplemented with variable concentration of copper chloride (1-10 mM) at 121°C and 15 psi for 30 min. The color of the reaction mixture was blue before autoclaving and turned brownish-black during autoclaving, confirming the synthesis of CuO NP [13] (inset of Figure 1). The CuO NP solutions synthesized at 1 mM and 10 mM were faint brown and dark blue, indicating low and incomplete synthesis. Further, these solutions were precipitated with time, and the corresponding UV-vis absorption spectra showed continuous wide absorption. While the CuO NP synthesized at 5 mM of copper chloride showed brownish-black coloration and remained stable in the solution with no precipitation (inset of Figure 1). The solutions of CuO NP synthesized at 5 mM concentration were diluted 4 times, and the collected UV-vis spectrum showed a prominent absorption peak at 275 nm (Figure 1). The recorded absorption peak concurs with earlier studies on CuO NP biosynthesized with leaf extracts [14,28].

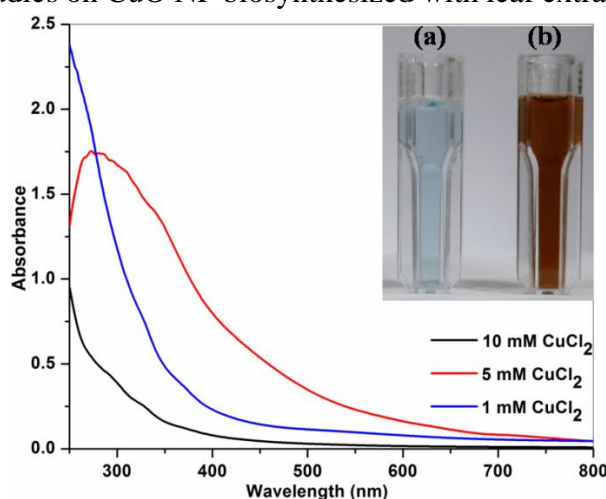


Figure 1. The UV-visible absorption spectra of copper oxide nanoparticles synthesized by autoclaving at different concentrations (1-10 mM) of copper chloride and 0.5% gum at pH 12. Inset: Color of the reaction mixture of 0.5% gum and 5 mM copper chloride (a) before and (b) after autoclaving.

The XRD pattern of CuO NP synthesized at an optimal concentration of 5 mM showed distinctive diffraction peaks at 35.2°, 38.7° and 49.0° corresponding to (022), (111), and (-202) planes of the characteristic monoclinic crystal structure of CuO (JCPDS-05-0661) (Figure 2) [13].

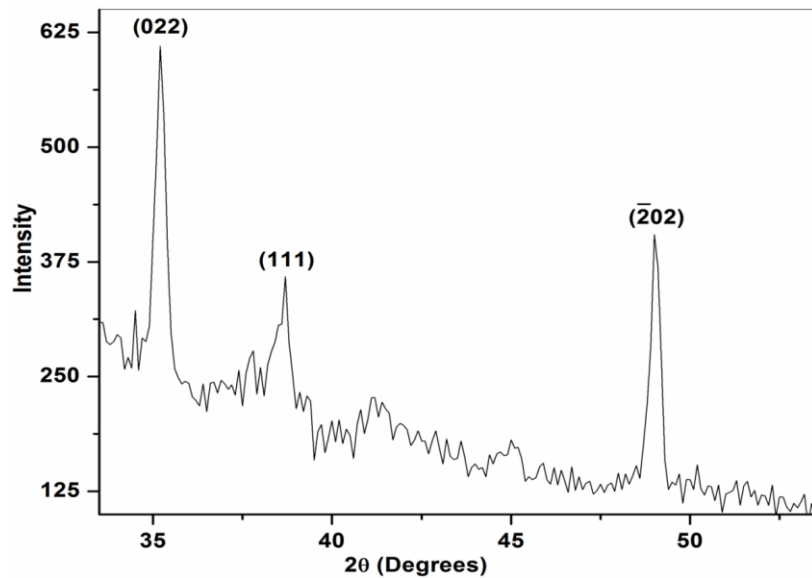


Figure 2. The XRD pattern of gum-capped copper oxide nanoparticles.

