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Synthesis of copper nanoparticles using *RaphanusSativus* (Radish) and its application in degradation of Methylene Blue dye

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Abstract : In the present work, copper nanoparticles (Cu NPs) have been synthesized by simpleand green technique by using *RaphanusSativus*(Radish) leaf extract. The formation of Cu NPs is monitored by recording the UV-Vis absorption spectra which showed surface Plasmon resonance at 320 nm. The green synthesized Cu NPs were further characterized by FT-IR, SEM and XRD. FT-IR identified the presence of active and phenolic groups. The crystalline morphology and size of the nanoparticles were determined by SEM and X-ray diffraction studies. The average particle size of Cu nanoparticles was found to be in the range of 71 nm. These biologically synthesized Cu NPs were tested for antimicrobial activity against five pathogens. The Cu Nps were also used for the remediation of a Methylene Blue dye. Approximately, 89% degradation of Methylene Blue was observed within 72 hours using Cu NPs. The mechanism involved in the degradation of dye and its phytotoxicity study has been presented. The overall outcome of this study suggests that the green synthesis of Cu NPs hold promise as a potent antimicrobial and antioxidant agent. The particles obtained were also found to degrade Methylene Blue dye.
Keywords : *RaphanusSativus* ,Cu NPs, Methylene Blue, Phytotoxicity, Antioxidant activity.

1. Introduction

Over the past few years, nanotechnology plays a very important role in modern research[1]. This technology deals with the synthesis of nanoparticles with controlled size, shape and dispersity of materials at the nanometer scale length [2]. Plant mediated synthesis of nanoparticles has achieved a keen interest due to its eco-friendly and low time consuming properties [3]. Biological methods of synthesizing nanoparticles using Plants, microorganisms and enzymes have several advantages over conventional physical and chemical methods [4]. Copper nanoparticles (Cu NPs) have recently attracted a particular attention because of their lower-cost, extensive availability and various applications in the field of sensors, catalysis, inkjet etc. [5-8]. However, these Cu NPs have relatively lower redox potential, and it is prone to oxidation when it is exposed to air. Hence, the synthesis of Cu NPs is a very challenging task. Cu NPs are generally synthesized by hydrothermal method, microwave assisted pylol method, thermal reduction etc. However these methods are costly and utilize harsh

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Dyes are a major class of synthetic organic compounds which are released by industries such as paper, leather, food, textile etc. Dyes may be of different types such as azo, basic, acidic, anthraquinoneetc[9]. These organic pollutants may induce skin irritation, blood disorder, liver and kidney damage. Thus, the consequence necessitates the development of methods for the degradation of synthetic dyes [10]. Degradation of these compounds to non-toxic products is very difficult because of their high stability [11]. Recently, nanoparticles of noble metals have gained importance in reduction and degradation of dye due to their stability and high catalytic activity

Thus, the aim of the present study is to develop a safe, cost-effective, non-hazardous method for the synthesis of Cu NPs using an aqueous extract of *RaphanusSativus*and its application in dye degradation.

Experimental details

Materials

All the reagents used in this experiment were obtained from Sigma Aldrich chemicals. Double distilled water was used throughout the experiments. Filtration was established by using Whatman No.1 filter papers. Glasswares used for the complete reactions were washed well, rinsed with double distilled water and dried in hot air oven.

Preparation of aqueous extract of *Raphanus sativus*

The fresh *RaphanusSativus*leaves were collected from Thiruvalluvar University, Vellore,Tamil Nadu, India. The leaves were thoroughly washed several times using distilled water to remove impurities. The cleaned leaves were dried subsequently under sunshade to remove the moisture completely and then it is powdered by using mechanical grinder. The 5g of powdered plant leaves were taken into a beaker along with 100 ml of distilled water and allowed to boil at 60˚C for 30 minutes. The prepared solution was filtered through Whatmann No.1 Filter paper. The extract was stored in a refrigerator at 4 °C for further use[10, 12-13]

Phytochemical analysis

The fresh leaf extract of *RaphanusSativus*was used for Phytochemical screening. It was been carried out by standard phytochemical methods [12, 14-15]

Biosynthesis of copper nanoparticles

For the synthesis of copper nanoparticles (Cu NPs) 25 ml of fresh leaf extract of *RaphanusSativus* was added to 75 ml of 1mM aqueous copper acetate. It was stirred for about 3 hours. Then, the solution was dried at 60ºC in the oven [13-14, 2]

