Antibacterial and nematicidal properties of biosynthesized Cu nanoparticles using extract of holoparasitic plant

Gulwaiz Akhter¹ · Afroz Khan² · Syed Ghazanfar Ali³ · Tabreiz Ahmad Khan¹ · Khwaja Salahuddin Siddiqi⁴ · Haris M. Khan³

Received: 5 March 2020 / Accepted: 12 June 2020 / Published online: 23 June 2020
© Springer Nature Switzerland AG 2020

Abstract

In this report, copper nanoparticles (Cu NPs) are successfully prepared using biosynthesis from stem extract of Holoparasitic plant (*Orobanchae aegyptiaca*). The structural and morphological properties of NPs were determined by using X-ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy, Scanning Electron Microscopy, energy dispersive x-ray spectroscopy (EDX) and Transmission Electron Microscopy (TEM). The optical study has been analyzed using Ultraviolet-Visible Spectroscopy and optical band gap was calculated 4.65 eV. The XRD and TEM patterns confirmed the formation of single phase Cu NPs having an average particle size less than 50 nm. Notable antimicrobial activity of Cu NPs was observed against *E. coli* & *S. aureus* and nematicidal properties was confirmed against *Meloidogyne incognita* in-vitro. This is the first report on biosynthesis of Cu NPs for its enhanced nematicidal activity.

Keywords Nanoparticles · Electron microscopy · Biological material · Biological application

1 Introduction

Nanotechnology is the science doing wonders at the level of a billionth of a meter. It is one of the most significant advancements which has revolutionized all fields of science i.e. chemistry, biology, physics, material science or engineering [1]. Due to the remarkable surface chemistry of the coinage metals (group IB), such as gold, silver and copper, they have notched top slots among many other metal nanoparticles. Despite being cheaper than gold and silver, Cu NPs have not garnered much attention in the past. This could be attributed to the complexity involved in synthesizing stable Cu NPs as Cu undergoes rapid oxidation [2]. To combat this, various physical and chemical methods have been adopted [3]. Although very promising but the cost to benefit ratio of these synthesis procedures are very high. The grave environmental damage posed by

these procedures cannot be seriously overlooked. They utilize expensive and hazardous chemicals toxic to living system and environment. Therefore, the green method of nanoparticles production serves as savior. The field of nanoparticles as nematicidal and antibacterial is new and growing area for the researcher in the field of agricultural and medicinal sciences. The *Meloidogyne* species are the most widespread agricultural pests in the world and in particular, *M. incognita*, is considered to be the most economically important pathogen [4]. In order to sustain the agricultural production, there is a need to develop cheap and eco-friendly methods to suppress nematode infestation and biogenic synthesis of nanoparticles found to be the best alternative. Previously, green synthesis of nanoparticles and their nematicidal / or ovicidal activity have been reported [5]. *Orobanchae aegyptiaca* is a root holoparasitic plant that parasitizes many dicotyledonous plants and

✉ Afroz Khan, akhan@myamu.ac.in | ¹Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India. ²Department of Physics, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India. ³Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India. ⁴Department of Chemistry, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India.

cause severe damage [6]. It is documented that this weed contains several polyphenol, tannins, alkaloids and peptide hence being used in skin cure. All the plant extracts play dual role of potential reducing and stabilizing agents for stabilization the metal nanoparticles [7]. However, to the best of our knowledge no other work on the synthesis of Cu NPs by using stem extract of *O. aegyptiaca* has been reported for their antibacterial and nematicidal activity.

2 Materials and methods

2.1 Synthesis of Cu NPs

Cu NPs were synthesized from the aqueous extract of *O. aegyptiaca*. Dried stems were grinded to make fine powder and then 10 g of this powder was refluxed in 100 mL distilled water for 30 min. Prepared broth was centrifuged at 10,000 rpm to settle down the solid mass. The supernatant (10 mL) at pH 7 was taken in an Erlenmeyer flask and 1 mL of 0.001 M solution of CuSO_4 was added to start the reduction of copper ions to Cu NPs. The mixture was stirred for 10–15 min and the resultant mixture was kept in dark for 72 h. The final product was again centrifuged at 12,000 rpm to separate Cu NPs from the liquid. The supernatant was discarded and the pellet was washed with deionized water, stirred and centrifuged at 12,000 rpm. This process was repeated thrice to obtain pure isolated Cu NPs. Copper (II) sulfate (159.609 g/mol) was purchased from the Sigma Aldrich. *Orobanche aegyptiaca* parasitizing *Solanum melongena* was collected from the Kalinjar village of Banda district, Uttar Pradesh, India and the voucher specimen (Accession No. 31024) of *O. aegyptiaca* was deposited in the Museum of Botany Department, Aligarh Muslim University, Aligarh India.