The IR spectrum of gum showed typical absorbance peaks at 3421 (O-H group) 2920, 2855 (asymmetric stretching, scissoring; and symmetric stretching vibrations of methylene groups), 2318 (different carbonyl species), 1736 (carbonyl stretching vibrations of aldehydes, ketones, and carboxylic acid), 1632 (asymmetrical carboxylate stretch and amide I linkage of protein), 1429 (the symmetrical stretch of carboxylate groups) and 1035 cm^{-1} (C-O stretch of the alcoholic group) [23]. The capped CuO NP revealed absorbance bands at 3437, 2922, 2853, 2359, 1738, 1635, 1457, 1417, 1318 and 1030 cm^{-1} (Figure 3). The NP demonstrated major peak shift (3421 to 3437 cm^{-1} , 2318 to 2359 cm^{-1} and 1429 to 1417 cm^{-1}), minor peak shift (1035 to 1030 cm^{-1}), and new peak emergence (1318 cm^{-1}), confirming the binding of copper ions with gum's hydroxyl, carbonyl and carboxylate groups [23,29].

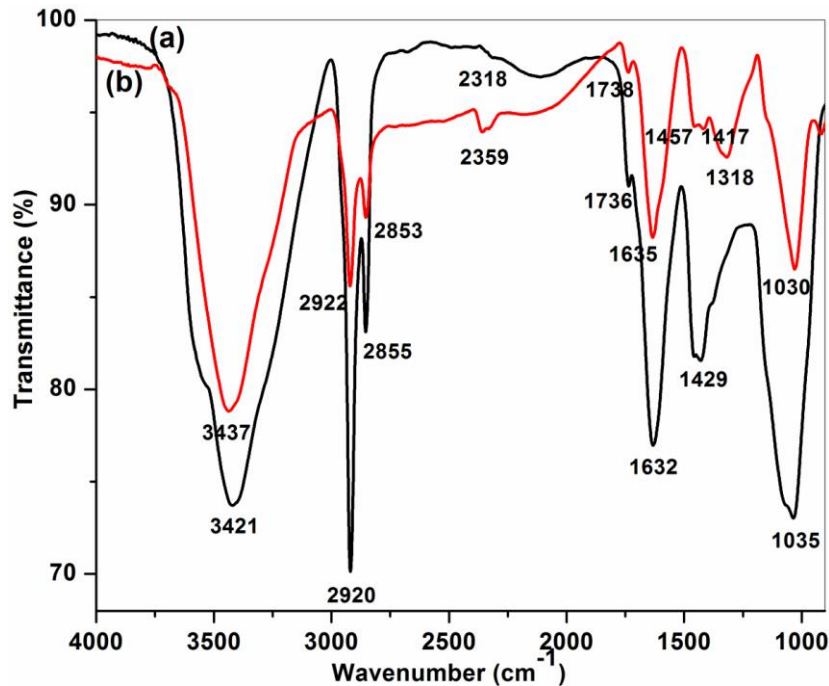


Figure 3. The FTIR spectra of (a) gum and (b) gum capped copper oxide nanoparticles.

The CuO NP showed a hydrodynamic radius of 80 nm and a zeta potential value of -25.7 mV, respectively (Figure 4).