Characterization of Copper nanoparticles

The formation of copper nanoparticles was confirmed by UV-Visible spectroscopy. Size of the Cu NPs was analyzed by UV in the range between 300-700 nm. FTIR analysis was carried out to find out the bio-molecules which is responsible for the reduction of copper ions. The structure of copper nanoparticles were determined by X–Ray diffraction analysis. Morphology and mean particle size were determined by SEM analysis. [10, 17]

Application Studies

Antioxidant activity (Hydrogen peroxide scavenging assay):

The hydrogen peroxide scavenging assay was assessed by the method using aqueous leaf extract of *RaphanusSativus.* Hydrogen peroxide (40mM) solution was prepared in phosphate buffer (pH 7.4). Extractsand Cu NPs (100 µg/ml) were added to a 40mM hydrogen peroxide solution. The reaction mixture was incubated

for 10 min at RT. After incubation, the reaction mixture read at 230 nm against the blank solution with phosphate buffer solution [18-19]. The inhibition percentage was calculated based on the formula % of inhibition = (A1 – A2) / A1 X 100

Where A1-absorbance of Hydrogen peroxide ; A2- absorbance of the reaction mixture with extract.

Antibacterial activity:

The antimicrobial study of Cu NPs was evaluated against bacterial pathogens such as *E. coli*, *S. aureus*, *P.aeurginosa*, *Listeria* sp, *Proteus* sp by disc diffusion method. The plant extract and the synthesized Cu NPswere added to the sterilized disc and carefully dispensed on Muller-Hinton agar plates and incubated for 24 hours at 37ºC. The Zone of inhibition was measured from the centre of the disc to the clear zone in millimeter and the results were recorded [20-21].

Catalytic reduction of Methylene Blue using Cu NPs and Plant extract:

*RaphanusSativus*mediated synthesized Cu NPs were tested against Methylene Blue in the presenceof UV light exactly at 365 nm. About 1 ml of Methylene Blue (1x $10^{-}4$ M) was mixed with 25, 50, 75, 100 µl of Cu NPs [22-24]. Every 24 hour interval sample was collected and subjected to UV- Visible Spectroscopy

% of dye degradation was estimated by

$$\% \text{ Decolourization} = \frac{100 \times (C0 - C)}{C0}$$

Where C0 is the initial dye concentration and is the concentration of dye after catalytic degradation

Phytotoxicity study:

The study was carried out with degraded dye (Methylene Blue) using the seeds of *Cicerarietinum*. The seeds were sterilized to discourage fungal growth by wiping them on the surface with 1.2% sodium hypochlorite solution [24-25]. Five seeds were placed in each petri plate and watered separately with 5ml samples of each degraded dye per day. Positive Control was set up with normal water and the untreated dye was used as negative control. The plates were regularly monitored regularly for germination. Seeds with emerging radical are considered to be germinated**.** The length of the radical (root) and plumule (shoot) and the germination rate (%) were recorded after 7 days.

Results and discussions:

Qualitative Phytochemical screening:

The results of phytochemical screening were represented in Table (1).

Table (1): Results of Phytochemical analysis

S. No	Phytochemical Test	Reagents used	Interference	Result
1.	Alkaloid	Mayer' Reagent	Appearance of yellow cream ppt.	Positive
		Hager's Reagent	Formation of yellowish white ppt	Positive
2.	Carbohydrate Test	Molish's Reagent	Formation of Violet ring	Positive
		Benedict's Reagent	Formation of orange red ppt	Positive
		Fehling's solution	Formation of red ppt.	Positive
3.	Saponin test	Foam Test	Produce foam that lasts for more than 10 minutes	Negative

4.	Glycoside test	Modified Brontrager's Reagent	No formation of pink colour	Negative
5.	Phytosterol test	Salkowski's Test	Golden brown colour obtained	Positive
6.	Fats and Fixed oil test	Filter Paper press Test	No oily stain was obtained	Negative
7.	Resin test	Acetone Water Test	No Appearance of Turbidity	Negative
8.	Phenol test	Ferric Chloride Test	Appearance of bluish black ppt	Positive
9.	Tannin test	Gelatin Test	No formation of white ppt.	Negative
10.	Diterpenes test	Copper Acetate Test	No formation of bright green colour	Negative
11.	Flavonoids test	Alkaline Reagent Test	No intense yellow colour obtained	Positive
		Lead Acetate Test	Yellow ppt. obtained	Positive
12.	Proteins and amino acids test	Xanthoproteic Test	Formation of yellow colour	Positive

Characterization of Copper nanoparticles:

UV-Visible Spectroscopy:

UV-Vis Spectrophotometer monitored the reduction of Cu+ ions for the metal ions stability. The characterization of copper nanoparticles by UV-Spectrophotometer from the range 200-800 nm. The broad peak was obtained at 320 nm which is represented in fig.1.

