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# SUSTAINABLE CHEMISTRY ROUTE SYNTHESIS AND CHARACTERIZATION OF *AZADIRACHTA INDICA* AND *OCIMUM SANCTUM* LEAF EXTRACT MEDIATED COPPER OXIDE NANOPARTICLES AND ITS ANTIFUNGAL ACTIVITY AGAINST *FUSARIUM MONILLIFORME.*

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**ABSTRACT**

Currently, the agricultural sector is facing various global challenges, including environmental problems, climate change, such as chemical fertilizer, pesticides, which will be food shortages and population growth. Thus, there is an essential need to transform organic farming and replace new technologies. Nanotechnology, particularly sustainable chemistry route of nano-biosynthesis, offers significant potential to address these challenges effectively. Nanotechnology can transform agriculture by advancing biosensors, food packaging, pesticides, organic fertilizers and other sectors. Due to their unique properties, nanomaterials are ideal carriers for stabilizing fertilizers and pesticides, enabling controlled nutrient transfer and enhancing crop protection. The physical and chemical synthesis of nanoparticles requires the use of hazardous materials, harmful for plant, animal and has a negative impact on the environment. But nanotechnology is one of the eco-friendly and cost effective methods for synthesis of nanoparticles. Sustainable chemistry route of biosynthesis of copper oxide nanoparticles which are generally synthesized by using *Azadirachta indica* (Neem) Leaf and *Ocimum sanctum* leaf Aqueous Extract. In this study, copper sulphate pentahydrate was used as a precursor and *Azadirachta indica* and *Ocimum sanctum* Aqueous Leaf Extract acts as reducing agent. The synthesized metal nanoparticles were characterized by UV– visible spectroscopy. The copper oxide nanoparticles showed characteristic peak at 250 nm and 330 nm. The synthesized nanoparticles from neem and tulsi extract have antifungal activity against *Fusarium moniliforme* Sheldon fungal disease of sugarcane crop plant with zone of inhibition 1.77 cm diameter at 1000 ppm Cu NP's Suspended in Distilled water. The synthesized copper oxide nanoparticles are expected to have potential antifungal activity. This approach to nanoparticle synthesis is regarded as more convenient than alternative methods.

***Key words*: ** Green chemistry, *Azadirachta indica*, *Ocimum sanctum*, copper sulphate pentahydrate, Metal nanoparticles, Antifungal activity.

## Introduction

In past few years, the agriculture sector facing major global concern is pollution. The farmers use chemical fertilizers and pesticides to boost crop production and control pests, but their excessive use has harmed the environment and human health. This degradation is exacerbated by the growing population in developing nations, leading to an imbalance between human needs and resource sustainability. Chemical fertilizers, while increasing yields, have caused significant environmental issues, notably water pollution from phosphates and nitrates. (R. Kumar *et al.*, 2017)

But currently few years, scientists and researchers interested towards nanotechnology for the formation of nanoscale materials by using different physico-chemical and biological method. Nanoparticles have unique applications in all fields such as agriculture, medicine, electronic, space due to their small size (1 to 100 nm) and their controllable properties. The nanoparticles exhibit changes in properties due to main two reasons as depend on; (i) Surface effects (the surface to volume ratio is high when particle size is reduced and easily penetrate to plant) ii) quantum confinement effect (Campos *et al.*, 2015; Kirdat *et al.*, 2020).

Metal nanoparticles can be synthesized using methods like gas-phase deposition, mechanical techniques, sol-gel, co-precipitation, hydrothermal, and thermal decomposition. However, these often use hazardous chemicals, raising biocompatibility concerns in biomedical applications. Therefore, sustainable green chemistry techniques offer an eco-friendly, low-cost, and controllable approach for synthesizing various metal nanoparticles. (Karade *et al*., 2018; Kirdat *et al*., 2020)

In green synthesis of metal nanoparticles by using biological materials such as plant extracts, bacteria, fungi, and algae, etc. due to their simplicity, speed, and cost-effectiveness. Also, they are called biogenic nanoparticles. (Indurkar *et al*., 2020; Kirdat *et al*., 2020)

The leaves of plants consist of various secondary metabolites and they act as reducing agents and capping agents. They reduce the charge on the metal surface i.e. gain of an electron to form metal nanoparticles. They are also called phytochemicals (Singh *et al*., 2018; Mirza *et al*., 2018; Taib *et al*., 2018)

Neem, scientifically known as *Azadirachta indica*, is predominantly found in India, Pakistan, Bangladesh, and Nepal. Different components of the neem tree—such as leaves, roots, shoots, and seeds—are harnessed to produce organic formulations aimed at disease control. They have various bioactive compounds including nimbin, nimbidin, nimbolide and limonoids plays a significant role in disease management by modulating various genetic pathways and other physiological activities. These compounds possess antifungal, antibacterial etc. (Alzohairy, *et al.*, 2016)

Tulsi, scientifically known as *Ocimum sanctum* to have found in north-central India. In Ayurveda, tulsi is known as the "Mother Medicine of Nature" and "The Queen of Herbs". (Cohen, *et al.,* 2014) The tulsi leaf extract has various secondary metabolites like carbohydrates, tannin, flavonoids, saponins, glycosides, terpenoids, fatty acids phenols, etc. Most of the *Ocimum sanctum* leaves consist as water-soluble phenolic compounds. (Borah *et al.*, 2018)

### How Nanoparticles are important in Plant Protection?

1. **Enhanced Nutrient Delivery:** Nano-fertilizers improve absorption and provide slow-release nutrients, reducing application frequency and nutrient loss.
2. **Disease and Pest Management:** Nano-pesticides and fungicides target infections directly, reducing chemical use and environmental impact. Metal nanoparticles control fungal and bacterial infections.
3. **Improved Growth and Development:** Nanoparticles efficiently deliver growth hormones and improve stress resistance, enhancing growth and yields.
4. **Sustainable Agriculture:** Reduced runoff and lower chemical dosages protect ecosystems and promote sustainability.
5. **Economic Benefits:** Improved delivery reduces costs, and enhanced growth increases yields and profitability.