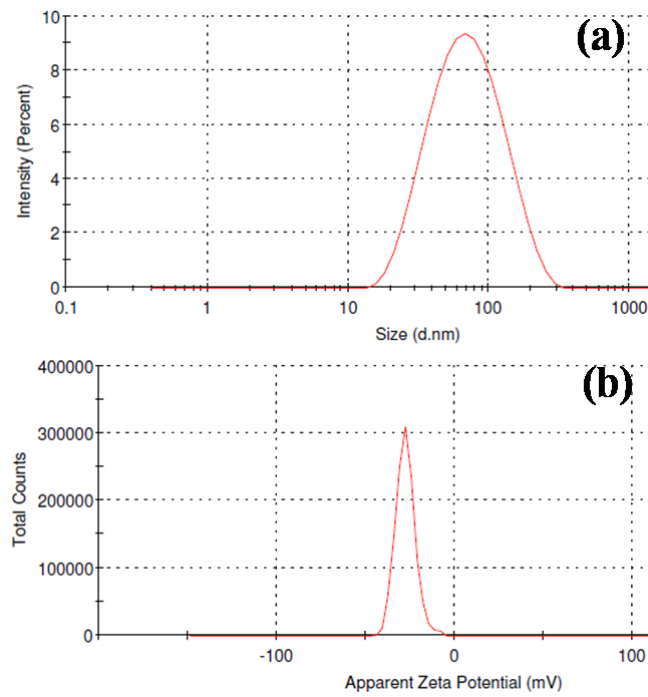


Figure 4. The (a) particle size distribution and (b) zeta potential of synthesized copper oxide nanoparticles.

The TEM photographs revealed the spherical morphology of the produced NP. The particle size ranged from 3.6 - 9.3 nm, and the mean particle size was 4.5 ± 1 nm. The crystal character of the fabricated CuO NP was also corroborated from the selected area electron diffraction (SAED) pattern (Figure 5). The particle size of CuO NP achieved in the present report is compared with the previous studies on biosynthesized CuO NP. The CuO NP of 60-100 nm size were fabricated with aqueous spinach leaf extract [30]. The *Piper betle* leaf extract synthesized CuO NP showed a particle size of 50-100 nm [31]. At the same time, the CuO NP of 10-70 nm were generated by bacterial-mediated synthesis with *Pseudomonas fluorescens*[32]. The leaf extract synthesized CuO NP of 34 nm and 68 nm were fabricated with *Prosopis juliflora* and *Pluchea sericea* [33]. The CuO NP of 20-35 nm were biosynthesized with flower extract of *Stachys lavandulifolia* [15]. The CuO NP with a mean size of 28 nm were biosynthesized with extract of *Eichhornia crassipes* leaves [34]. The CuO NP of 27.5 nm size were fabricated employing leaf and stem ash of *Seidlitzia rosmarinus* [35]. The CuO NP of 20 nm were green synthesized by the leaf extract of *Aloe barbadensis* [14]. The cell-free culture supernatant of *Streptomyces* produced the biogenic CuO NP in the range of 5-14 nm [16]. The CuO NP of 7.8 nm size were prepared with another tree gum, karaya [13]. While in the current study CuO NP of 4.5 nm were synthesized.

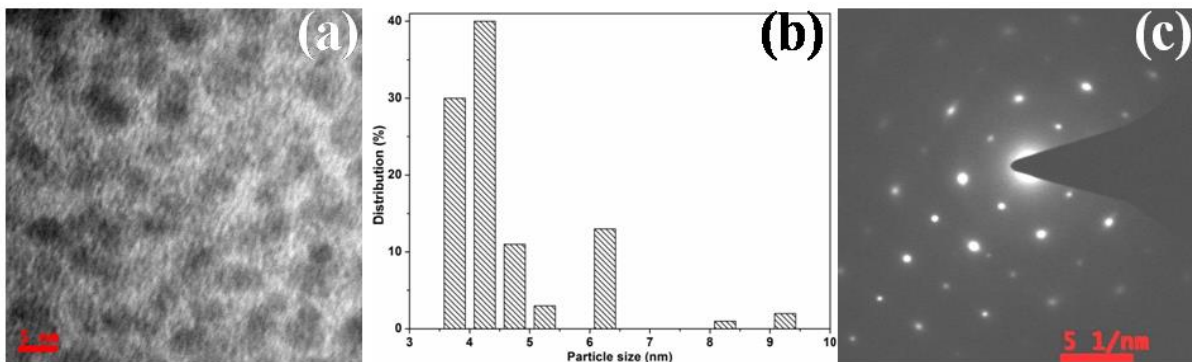


Figure 5. The TEM images of gum-capped copper oxide nanoparticles at (a)5 nm, (b) particle size histogram, and (c) corresponding selected area electron diffraction (SAED) pattern.