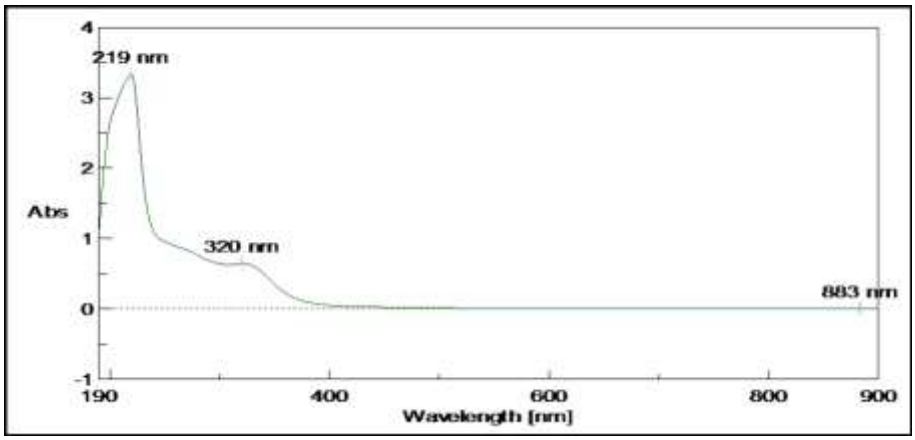


Fig.1. UV-Visible Spectroscopy

FT-IR Analysis:

The FT-IR analysis is used to identify the capping and stabilizing capacity of the leaf extract. The FTIR bands of biosynthesized Cu NPs using leaf extract of *RaphanusSativus*were indicated at 3441.65 (Bond: N– H stretch) ,3002.02 (Bond: C–H stretch). The present study revealed that the FTIR band proved the appearance of

carboxylic acid, and strong aromatic ring, which may be responsible for the synthesis of copper Nanoparticles using *RaphanusSativus***(Fig.2).**

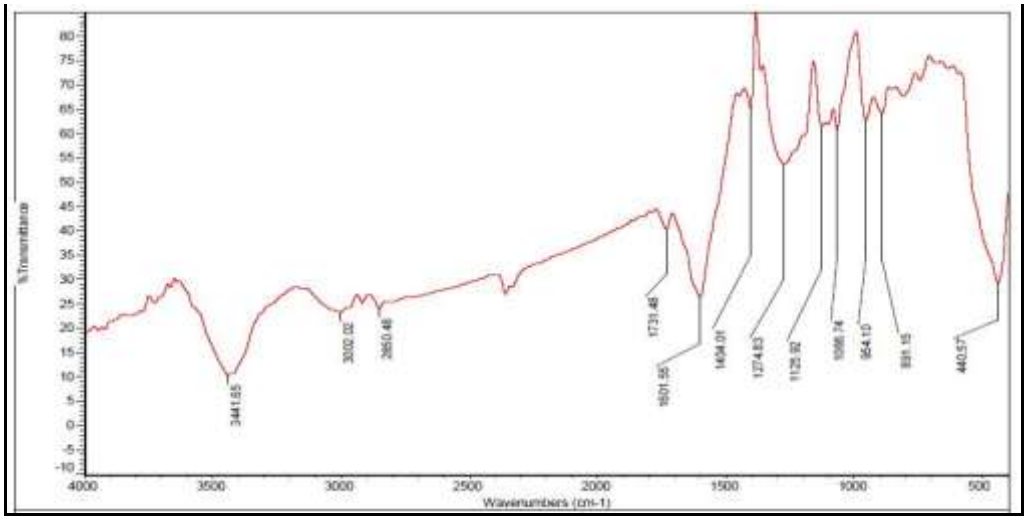


Fig.2 FTIR Analysis

XRD Analysis:

XRD pattern with a sharp peaks at 2h = 40.52, 53.31 and 71.69corresponding to (111), (200) and (220) representing face centered cubic structure of copper and are obtained (JCPDS No. 851554). The average crystallite size estimated using the Debye-Scherrer formula was found to be about 87 nm. This is related to the polycrystalline nature of the nanoparticles. XRD pattern revealed the structure of the synthesized Cu NPs (Fig. 3).

