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# Green synthesis of CuO nanoparticles via Allium Cepa extract and its characterizations on dye degradation and antimicrobial activity

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**Key words:** Copper nanomaterial (CuO), Malachite green (MG), Photo catalytic degradation, Antimicrobial activity

## Abstract

Industries use dyes in order to colour their products. The discharge of dye bearing wastewater into natural streams, rivers from textile, leather industries make severe environmental problem of the present day. The Heterogeneous photocatalytic process is a high efficiency technique, which is used to oxidize the organic pollutants present in aqueous system. This report aimed to elucidate the Photocatalytic degradation of Malachite green dye in aqueous medium in presence of UV light irradiation using Allium cepa mediated (CuO) Nanoparticle prepared by Bio-method. The as-synthesized copper Nanoparticle was characterized with various techniques UV, FT-IR, XRD, SEM and DLS which reveals many possible interactions of Dye-Nanoparticle system. The maximum photo degradation was obtained at 120min of irradiation time due to their higher surface sites and surface defects. Although the photostablity of Nano copper material (CuO) was also assessed for 4 cycles which ascertained the potential photo catalytic activity and it also proves excellent microbial activity against *E-coli and Bacillus*. Thus, the Allium cepa mediated Nano copper could play a vital role in environmental remediation of polluted water.

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## Introduction

The startling technological development has increased the large-scale industrial production of much of the daily-used products with a broad range of applications (Hamza *et al.,* 2018; Meaty *et al.,* 2020; Amr Fouda *et al.,* 2020). Among these products, dyes are crucial in the textile and fashion industries. However, with intensive applications, dyes transform into dangerous pollutants when they are improperly handled and disposed of causing serious environmental and public health hazards (Yoga Lakshmi *et al.,* 2020; Amr Fouda *et al.,* 2020). The increased usage of nanoparticles especially for medical purposes created a new challenge of synthesizing them by untraditional methods to overcome the undeniable disadvantages of other physical and chemical methods (Murali Sastry *et al.,* 2003; Amr Fouda *et al.,* 2020). Plant mediated nanoparticles synthesis (green method) is preferred as it is clean, cost effective, nontoxic and safe for human therapeutic use (Renata Dobrucka and Jolanta Długaszewska, *2015*). Onion (Allium cepa) is a widely cultivated plant all over the world. It is rich in carbohydrates, proteins, sodium, potassium and phosphorus. It has been reported to have numerous important properties such as antimicrobial, antioxidant, antiparasitic and anti-inflammatory activities (Johanna, *1999*; Eman, *2017;* Philip and Unni, *2011*). Recently, it is found that onion can be used as a potential candidate for synthesis of CuO nanoparticles.

CuO nanoparticles as a narrow band gap semiconductor was investigated extensively due to its promising applications towards sensors, superconductors, solar cells, catalysis, lithium-ion battery and super capacitors, (Zhang *et al.,2014*). In addition, US Environmental protection agency recognized copper based materials as antimicrobial materials (Dollwet and Sorenson, *2001).* The use of CuO nanomaterial in prevention of bacterial infection in medical devices and enhancement of antimicrobial activity towards pathogenic microorganisms in nano form displays is also recorded. CuO nanomaterials were applied in fabrics, agriculture, paints and also in hospitals due to its desirable properties (Amr Fouda *et al., 2020;* Kumar *et al., 2012*). Due to its low cost and toxicity, good chemical and thermal stability with high surface area, CuO based nanomaterials have received considerable attention. In particular, semiconductor nanostructured-based photo catalysis displayed high potential for degradation of various organic pollutants including dyes, surfactants, solvents, pesticides, phenolic compounds, etc. into harmless products under light irradiation. Among nanostructured oxides, copper (II) oxide (CuO) is classified as a semiconductor material type-p with a narrow band gap energy of 1.2 eV and possessing excellent optical, electrical, magnetic, catalytic, and biological properties. In this study, we report the synthesis of CuO nanoparticle by green syn- thesis process using Allium cepa extract as reducing agent. The final products characteristics were studied - SEM, XRD, UV, DLS and FTRI analysis. Along with these characteristics Photocatalytic degradation the antibacterial ability against *E. coli and S. Bacillus* was examined and reported.