3.2. Antifungal evaluation of CuO NP.

The *in vitro* antifungal activity of the produced CuO NP (25-100 µg/mL) was evaluated with the resazurin broth method towards the mycelia and sclerotia of different strains of *Rhizoctonia solani*, a soil and seed-borne fungal plant pathogen causing sheath blight disease in rice. Among the other antifungal evaluation techniques, the resazurin reduction method is a simple, rapid, sensitive, visual colorimetric, non-toxic, stable, liquid-based viable cell detection assay that is amenable to automation and serves as an indicator of microbial respiration [27]. The minimum inhibitory concentration (MIC) values were obtained from the PDB color change after 48 h incubation. The values ranged from 50-75 and 75-100 µg/mL, correspondingly towards mycelia and sclerotia of various *R. solani* strains (Figure 6). The NP inhibited both mycelial growth and sclerotial germination. The inhibition (%) ranged from 41.4-59% and 46.8-69.3% at 50-75 and 75-100 µg/mL for the respective mycelia and sclerotia. The positive control, propiconazole (100 µg/mL), completely inhibited hyphal growth and sclerotial germination (Table 1).

The utilization of elemental copper and copper-based compounds as antimicrobials were approved by the US Environmental Protection Agency (EPA), and the copper nanocomposites are commercially utilized in food packaging [36], paints, textiles, fabrics, agriculture, etc. [11]. The copper-based nanocomposites are exploited as bactericides; and fungicides towards various phytopathogenic fungi, including *Alternaria*, *Macrophomina*, *Fusarium*, *Penicillium*, *Colletotrichum*, *Rhizoctonia*, *Phytophthora*, *Botrytis* etc. because of their capability to cross biological barriers interact with target organelles [14,20,28,29]. The antifungal results obtained in the present report study were weighed against earlier research carried out with CuO NP. In the case of *Penicillium*, the CuO NP of 40 nm inhibited mycelial growth at 150 mg/mL [37]. The CuO NP (30 nm) treatment at 10000 µg/mL inhibited the wood decay caused by *Trametes versicolor* [38]. At 100 µg/mL, the 28 nm-sized CuO NP inhibited the phytopathogenic fungi, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium oxysporum*, and *F. culmorum* [34]. In-field studies, 14 nm-sized CuO NP decreased the necrosis of tomato after 10 days of treatment at 277.8 µg/mL [1]. Thus, the results achieved in the present report are comparable with previous antifungal studies. In sclerotia-forming phytopathogenic fungi such as *R. solani* and *Sclerotium rolfii*, the fungicidal action of copper nanomaterials was attributed to cytoplasmic loss cytoplasmic coagulation, deformation, and destruction of fungal hyphae [39].

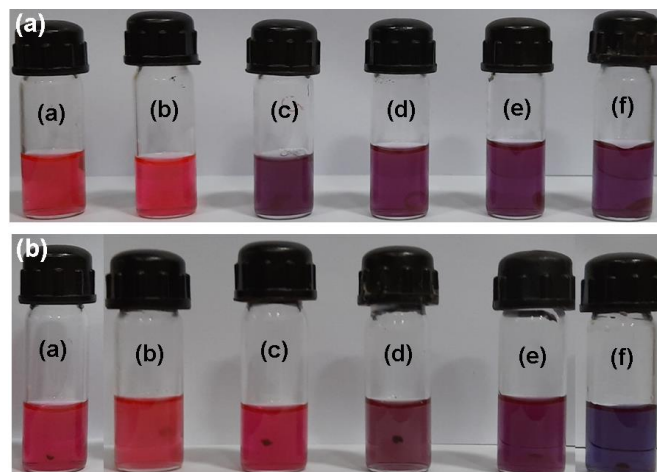


Figure 6. The antifungal action of CuO NP towards (a) mycelia and (b) sclerotia of *R. solani* TS-20 strain in resazurin broth assay at, (a) 0, (b) 25, (c) 50, (d) 75 and (e) 100 µg/mL; and (f) propiconazole (100 µg/mL).