2.2 Characterization techniques

Cu nanoparticles (Cu NPs) were synthesized using biosynthesis. The X-ray diffraction (XRD) measurement of Cu NPs was performed on Shimadzu Lab XRD-6100 using Cu-K α radiation ($\lambda = 1.54 \text{ \AA}$) from 20° to 80° with step size 0.02° and scanning rate of 4° per minute at room temperature to check the phase purity and to determine crystal symmetry. FT-IR spectra were carried out using Perkin Elmer Spectrum-2 FT-IR spectrophotometer with wavenumber range $400\text{--}4000 \text{ cm}^{-1}$. UV-Vis absorbance spectra and band gap analysis had been studied using Perkin Elmer Lambda-36 UV-Vis Spectrophotometer. Scanning Electron Microscope (SEM) was used for the surface morphology of sample using a JEOL JSM-6510LV SEM at 50 kV (JEOL Co. Ltd. Japan). Transmission Electron Microscope (TEM) was used for the internal

structure of the sample at 200 kV using a JEOL JEM-2100 TEM (JEOL Co. Ltd. Japan).

2.3 Antibacterial effect of Cu NPs

The antibacterial activity of green synthesized Cu NPs were tested using well diffusion assay. For this purpose overnight grown culture of *E. coli* ATCC25922 and *S. aureus* ATCC25923 were spread on nutrient agar plates, and the plates were allowed to dry for few minutes. After then wells of equal diameter were cut and the gaps were sealed using soft agar (100 μL in each well). Cu NPs of varying concentration (25, 50, 100, 200 and 400 $\mu\text{g/mL}$) were poured into each well along with the control (distilled water) and plates were allowed to incubate for 18–24 h at 37°C .

2.4 Nematicidal properties of Cu NPs against *Meloidogyne incognita*

The nematicidal properties of Cu NPs on *M. incognita*, second stage juveniles (J_2) were treated with various concentrations (50, 100, 200, 400 and 800 $\mu\text{g/mL}$) of Cu NPs in Petri plates. The sterilized Petri plates of 5 cm diameters were separately pipette with 5 mL of each concentration of Cu NPs. In each Petri plates approximately one hundred freshly hatched second stage juveniles of *M. incognita* were also added. The Petri plates containing distilled water served as a control and there were five replications for each treatment. After 1, 2, 4, 8 and 16 h, the number of immobilized nematodes were counted under stereo-microscope. The mortality of nematode was checked by the methods of El-Rokiek and El-Nagdi [8] and Aissani et al. [9].

2.5 Egg hatchability assay of *M. incognita*

For determining the effect of Cu NPs on cumulative emergence of second stage juveniles from the eggs of root-knot nematode, 5 sterilized healthy eggs masses of nearly uniform size were transferred to 5 cm diameter Petri plates containing 5 mL Cu NPs of different dilutions (50, 100, 200, 400 and 800 $\mu\text{g/mL}$). The egg masses placed in sterilized distilled water served as control. All Petri plates including those of mortality test were kept at $28 \pm 2^\circ\text{C}$ in an incubator. The total number of juveniles hatched out from eggs in each Petri plate was counted after 6 days under stereo-microscope.

3 Results and discussion

3.1 Structural and morphological analysis

The XRD spectrum of biosynthesized Cu NPs is shown in Fig. 1a. The Characteristics peaks of Cu NPs using XRD

referring to the (111), (200), and (220) Miller planes of fcc crystal structure at 2θ value of 44° , 50° and 77° respectively [10]. The XRD pattern shows no impurity peaks in the typical XRD spectrum and the intense peaks which are shown in the spectrum attributed to the high degree of crystallinity of the sample. The crystallite size of the Cu NPs which is found to be >30 nm, was calculated by Scherrer's formula ($D = \frac{0.9\lambda}{\beta \cos \theta}$) [11]. Where λ is the wavelength of the Cu K α radiation, β is full-width half maxima, θ is the glancing angle and D is the crystallite size of the Cu NPs. The output from FT-IR spectrum as shown in Fig. 1b confirms the composition of the Cu NPs sample. A broad peak at 3400 cm^{-1} corresponds to O–H stretching vibration due to the water present in the sample. The bands at 2917 cm^{-1} and 2829 cm^{-1} are attributed to symmetric and asymmetric stretching of $-\text{CH}_2$ group. Other absorption bands at 1634 cm^{-1} and 1410 cm^{-1} indicated to asymmetric stretching of COO^- and bands located at 1110 cm^{-1} and 600 cm^{-1} corresponding to symmetric stretching of COO^- group [12].