## Materials and methods

*Preparation of Allium Cepa Extract*

The Allium Cepa (onion) was collected from local market and washed with distilled water thoroughly. After that it was cut into small pieces and grinded in mortar and pestle to get a paste formation. Then the pasty formation was mixed with 100ml distilled water and heated for about 30mins to 1hour. After 1 hour it was filtered using whatmman no.1 filter paper and stored in refrigerator for further use.

*Synthesis of Allium Cepa mediated CuO Nanoparticle (Bio-Method)*

1.5g of copper nitrate was dissolved in 100ml distilled water and stirrer continuously for about 30mins. Then the onion extract was added drop by drop to the above solution suddenly the colour changes from blue to green indicates the reduction process. Then it was kept under the heat 60°C for 1 hour, the colour again changes from green to brown indicates the formation of CuO Nanoparticle. The formed CuO Nanoparticle was centrifuged at 3000rpm for 30mins to collect the CuO Nano pellet. The collected Nano pellet was dried at oven for 24 hours to further studies.

*Photo degradation Studies*

The Photocatalytic degradation of Malachite green dye was performed using Allium cepa mediated CuO Nanoparticle in a photocatalytic reactor and the absorbance was measured using UV-Visible spectrophotometer at 620nm. The concentrations of dyes were measured with an UV/visible spectrophotometer. Each experiment was at least duplicated under identical conditions. The amount of adsorption at time t, qt (mg/g), was obtained as follows: The percentage of degradation was calculated by

$$\% = \frac{(CI - Cf)}{CI} X\ 100 \qquad (1)$$

Where $C_I$ (mg/L) and $C_F$ (mg/g) are the liquid-phase concentrations of solutes at Initial and final dye solution.

*Antimicrobial Studies*

The antibacterial analysis was performed against bacterial pathogens using Allium cepa mediated CuO Nanoparticle by agar well diffusion method.

**Result and discussion**

*Ultraviolet-visible spectroscopy*

The UV-vis absorption spectrum of the biosynthesized CuO NPs was recorded at room temperature by dispersing the CuO NPs in deionized water with a concentration of 0.1 wt% as shown in Fig. 1. The spectrum showed an absorption peak at 270nm. This indicated a blue shift occurred when compared to the value of 375nm reported by Xu and co-workers (Xu *et al., 2008*; Mohammod Aminuzzaman *et al., 2021*). Absorption of the CuO NPs at 270nm resulted from the resonant oscillating of electrons at the conduction band triggered by the incident electromagnetic radiation which is known as surface plasmon resonance (Suresh *et al., 2015*; Mohammod Aminuzzaman *et al., 2021*). In this report CuO Nanoparticle was prepared by green method (Allium cepa extract). The synthesized nano particle was analysized by UV - spectro photo meter in the range from 200 to 500nm. The major absorption peak occurs at 250nm confirms the formation of CuO Nanoparticle Which was shown in Fig.1. This result was well similar with studies of (Udhaya banu *et al.*, 2016). The feature of the plot indicated that it had a direct transition due to the linear absorption at the end of the plot (Mokhtari *et al., 2016*; Mohammod Aminuzzaman *et al., 2021*). The higher band gap energy of the CuO NPs than the reported value (1.9-2.1 eV) could be related to the quantum confinement effect that is the band gap increase with a reduction in particle size (Das *et al., 2013*; Mohammod Aminuzzaman *et al., 2021*).