Table 1. The minimum inhibitory concentration (MIC) values of CuO NP towards mycelia and sclerotia of *R. solani* strains obtained from the resazurin broth assay.

Strain	Mycelia		Sclerotia	
	MIC ($\mu\text{g/mL}$)	Mycelial inhibition (%)	MIC ($\mu\text{g/mL}$)	Sclerotial germination inhibition (%)
TS-06	50	50.8 \pm 0.7	100	69.3 \pm 1.0
TS-10	75	44.2 \pm 0.6	100	48.4 \pm 0.7
TS-14	75	59.0 \pm 0.8	100	60.4 \pm 0.9
TS-20	50	54.7 \pm 0.8	75	67.4 \pm 1.0
TS-22	50	41.4 \pm 0.6	100	54.1 \pm 0.8
TS-24	50	44.1 \pm 0.6	100	46.8 \pm 0.7

4. Conclusions

The current paper synthesized gum ghatti capped CuO NP of 4.5 nm by a facile autoclaving method. The *in vitro* antifungal action of synthesized CuO NP was determined towards mycelia and sclerotia of various *R. solani* strains using the facile, visual resazurin method. At MIC values of 50-75 and 75-100 $\mu\text{g/mL}$, the NP inhibited the mycelial growth (41.4-59%) and the sclerotial germination (46.8-69.3%), respectively. Thus, the present report serves as a green method for producing CuO NP-based fungicides towards the control and management of different fungal diseases in a broad range of crops. However, further studies are warranted on the role of NP particle size, shape, capping, oxidation state, etc., on antifungal action and mode of antifungal activity.

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Conflicts of Interest

The author declares no conflict of interest.

References

1. Giannousi, K.; Avramidis, I.; Dendrinou-Samara, C. Synthesis, characterization and evaluation of copper based nanoparticles as agrochemicals against *Phytophthora infestans*. *RSC Advances* **2013**, *3*, 21743, <https://doi.org/10.1039/c3ra42118j>.
2. Awwad, A.M.; Albiss, B. Biosynthesis of colloidal copper hydroxide nanowires using *Pistachio* leaf extract. *Advanced Materials Letters* **2015**, *6*, 51-54, <https://doi.org/10.5185/amlett.2015.5630>.
3. Ighalo, J.O.; Sagboye, P.A.; Umenweke, G.; Ajala, O.J.; Omoarukhe, F.O.; Adeyanju, C.A.; Ogunniyi, S.; Adeniyi, A.G. CuO nanoparticles (CuO NPs) for water treatment: A review of recent advances. *Environmental Nanotechnology, Monitoring & Management* **2021**, *15*, 100443, <https://doi.org/10.1016/j.enmm.2021.100443>.
4. Wang, R.; Li, X.; Nie, Z.; Zhao, Y.; Wang, H. Metal/metal oxide nanoparticles-composited porous carbon for high-performance supercapacitors. *Journal of Energy Storage* **2021**, *38*, 102479, <https://doi.org/10.1016/j.est.2021.102479>.
5. Attia, N.F.; Elashery, S.E.A.; Oh, H.; Ehrmann, A.; Nguyen, T.A.; Nguyen Tri, P. Chapter 9 - Nanomaterials-based antibacterial textiles. In *Nanosensors and nanodevices for smart multifunctional textiles* **2021**, *2021*, 135-147, <https://doi.org/10.1016/B978-0-12-820777-2.00009-1>.