The morphology of Cu NPs powder has been investigated by using SEM, EDX, and TEM. SEM analysis was used to investigate the surface morphology of the well prepared Cu NPs, shown in the Fig. 1c which depicts the

spherical shape of the Cu NPs [13]. These stabilized NPs also form the clusters in spite of close to each other. Moreover, discrete NPs are captured by each other by stabilizing agent, which play an important role in controlling the distribution of particle size and limiting the aggregation [14]. This study exhibits that the extract of *O. aegyptiaca* is a good capping agent, results in formation of small sized NPs. EDX spectroscopy is used to identify the elemental composition of the synthesized Cu NPs. The recorded EDX spectrum of Cu NPs is shown in Fig. 1d, confirming the presence of Cu, O and C at their respective energy level. The elemental analysis of the synthesized Cu NPs is also represented in the inset of Fig. 1d. The observed spectrum of Cu NPs is similar as previously reported by A. Khan et al. [3], except for slightly enhanced peak of C. The C and O peaks verified the existence of carbon based stabilizers in the sample. The appearance of the C and O peaks may attribute to the carbon tape used to mount the sample during measurement and surface oxidation, respectively [15]. TEM micrograph of Cu NPs and their size distribution is shown in Fig. 1e, which also frame up the spherical NPs with particle sizes less than 50 nm. The distribution uniformity of NPs due to *O. aegyptiaca* extract chemically bound at their surface is also presented [13]. The results

Fig. 1 Showing **a** XRD spectrum **b** FTIR spectrum **c** SEM micrograph **d** EDX analysis **e** TEM micrograph **f** absorbance spectrum; inset shows the Tauc plot of Cu NPs

Fig. 2 Showing antibacterial activity of Cu NPs against a *S. aureus* and b *E. coli*

obtained from the TEM have good agreement with the XRD analysis.

3.2 Optical analysis

The absorption spectra of Cu NPs and Oro extract solution are shown in Fig. 1f. There are two absorbance peaks have been found at 280 nm and 327 nm in the Cu NPs and *O. aegyptiaca* extract solution respectively. The optical band gap of the Cu NPs sample has been calculated by Tauc plot [16, 17] using relation (Eq. 1) as presented in the inset of Fig. 1f.

$$\alpha h\nu = A(h\nu - E_g)^n \quad (1)$$

where $n = \frac{1}{2}$ is used for direct band gap semiconductor, h is Planck constant, ν is the frequency of the radiation which is used in the experiment and E_g is the optical band gap. The intercept on the x-axis by linear extrapolation of the graph between $(\alpha h\nu)^2$ and $h\nu$ gives the optical band gap of the sample which is found to be 4.65 eV. This wide band gap of the sample may be due to the less intermediate energy levels and small average crystallite size of the sample. This property of wide band gap of Cu NPs attributed its application in antibacterial and nematicidal activities [10].

3.3 Antibacterial effect of Cu NPs

Cu NPs as an antimicrobial agent were evaluated against *E. coli* and *S. aureus*. The results showed that Cu NPs possess antibacterial activity both for *E. coli* and *S. aureus*. Zones of inhibition could be clearly seen at concentration of 200 µg/mL and below to this concentration 100 µg/mL no zone of inhibition was seen in Fig. 2a, b. Cu NPs with larger surface area endow a better contact

with bacteria [1, 18]. Therefore, Cu NPs may penetrate the bacterial cell membrane or intracellular loading or attached to the bacterial cells and inhibited their replication [19–21].

3.4 Nematicidal effect of Cu NPs

It is clear from the data illustrated in Fig. 3a that the Cu NPs nematicidal effect of varying degree on Juveniles (J_2) of *M. incognita*. Percentage mortality of nematode was directly proportional to the concentration of NPs and exposure time. All the concentrations of Cu NPs (50 µg/mL to 800 µg/mL) were toxic to J_2 of *M. incognita*, causing mortality up-to 91.5% at 16 h. The highest mortality % was recorded at 800 µg/mL, whereas the lowest was at 50 µg/mL.