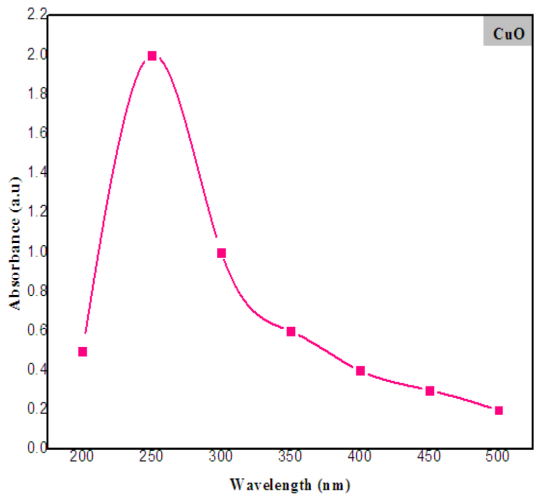


**Fig. 1.** UV Spectral image of allium cepa mediated CuO Nanoparticle.

*Fourier-transform infrared spectroscopy*

FTIR measurements were used to identify the possible biomolecules associated with CuO formation. The FTIR spectra were recorded by Shimadzu. The FTIR analysis was used to identify the possible bio-molecules responsible for capping and efficient stabilization of copper oxide synthesized by onion extract. (Jha *et al., 2009*; Vitthalraj Chandrakant Gosavi *et al., 2020*).

The Functional group of synthesized allium cepa mediated CuO nano particle was recorded by FT-IR shown in Fig. 2. The formation of allium cepa mediated CuO nano particle was observed in range from 400-4000cm-1. The peak at 3391cm$^{-1}$ is OH stretch vibration. 2920 cm$^{-1}$ is attributed to C-H stretching vibration.1641. cm$^{-1}$ .is C=C.The peak at 1501cm$^{-1}$ is aromatic N=O bending. 1289 cm$^{-1}$peak is C-O stretch of metal oxygen peak observed at 817 cm$^{-1}$,604 cm$^{-1}$,583cm$^{-1}$ of allium cepa mediated CuO Nanoparticle.

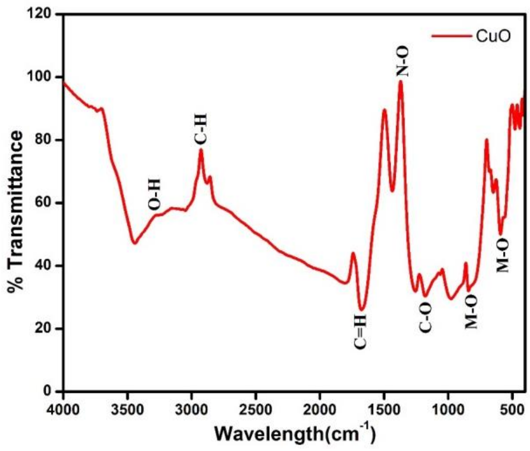


**Fig. 2.** FT-IR analysis of allium cepa mediated CuO Nanoparticle.

*X-ray Powder Diffraction (XRD)*

Pattern with a sharp peak at 2Θ =25.5, 42.3, 50.1, 79.1corresponding to (110), (111), (200), (004) representing face centered cubic structure of copper and are obtained (JCPDS No. 05- 0661). The average crystallite size estimated using the Debye-Scherrer formula was found to be about 20nm. This is related to the polycrystalline nature of the nanoparticles. These peaks indicated that some crystalline compounds other than copper were also present in the final system. These products probably originated from the raw material used. These unidentified Crystalline peaks are also apparent in many works (Abboud *et al., 2013*; Arockiya Aarthi Rajathi *et al., 2012*). Therefore, the formation of copper oxide nano particle was confirmed by the above XRD results. XRD pattern revealed the structure of the synthesized allium cepa mediated CuO Nanoparticle Fig.3.