6. Nasrollahzadeh, M.; Akbari, R.; Sakhaei, S.; Nezafat, Z.; Banazadeh, S.; Orooji, Y.; Hegde, G. Polymer supported copper complexes/nanoparticles for treatment of environmental contaminants. *Journal of Molecular Liquids* **2021**, *330*, 115668, <https://doi.org/10.1016/j.molliq.2021.115668>.
7. Mondal, A.; Debnath, P.; Mondal, N.K.; Bhattacharyya, S.; Mondal, N.K.; Platos, J.; Snášel, V.; Krömer, P. 3 - Nanoparticles: A new tool for control of mosquito larvae. In *Intelligent Environmental Data Monitoring for Pollution Management*, Eds. Academic Press: **2021**, *2021*, 49-70, <https://doi.org/10.1016/B978-0-12-819671-7.00003-8>.
8. Keller, A.A.; Adeleye, A.S.; Conway, J.R.; Garner, K.L.; Zhao, L.; Cherr, G.N.; Hong, J.; Gardea-Torresdey, J.L.; Godwin, H.A.; Hanna, S., et al. Comparative environmental fate and toxicity of copper nanomaterials. *NanoImpact* **2017**, *7*, 28-40, <https://doi.org/10.1016/j.impact.2017.05.003>.
9. Ponnurugan, P.; Manjukarunambika, K.; Elango, V.; Gnanamangai, B.M. Antifungal activity of biosynthesised copper nanoparticles evaluated against red root-rot disease in tea plants. *Journal of Experimental Nanoscience* **2016**, *11*, 1019-1031, <https://doi.org/10.1080/17458080.2016.1184766>.
10. Kanhed, P.; Birla, S.; Gaikwad, S.; Gade, A.; Seabra, A.B.; Rubilar, O.; Duran, N.; Rai, M. *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Materials Letters* **2014**, *115*, 13-17, <https://doi.org/10.1016/j.matlet.2013.10.011>.
11. Rai, M.; Ingle Avinash, P.; Pandit, R.; Paralikar, P.; Shende, S.; Gupta, I.; Biswas Jayanta, K.; da Silva Silvio, S. Copper and copper nanoparticles: Role in management of insect-pests and pathogenic microbes. *Nanotechnology Reviews* **2018**, *7*, 303-315, <https://doi.org/10.1515/ntrev-2018-0031>.
12. Waris, A.; Din, M.; Ali, A.; Ali, M.; Afridi, S.; Baset, A.; Ullah Khan, A. A comprehensive review of green synthesis of copper oxide nanoparticles and their diverse biomedical applications. *Inorganic Chemistry Communications* **2021**, *123*, 108369, <https://doi.org/10.1016/j.inoche.2020.108369>.
13. Padil, V.V.T.; Černík, M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. *International Journal of Nanomedicine* **2013**, *8*, 889-898, <https://doi.org/10.2147/IJN.S40599>.
14. Gunalan, S.; Sivaraj, R.; Venkatesh, R. *Aloe barbadensis* Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: Optical properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2012**, *97*, 1140-1144, <https://doi.org/10.1016/j.saa.2012.07.096>.
15. Veisi, H.; Karmakar, B.; Tamoradi, T.; Hemmati, S.; Hekmati, M.; Hamelian, M. Biosynthesis of CuO nanoparticles using aqueous extract of herbal tea (*Stachys Lavandulifolia*) flowers and evaluation of its catalytic activity. *Scientific Reports* **2021**, *11*, 1983, <https://doi.org/10.1038/s41598-021-81320-6>.