3.5 Ovicidal effect of Cu NPs

The data in Fig. 3b showed the inhibition in hatching of eggs. There was a relative significant decrease in the eggs hatching with the corresponding increase in the concentration of extract of Cu NPs. In 800 µg/mL concentration, highest inhibition in juveniles emergence of *M. incognita* was recorded followed by 400, 200, 100, and 50 µg/mL. In the corresponding treatments, the per cent inhibition was recorded as 83%, 68%, 53%, 39% and 26% respectively. Our in-vitro studies showed that all tested concentrations of Cu NPs increased J_2 mortality and inhibition rate of *M. incognita* after exposure to Cu NPs. Similar nematicidal and ovicidal properties of ZnO NPs, ZnO and GO NPs, Ag NPs, Cu NPs and SiO₂ NPs have been reported [5, 22–25].

Fig. 3 Showing **a** nematicidal and **b** ovicidal Cu NPs against of *M. incognita*

4 Conclusions

In summary, we have reported a new route for biological synthesis of Cu NPs. The XRD pattern confirms the single phase structure of the Cu NPs. SEM and TEM images revealed the morphology and size of the Cu NPs which are found to be less than 50 nm. The toxicity of Cu NPs against *M. incognita* was found to be dependent on its concentration and exposure time. Again, Cu NPs have been also found to be very effective against *E. coli* and *S. aureus* at higher concentration. However, further studies need to be carried out to understand the specific mode of action of Cu NPs against nematodes activities.

Acknowledgements The authors are thankful to Chairpersons, Department of Botany, Physics, Applied Physics, Chemistry and Saidla, AMU, Aligarh for providing the necessary support for experiments. GA and AK thanked to *Mohd Shameem* for his help during SEM and TEM measurements from USIF, AMU, Aligarh.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Siddiqi KS, Husen A, Rao RAK (2018) A review on biosynthesis of silver nanoparticles and their biocidal properties. *J Nanobiotechnol*. <https://doi.org/10.1186/s12951-018-0334-5>
- Shah AA, Khan A, Dwivedi S, Musarrat J, Azam A (2018) Antibacterial and antibiofilm activity of barium titanate nanoparticles. *Mater Lett* 229:130–133. <https://doi.org/10.1016/j.matlet.2018.06.107>
- Khan A, Rashid A, Younas R, Chong R (2016) A chemical reduction approach to the synthesis of copper nanoparticles. *Int Nano Lett* 6:21–26. <https://doi.org/10.1007/s40089-015-0163-6>
- Akhter G, Zafar A, Khan W, Jamshed M (2018) In vitro nematotoxic potential of some leaf extracts on Juvenile mortality of *Meloidogyne incognita* race-3. *Arch Phytopathol Plant Prot* 51:399–407. <https://doi.org/10.1080/03235408.2018.1490238>
- Kalaiselvi D, Mohankumar A, Shanmugam G, Nivitha S, Sundararaj P (2019) Green synthesis of silver nanoparticles using latex extract of *Euphorbia tirucalli*: a novel approach for the management of root knot nematode, *Meloidogyne incognita*. *Crop Prot* 117:108–114. <https://doi.org/10.1016/j.cropro.2018.11.020>
- Akhter G, Khan TA (2018) First report of *Orobanche cernua* parasitism on *Allium sativum* in Banda district of Uttar Pradesh, India. *Indian Phytopathol* 71:463–464. <https://doi.org/10.1007/s42360-018-0057-4>
- Siddiqi KS, Husen A (2017) Plant response to engineered metal oxide nanoparticles. *Nanoscale Res Lett*. <https://doi.org/10.1186/s11671-017-1861-y>
- El-Rokiek K, El-Nagdi W (2011) Dual effects of leaf extracts of *eucalyptus citriodora* on controlling purslane and root-knot nematode in sunflower. *J Plant Prot Res* 51:121–129. <https://doi.org/10.2478/v10045-011-0021-0>
- Aissani N, Urgheghe PP, Oplos C, Saba M, Tocco G, Petretto GL, Eloh K, Menkissoglu-Spiroudi U, Ntalli N, Caboni P (2015) Nematicidal activity of the volatiles of *Eruca sativa* on *Meloidogyne incognita*. *J Agric Food Chem* 63:6120–6125. <https://doi.org/10.1021/acs.jafc.5b02425>
- Usman MS, Ibrahim NA, Shameli K, Zainuddin N, Yunus WMZW (2012) Copper nanoparticles mediated by chitosan: synthesis and characterization via chemical methods. *Molecules* 17:14928–14936. <https://doi.org/10.3390/molecules171214928>
- Patterson AL (1939) The scherrer formula for X-ray particle size determination. *Phys Rev* 56:978–982. <https://doi.org/10.1103/PhysRev.56.978>
- Pashchanka M, Hoffmann RC, Gurlo A, Schneider J (2010) Molecular based, chimie douce approach to 0D and 1D indium oxide nanostructures. Evaluation of their sensing properties towards CO and H₂. *J Mater Chem*. <https://doi.org/10.1039/c0jm01490g>
- Habibi MH, Kamrani R, Mokhtari R (2010) Fabrication and characterization of copper nanoparticles using thermal reduction: the effect of nonionic surfactants on size and yield of nanoparticles.