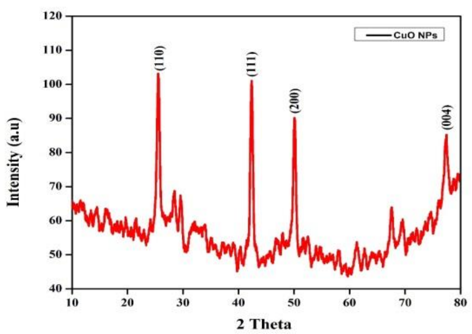


**Fig. 3.** XRD Pattern of allium cepa mediated CuO Nanoparticle.

*Scanning electron microscope (SEM)*

The images show that the copper nanoparticles are evenly distributed and homogeneous in nature. This result is similar to those in previous reports on the morphology of CuO Nps. It can be seen CuO Nanoparticles exclusively consist of Flower lamellar-like structures and the sizes of CuO Nanoparticles are about diameter of 40μm. Based on Fig. 4. The elemental composition of the prepared nanoparticles shows prominently larger peaks for copper (Cu) and oxygen (O) and much smaller peaks corresponding to impurities, aluminum (Al), silicon (Si), iron (Fe), and nickel (Ni). The weight percentage of copper and oxygen was calculated to be 73.15 and 22.17%, respectively (Das *et al.*, 2013; Ananth *et al.*, 2015, Saranya Sukumar *et al.*, 2020). The strong and narrow diffraction peaks of CuO Nps suggest that the resultant particles are highly crystalline in nature (Saranya Sukumar *et al.*, 2020). The agglomeration of the nanoparticles was due to the high surface tension (Son *et al.*, 2015; lalau *et al.*, 2015; Mello *et al.*, 2020; You-Kang Phang *et al.*, 2021). As reported in the literature, CuO NPs have a high tendency to aggregate in ultrapure water during the preparation step before being subjected to high surface measurement (Son *et al.*, 2015; lalau *et al.*, 2015; Mello *et al.*, 2020; You-Kang Phang *et al.*, 2021).

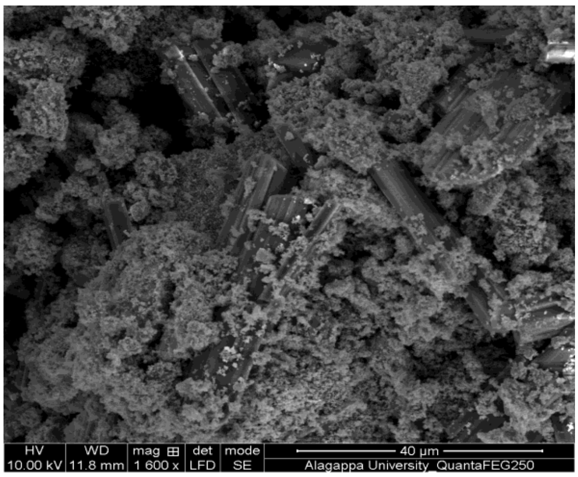


**Fig. 4.** SEM allium cepa mediated CuO Nanoparticle.

*Dynamic Light scattering (DLS)*

Dynamic light scattering (DLS) was performed to determine the hydrodynamic size (diameter) of the synthesized allium cepa mediated CuO Nanoparticle shown in Fig. 5. The particle size of formed CuO nanoparticles by using Allium cepa extract was analysed by DLS and which shown in the Fig. 5. However, the CuO Nanoparticle used in this study has the average diameter 2.6nm corresponds to 0.491 (PDI) which is monodispersed. The predominant particle size of synthesized CuO nanoparticles stands in the 25–65nm range. Less quantity of particles was seemed to have a particle size greater than 60nm in the distribution curve. This existence of larger sized nanoparticles was attributed to the retention of water ($H_2O$) molecules from the surrounding atmosphere. Moreover, it ordinarily occurred due to the synthesis procedure lies in the water medium (Danaei *et al., 2018*; Velsankar *et al., 2019*; Velsankara *et al., 2020*). This, in turn, notified for an earlier implication of the synthesized CuO nanoparticles must have the potential to drug delivery and biological applications (Danaei *et al., 2018;* Velsankar *et al., 2019*; Velsankara *et al., 2020*).