16. Bukhari, S.I.; Hamed, M.M.; Al-Agamy, M.H.; Gazwi, H.S.S.; Radwan, H.H.; Youssif, A.M. Biosynthesis of copper oxide nanoparticles using *Streptomyces* MHM38 and its biological applications. *Journal of Nanomaterials* **2021**, *2021*, 6693302, <https://doi.org/10.1155/2021/6693302>.
17. Priyadarshini, E.; Priyadarshini, S.S.; Cousins, B.G.; Pradhan, N. Metal-fungus interaction: Review on cellular processes underlying heavy metal detoxification and synthesis of metal nanoparticles. *Chemosphere* **2021**, *274*, 129976, <https://doi.org/10.1016/j.chemosphere.2021.129976>.
18. Paiva-Santos, A.C.; Herdade, A.M.; Guerra, C.; Peixoto, D.; Pereira-Silva, M.; Zeinali, M.; Mascarenhas-Melo, F.; Paranhos, A.; Veiga, F. Plant-mediated green synthesis of metal-based nanoparticles for dermatopharmaceutical and cosmetic applications. *International Journal of Pharmaceutics* **2021**, *597*, 120311, <https://doi.org/10.1016/j.ijpharm.2021.120311>.
19. Kora, A.J. Gum ghatti (*Anogeissus latifolia*), a proteinaceous edible biopolymer and its multifaceted biological applications, https://www.researchgate.net/publication/317277268_Gum_ghatti_Anogeissus_latifolia_a_proteinaceous_edible_biopolymer_and_its_multifaceted_biological_applications. In *Biopolymers: Structure, Performance and Applications*, A. K. Mishra, Hussain, C.M., Mishra, S.B., Eds. Nova Science Publishers Inc.: USA, **2017**, 155-172, <https://www.amazon.com/Biopolymers-Applications-Ajay-Kumar-Mishra/dp/153611846X>.
20. Kora, A.J.; Beedu, S.R.; Jayaraman, A. Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (*Anogeissus latifolia*) and its biological activity. *Organic and medicinal chemistry letters* **2012**, *2*, 17, <https://doi.org/10.1186/2191-2858-2-17>.
21. Alam, M.S.; Garg, A.; Pottoo, F.H.; Saifullah, M.K.; Tareq, A.I.; Manzoor, O.; Mohsin, M.; Javed, M.N. Gum ghatti mediated, one pot green synthesis of optimized gold nanoparticles: Investigation of process-variables impact using Box-Behnken based statistical design. *International Journal of Biological Macromolecules* **2017**, *104*, 758-767, <http://dx.doi.org/10.1016/j.ijbiomac.2017.05.129>.
22. Kora, A.J.; Rastogi, L. Green synthesis of palladium nanoparticles using gum ghatti (*Anogeissus latifolia*) and its application as an antioxidant and catalyst. *Arabian Journal of Chemistry* **2018**, *11*, 1097-1106, <http://dx.doi.org/10.1016/j.arabjc.2015.06.024>.
23. Kora, A.J. Multifaceted activities of plant gum synthesised platinum nanoparticles: catalytic, peroxidase, PCR enhancing and antioxidant activities. *IET nanobiotechnology* **2019**, *13*, 602-608, <https://doi.org/10.1049/iet-nbt.2018.5407>.
24. Mittal, H.; Ballav, N.; Mishra, S.B. Gum ghatti and Fe₃O₄ magnetic nanoparticles based nanocomposites for the effective adsorption of methylene blue from aqueous solution. *Journal of Industrial and Engineering Chemistry* **2014**, *20*, 2184-2192, <http://dx.doi.org/10.1016/j.jiec.2013.09.049>.