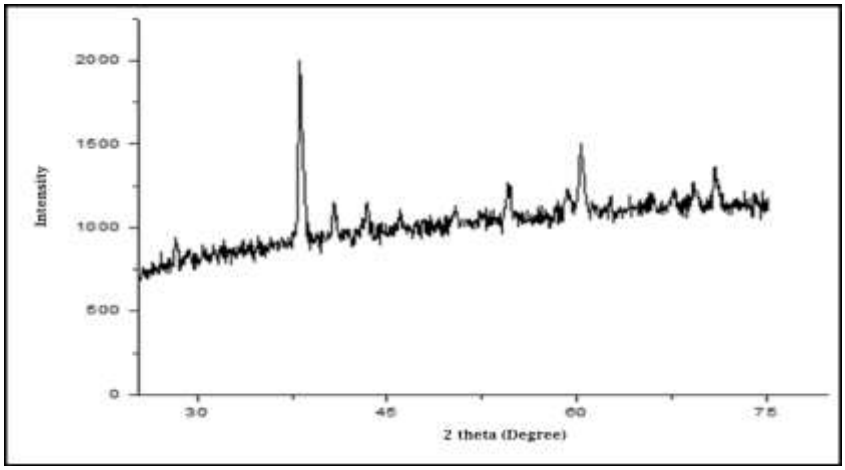


Fig.3. XRD

SEM Analysis:

The typical SEM image shows that the product mainly consists of particle-like spherical and monodispersed distribution of particle sizes and the size ranges from 85 to 118 nm. However, further observation with high magnification reveals that these Cu nanoclusters are assembled by smaller nanoparticles, which exhibit good uniformity and the average diameter is about 54 nm **(Fig.4)**.

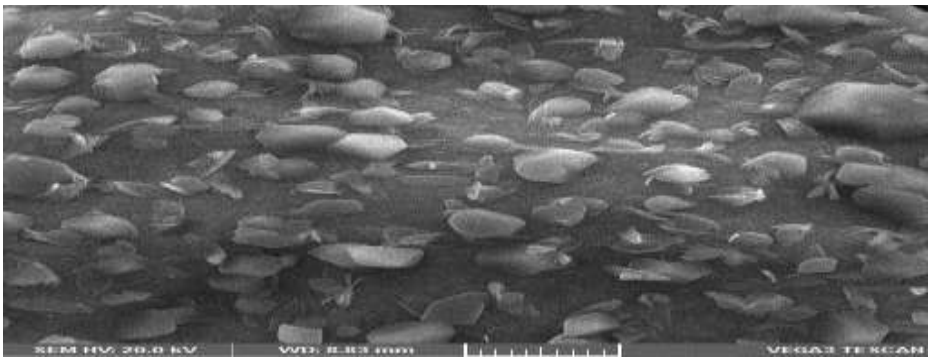


Fig.4 SEM Analysis

Antioxidant activity:

Hydrogen peroxide scavenging assay:

100 µg of aqueous extracts of *RaphanusSativus*exhibited 29% scavenging activity on hydrogen peroxide and Cu NPs exhibits 38%. On the other hand, α- tocopherol exhibited 46.77% hydrogen peroxide scavenging activity **(Table 1)**.

Table 1: Antioxidant activity

Samples	**% H_2O_2 Scavenging Activity (100 µg/mL)**
Control	0
α-tocopherol	46.77
Tridaxprocumbens(leaves extract)	29
Copper Nanoparticles	38

Antibacterial activity:

The synthesized Cu NPs and aqueous plant extract *RaphanusSativus*had tested for its ability to inhibit the growth of *E. coli, S. aureus, P. aeurginosa*, *Listeria*sp, *Proteus* sp. The nanoparticles showed maximum inhibition against *E.coli* followed by *S.aureus ,P. aeruginosa, Listeria sp, Proteus sp.* The plant extract showed maximum inhibition against *Pseudomonas aeruginosa followed by Listeria sp, S.aureus,Proteus sp, E. coli.* The zones are presented in Table**.**This result suggests that the copper nanoparticles and theplant extract have the potential against several bacteria species (Table 2)

Table 2. Antibacterial activity

Microorganism	**Cu NPs (mm)**	**Plant Extract (mm)**
E. coli	24± 0.36	6±0.47
S. aureus	18±0.87	10±0.91
P.aeurginosa	13±0.1	17±0.14
Listeria	9±0.41	13±0.23
Proteus sp	6±0.25	8±0.60

Catalytic reduction of Methylene Blue using Plant extract and Cu NPs*:*

Dyes with different concentration of Plant extract were observed from Fig. 5 (a) and (b) for decolourization. Methylene Blue showed maximum degradation only at 72 hours (100µl Plant extract showed 71% decolourization, 75 µl Plant extract showed 52% decolourization, 50 µl plant extract showed 43% decolourization and 25 µl plant extract showed only 32% decolourization).