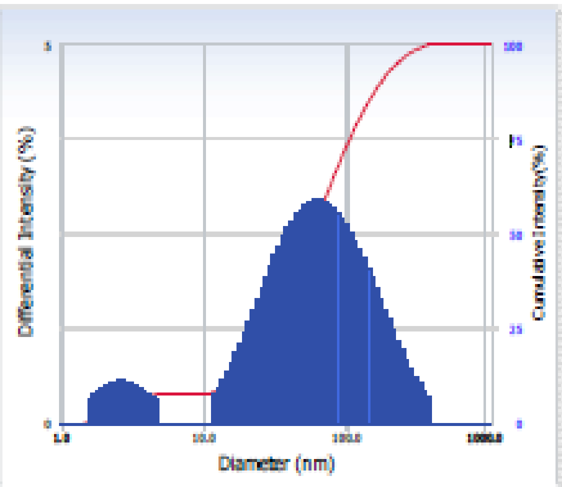


**Fig. 5.** DLS allium cepa mediated CuO Nanoparticle.

*Photocatalytic Studies of Allium Cepa CuO Nanoparticle*

The potential application of allium cepa mediated CuO nanoparticle towards the treatment of dye in waste water can be done by heterogeneous photo catalysis route. In this system, reactive dye such as Malachite green (MG) was used as a pollutant. The removal of MG dye by using allium cepa mediated CuO was carried out under UV light irradiation. The effect of photo catalyst on the removal of MG dye was monitored by the changes in the absorption spectrum of respective dye with its timescale. To study the activity of the catalyst, control experiments on MG dye in the absence of catalyst were done under visible light irradiation for 120 min. No considerable change is observed in the absorption spectra of dye molecules, which clearly indicates the dye molecules remain stable during the course of the reaction time. Approximately 90% of the MG dye was degraded by allium cepa mediated CuO within 120min shown in Fig. 6.

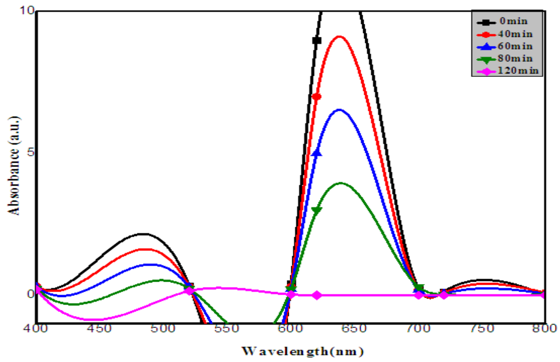


**Fig. 6.** Photo studies of Dye using allium cepa mediated CuO Nanoparticle.

*Photo stability of Allium Cepa Mediated CuO*

After four recycles, the catalyst did not exhibit any significant loss of activity, indicating its high stability during photo degradation process. The high efficiency of catalyst proved that it has relatively a longer shelf-life shown in Fig. 7 as per Equation (1). The use of allium cepa mediated CuO helped in recollection and reuse of photo catalyst. With respect to clean technology and green chemistry, the low cost, catalytic efficiency and excellent reuse, CuO nanopowder could be a good candidate for the environmental-friendly photo catalyst.

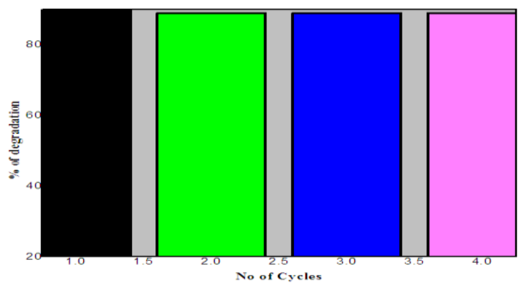


**Fig. 7.** Photo stability of Dye using allium cepa mediated CuO Nanoparticle.

*Photocatalytic Mechanism*

Photocatalytic decolourization resulted with the rapid cleavage of a chromophoric group of dye molecules, which is responsible for imparting colour to the dye solution. Moreover, the nanometric size of allium cepa mediated CuO adds to improve the light absorbing capacity of the catalyst and higher degree of photocatalytic activity of the catalyst in the UV Light region. The higher percentage of decolourization of MG is identified due to the interaction of negative charge of CuO with cationic MG which was shown in Fig. 8. The mechanism involved in the photocatalytic reactions leads to the generation of electron-holes pair by the absorption energy, which capable of involving oxidation and reduction process for the treatment of waste water. The strong oxidative power of photo generated holes on the surface of the semiconductor has made them a most suitable photocatalytic material by absorption of light for environmental remediation. The electron transfers directly into the conduction band of CuO, reducing $O_2$ to form superoxide ions ($O_{2+}$). The hole (h+) then reacts with OH- from water molecule to produce OH• radical. These active radicals are responsible for the decolourization of MG dye under UV light irradiation.