- Microchim Acta 171:91–95. <https://doi.org/10.1007/s00604-010-0413-2>
14. Feng L, Zhang C, Gao G, Cui D (2012) Facile synthesis of hollow Cu₂O octahedral and spherical nanocrystals and their morphology-dependent photocatalytic properties. *Nanoscale Res Lett*. <https://doi.org/10.1186/1556-276x-7-276>
 15. Abdul M, Khan M, Kumar S, Ahamed M, Alrokayan SA (2011) Structural and thermal studies of silver nanoparticles and electrical transport study of their thin films. *Nanoscale Res Lett*. <https://doi.org/10.1186/1556-276x-6-434>
 16. Raghavender AT, Hoa N, Joon K, Jung M, Skoko Z, Vasilevskiy M, Cerqueira MF, Samantilleke AP (2013) Nano-ilmenite FeTiO₃: synthesis and characterization. *J Magn Magn Mater* 331:129–132. <https://doi.org/10.1016/j.jmmm.2012.11.028>
 17. Khan A, Rahman F (2019) Study of microstructural and optical properties of nanocrystalline indium oxide: a transparent conducting oxide (TCO) 030091, 99 1–5. <https://doi.org/10.1063/1.5112930>
 18. Ali SG, Ansari MA, Khan HM, Jalal M, Mahdi AA, Cameotra SS (2017) Crataeva nurvala nanoparticles inhibit virulence factors and biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. *J Basic Microbiol* 57:193–203. <https://doi.org/10.1002/jobm.201600175>
 19. Tashi T, Vishal Gupta N, Mbuya VB (2016) Silver nanoparticles: synthesis, mechanism of antimicrobial action, characterization, medical applications, and toxicity effects. *J Chem Pharm Res* 8:526–537
 20. Amatya SP, Joshi LP (2020) Bio-synthesis of copper nanoparticles (CuNPs) using garlic extract to investigate antibacterial activity. *BIBECHANA* 17:13–19. <https://doi.org/10.3126/bibechana.v17i0.23485>
 21. Bastos CAP, Faria N, Wills J, Malmberg P, Scheers N, Rees P, Powell JJ (2020) Copper nanoparticles have negligible direct antibacterial impact. *NanolImpact* 17:100192. <https://doi.org/10.1016/j.nanol.2019.100192>
 22. Gupta S, Kushwah T, Vishwakarma A, Yadav S (2015) Optimization of ZnO-NPs to investigate their safe application by assessing their effect on soil nematode *Caenorhabditis elegans*. *Nanoscale Res Lett*. <https://doi.org/10.1186/s11671-015-1010-4>
 23. Siddiqui ZA, Parveen A, Ahmad L, Hashem A (2019) Effects of graphene oxide and zinc oxide nanoparticles on growth, chlorophyll, carotenoids, proline contents and diseases of carrot. *Sci Hortic* 249:374–382. <https://doi.org/10.1016/j.scienta.2019.01.054>
 24. Mohamed EA, Elsharabasy SF, Abdulsamad D (2019) Evaluation of in vitro nematicidal efficiency of copper nanoparticles against root-knot nematode *Meloidogyne incognita*. *South Asian J Parasitol* 2:1–6. <https://doi.org/10.9734/SAJP/2019/46065>
 25. Khan MR, Siddiqui ZA (2020) Scientia Horticulturae Use of silicon dioxide nanoparticles for the management of *Meloidogyne incognita*, *Pectobacterium betavascularum* and *Rhizoctonia solani* disease complex of beetroot (*Beta vulgaris* L.). *Sci Hortic* 265:109211. <https://doi.org/10.1016/j.scienta.2020.109211>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.