Dyes with different concentration of Copper NPs were observed from Fig.6 (a) and (b) for decolourization. Methylene Blue showed maximum degradation only at 72 hours (100µl Copper Nps showed

89% decolourization, 65 µl Copper Nps showed 72% decolourization, 50 µl Copper Nps showed 52% decolourization and 25 µl Copper Nps showed only 38% decolourization).

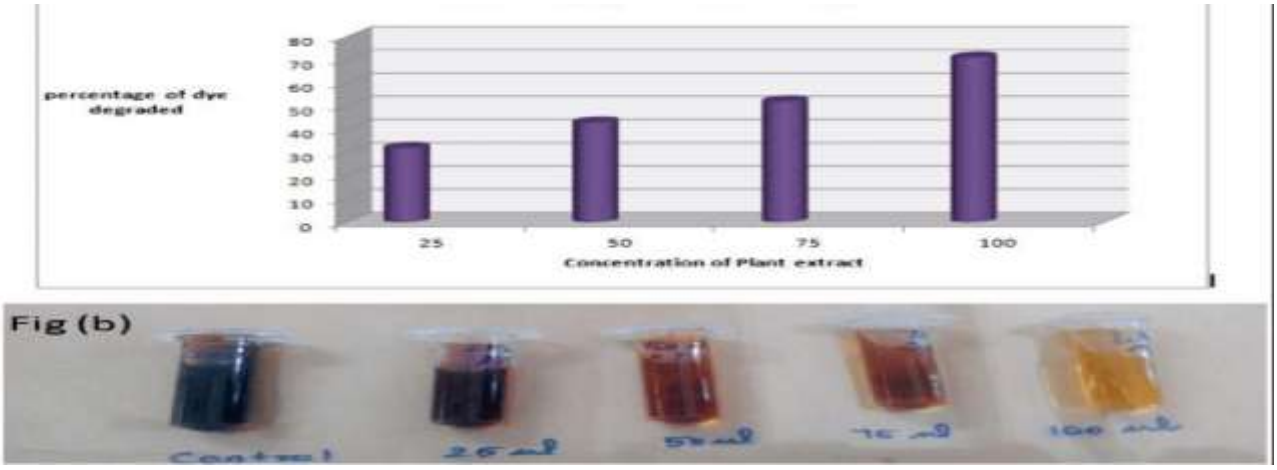


Fig 5 Catalytic reduction of Methylene Blue using Plant extract

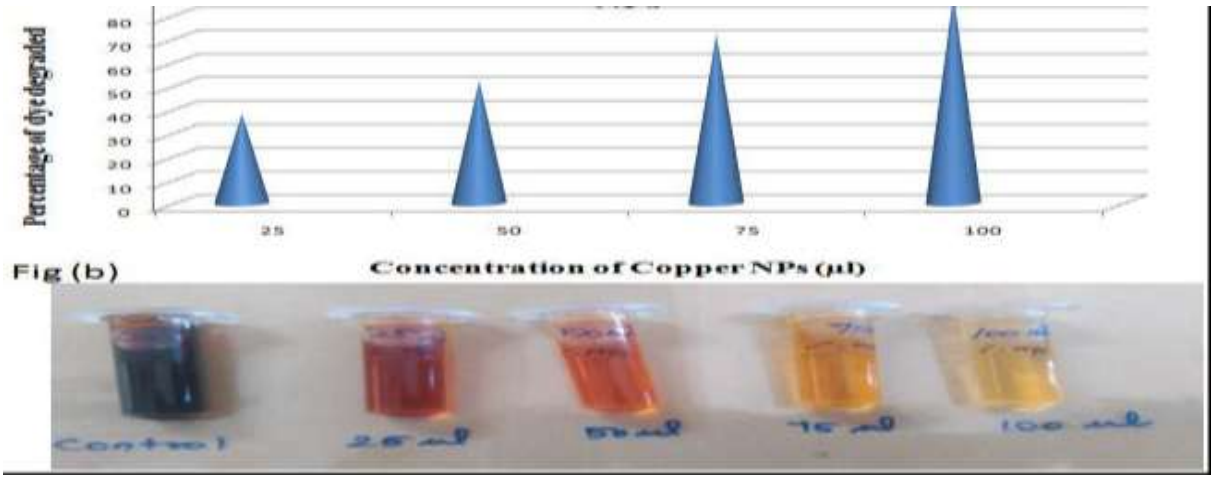


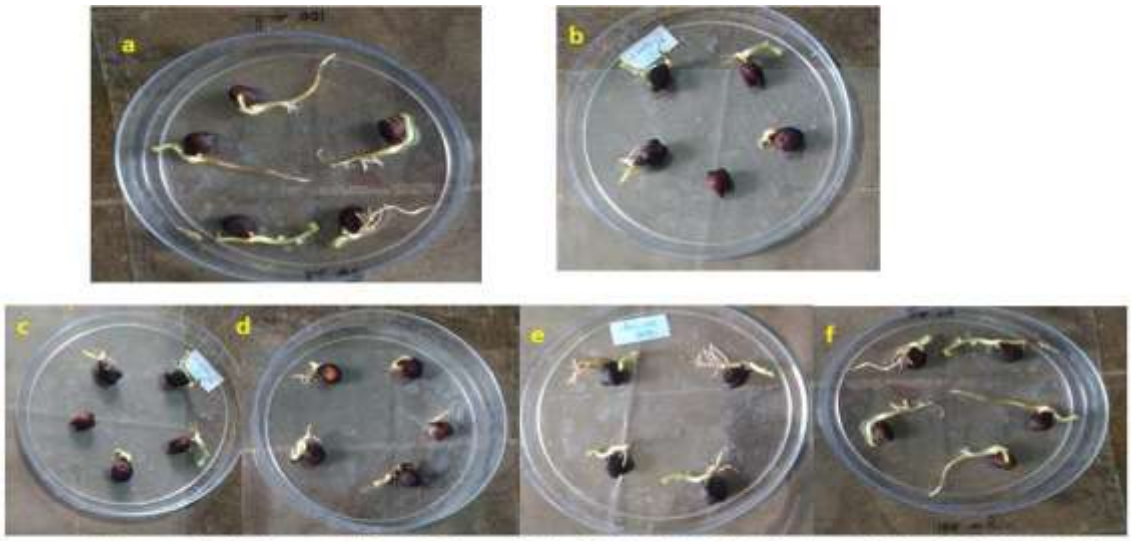
Fig 6 Catalytic reduction of Methylene Blue using Cu NPs

Phytotoxicity study:

This study was carried out to evaluate the relative sensitivities of *Cicerarietinum* towards the dye and their degradation products using seed germination and plant growth assays. Germination (%) of plant seeds was less with the raw dye when compared to the treatment of dye with Copper NPs . There was 100% Germination in control (Water). The germination (%) of *Cicerarietinum* was also found to be 100% when treated with degraded dye and the untreated dye showed only 40% germination (Fig.7) and **Table 3**. [26]conducted similar studies using *Aloe barabadensis* extract to degrade two