25. Mittal, H.; Ray, S.S. A study on the adsorption of methylene blue onto gum ghatti/TiO₂ nanoparticles-based hydrogel nanocomposite. *International Journal of Biological Macromolecules* **2016**, *88*, 66-80, <http://dx.doi.org/10.1016/j.ijbiomac.2016.03.032>.
26. Kora, A.J.; Mounika, J.; Jagadeeshwar, R. Rice leaf extract synthesized silver nanoparticles: An *in vitro* fungicidal evaluation against *Rhizoctonia solani*, the causative agent of sheath blight disease in rice. *Fungal biology* **2020**, *124*, 671-681, <https://doi.org/10.1016/j.funbio.2020.03.012>.
27. Cox, K.D.; Quello, K.; Deford, R.J.; Beckerman, J.L. A rapid method to quantify fungicide sensitivity in the brown rot pathogen *Monilinia fructicola*. *Plant disease* **2009**, *93*, 328-331, <https://doi.org/10.1094/pdis-93-4-0328>.
28. Sankar, R.; Maheswari, R.; Karthik, S.; Shivashangari, K.S.; Ravikumar, V. Anticancer activity of *Ficus religiosa* engineered copper oxide nanoparticles. *Materials Science and Engineering: C* **2014**, *44*, 234-239, <https://doi.org/10.1016/j.msec.2014.08.030>.
29. Kora, A.J.; Beedu, S.R. Antibacterial activity of biogenic silver nanoparticles synthesized with gum ghatti and gum olibanum: a comparative study. *The Journal of antibiotics* **2015**, *68*, 88-97, <https://doi.org/10.1038/ja.2014.114>.
30. Ibrahim, A.M.; Munshi, G.H.; Al-Harbi, L.M. Copper(II) oxide nanocatalyst preparation and characterization: Green chemistry route. *Bulletin of the National Research Centre* **2018**, *42*, 6, <https://doi.org/10.1186/s42269-018-0006-5>.
31. Praburaman, L.; Jang, J.-S.; Muthusamy, G.; Arumugam, S.; Manoharan, K.; Cho, K.-M.; Min, C.; Kamala-Kannan, S.; Byung-Taek, O. *Piper betle*-mediated synthesis, characterization, antibacterial and rat splenocyte cytotoxic effects of copper oxide nanoparticles. *Artificial Cells, Nanomedicine, and Biotechnology* **2016**, *44*, 1400-1405, <https://doi.org/10.3109/21691401.2015.1029630>.
32. El-Saadony, M.T.; El-Hack, M.E.A.; Taha, A.E.; Fouda, M.M.G.; Ajarem, J.S.; Maooda, S.N.; Allam, A.A.; Elshaer, N. Ecofriendly synthesis and insecticidal application of copper nanoparticles against the storage pest *Tribolium castaneum*. *Nanomaterials* **2020**, *10*, 1-16, <https://doi.org/10.3390/nano10030587>.
33. Leon-Jimenez, E.; Valdez-Salas, B.; Gonzalez-Mendoza, D.; Tzintzuncamacho, O. Synthesis and insecticide activity of Cu-nanoparticles from *Prosopis juliflora* (Sw) DC and *Pluchea sericea* (Nutt) on *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). *Revista de la Sociedad Entomológica Argentina* **2019**, *78*, 12-21, <https://www.biotaxa.org/RSEA/article/view/53445>.
34. Vanathi, P.; Raju, R.; Sivaraj, R. Synthesis and characterization of *Eichhornia*-mediated copper oxide nanoparticles and assessing their antifungal activity against plant pathogens. *Bulletin of Materials Science* **2016**, *39*, 1165-1170, <https://doi.org/10.1007/s12034-016-1276-x>.
35. Rezaie, A.B.; Montazer, M.; Rad, M.M. Photo and biocatalytic activities along with UV protection properties on polyester fabric through green *in-situ* synthesis of cauliflower-like CuO nanoparticles. *Journal of Photochemistry and Photobiology B: Biology* **2017**, *176*, 100-111, <https://doi.org/10.1016/j.jphotobiol.2017.09.021>.
36. Arfat, Y.A.; Ejaz, M.; Jacob, H.; Ahmed, J. Deciphering the potential of guar gum/Ag-Cu nanocomposite films as an active food packaging material. *Carbohydrate Polymers* **2017**, *157*, 65-71, <https://doi.org/10.1016/j.carbpol.2016.09.069>.
37. Chalandar, H.E.; Ghorbani, H.R.; Attar, H.; Alavi, S.A. Antifungal effect of copper and copper oxide nanoparticles against *Penicillium* on orange fruit. *Biosciences Biotechnology Research Asia* **2017**, *14*, 279-284, <http://dx.doi.org/10.13005/bbra/2445>.
38. Kartal, S.N.; Green, F.; Clausen, C.A. Do the unique properties of nanometals affect leachability or efficacy against fungi and termites? *International Biodeterioration & Biodegradation* **2009**, *63*, 490-495, <https://doi.org/10.1016/j.ibiod.2009.01.007>.
39. Rubina, M.S.; Vasil'kov, A.Y.; Naumkin, A.V.; Shtykova, E.V.; Abramchuk, S.S.; Alghuthaymi, M.A.; Abd-El salam, K.A. Synthesis and characterization of chitosan-copper nanocomposites and their fungicidal activity against two sclerotia-forming plant pathogenic fungi. *Journal of Nanostructure in Chemistry* **2017**, *7*, 249-258, <https://doi.org/10.1007/s40097-017-0235-4>.