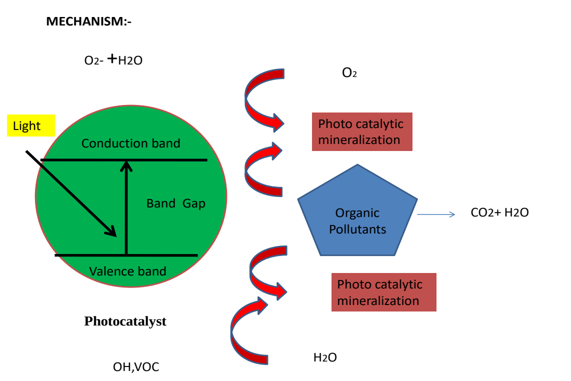


**Fig. 8.** Mechanism of Photo Studies.

*Antibacterial Studies of Allium Cepa Mediated CuO*

The antibacterial activity of allium cepa mediated CuO against *E. coli* and *B. subtilis* were Investigated by using standard Zone of Inhibition (ZOI) microbiology assay. The maximum ZOI around 9 mm was observed at the concentration of 120µL, whereas 20, 40, 60, 80 and 100µL concentration showed moderate activity (2, 3, 5,7 and 7mm) against *E. coli.* Likewise, the maximum ZOI around 8 mm was observed at the concentration of 120µL, whereas 20, 40, 60 80µL and 100µL concentration showed standard activity of (3, 4, 3, 2 and 5) mm against *B. subtilis* respectively Fig. 9. It is evident from the fig. that the increasing of the allium cepa mediated CuO concentration increases the ZOI against *E. coli* and *B. subtilis* shown in Table 1. On other handallium cepa mediated CuO at the concentration of 120µL showed significant morphological changes against both tested organisms. This may be due to the direct interaction of prepared particles on the membrane surface of *E. coli* cell causing disruption of cell membrane and finally lead to the cell death These results confirmed that the allium Cepa mediated CuO showed good antibacterial activity against both the microbes.

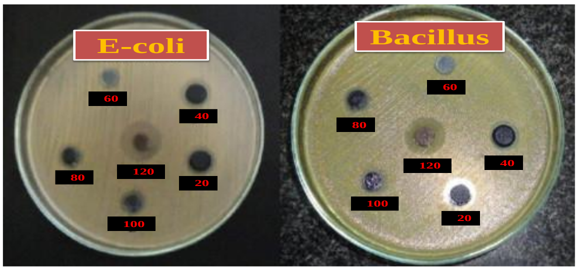


**Fig. 9.** Anti-Bacterial Activity.

## Conculsion

The present investigation deals about the synthesis of CuO Nanoparticle using Allium cepa extract (ONION). The synthesized CuO Nanoparticle is characterized by UV, FTIR, XRD, DLS and SEM analysis. The CuO Nanoparticle were synthesized by using onion extracts within 24 h and the size of the CuO nanoparticles ranges from 40µm and predominantly Flower lamellar like shapes. Functional groups of plant extracts involved in the bio-reduction process of CuO Nanoparticle were characterized by FT-IR. The crystalline nature of the synthesized particles is identified by XRD analysis. The synthesized Allium cepa mediated CuO Nanoparticle is to be used for photo catalytic degradation of Model dye (Malachite green). Effective destruction of (Malachite green) belonging to toxic chemical groups is possible by photo catalysis in the presence of UV visible light. The great part of the studies on the photocatalytic degradation of (Malachite green) dye relies only on the monitoring of solution decolorization, only few studies reported thorough mechanisms with detailed reaction steps of the different pathways leading to several photoproducts. It shows about 90% of the degradation capacity within 120min of irradiation. The synthesized CuO Nanoparticle by using onion extracts were tested for antibacterial activity against Escherichia coli and Bacillus sp which shows excellent zone formation in 120µl concentration in both the organism. The better understanding of the photo catalytic process and the operative conditions could give great opportunities for its application for the destruction of environmental organic contaminants.

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