different dyes (malachite green and congo red), where the authors found the marked difference in the toxic effect of dyes on seedling compared with plant extract treated dye products.

Table 3: The germination (%) of *Cicerarietinum*

Treatments	*Cicerarietinum*	
	Plumule (cm)	**Radicle (cm)**
Control (Water)	24±0.67	8±0.70
Untreated Dye (Bismarck Brown)	8.5±0.55	4±0.04
Treated Dye (Bismarck Brown+ Cu NPs)	16±0.69	7.2±0.17



(a) Control (b) Untreated dye (c) Treated dye(25µl of Cu NPs) (d) Treated dye(50µl of Cu NPs) (e) Treated dye(75µl of Cu NPs) (f) Treated dye(100 µl of CuNPs)

Fig.7 Phytotoxicity study - *Cicerarietinum*

Conclusion:

In the present work, we report an eco-friendly and simple method for the synthesis of Copper nanoparticles (Cu NPs) using *RaphanusSativus*leaf extract. Cu NPs has been synthesized using copper acetate as a precursor. The biosynthesized nanoparticles have been characterized by UV-VIS spectroscopy, FTIR, XRD and SEM. The biosynthesized Cu NPs showed excellent antimicrobial activity. Cu NPs also showed efficient catalytic activity towards degradation of Methylene Blue thus having potential for industrial application.

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