## Materials and Methods

### Materials

The fresh raw material of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) leaf was collected from campus of Vasantdada sugar institute, Manjari Budruk, Haveli, Pune Maharashtra State, India. The fresh chemical (Copper (II) sulfate pentahydrate salt CuSO4.5H2O) and fungal nutrient media used for this study were purchased from Hi-Media. The Pure Fungal culture of *Fusarium moniliforme Sheldon* was collected from Department of Plant Pathology Section, Vasantdada sugar institute, Manjari Budruk, Haveli, Pune Maharashtra State, India and the distilled water was used for entire procedure.

### Preparation of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) leaf extract

The fresh leaf of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) separately was washed thoroughly with distilled water. Both 100 g of washed leaf were added into 100 ml water in a 250 ml Erlenmeyer flask and kept in hot water bath for 15 min at $80^{o}$C. The extract was cooled and filtered and used using Whatmann No.1 filter paper to obtain the aqueous solution of extract. This solution was used for green synthesis copper oxide nanoparticles (Gopalakrishnan *et al*., 2020; Kirdat *et al.*, 2020).

### Preparation of 1 Mm Copper Sulphate Solution

For the green synthesis of metal oxide nanoparticles Copper (II) sulfate pentahydrate salt CuSO4.5H2O was used as precursor. 1M CuSO4.5H2O was weighed accurately and dissolved in 250ml distilled water on magnetic stirrer for 1h at room temperature. The after 1h the transparent solution of CuSO4.5H2O was formed. (Ansilinet *et al.*, 2016; Kirdat *et al.*, 2020)

### Synthesis of copper oxide nanoparticle

In green synthesis of metal oxide nanoparticle, 1mM of Copper (II) sulfate pentahydrate salt and 25 mL of

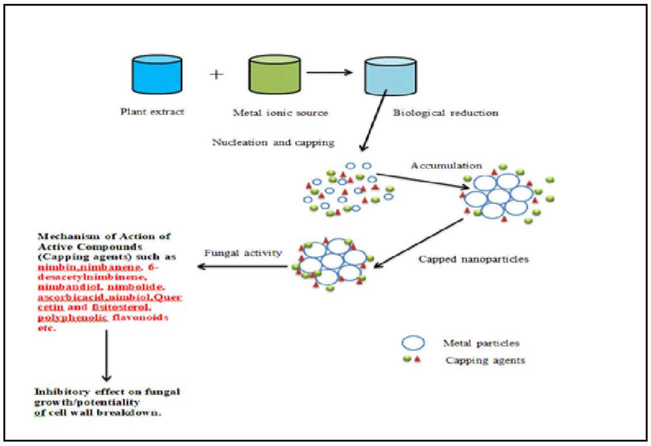


**Fig. 1:** Mechanism of plant extract mediated metal oxide nanoparticle synthesis to fungal activity.

*Azadirachta indica* (Neem) is taken two beakers. In this reaction the Copper (II) sulfate pentahydrate salt solution act as precursor and Neem leaf extract acts as reducing agent. 25ml of Neem leaf extract was kept for stirring on magnetic stirrer and drop wise addition of 100 ml Copper (II) sulfate pentahydrate salt solution for 1h was carried out. After complete addition of solution, the solution are kept in overnight incubation. The green to dark green colour was observed after incubation which indicates formation of copper oxide nanoparticles. This synthesized nanoparticle centrifuge for 15 min at 12000 rpm. The obtained precipitate was collected and kept sample for dry at 80°C in hot air oven. The resulting dark green coloured powder was used for characterization and *in vitro* application study. The note that, the same above reaction as follow for synthesis of copper oxide nanoparticle by using *Ocimum sanctum* (Tulsi) leaf extract. (Gopalakrishnan *et al.*, 2020. Kirdat *et al.*, 2020; Ansilinet *et al.*, 2016).

## Characterization of metal oxide nanoparticle

In synthesis of nanoparticle, the characterization as

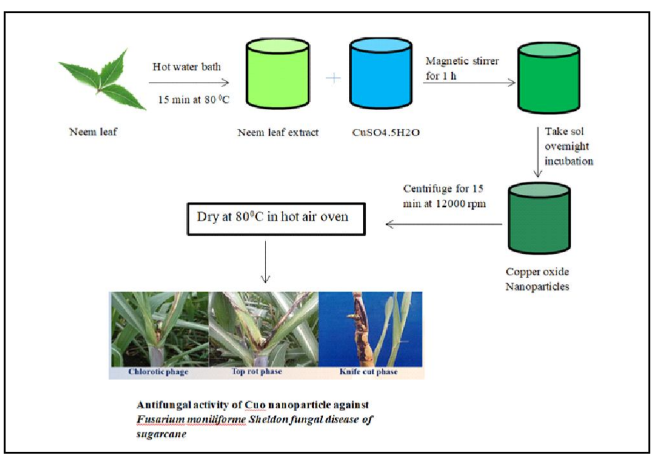


**Fig. 2:** Green synthesis of copper oxide nanoparticle by using neem leaf extract.

only tool to use and identify the synthesized materials properties such as size, shape, Structural morphology, nature, surface charge etc. In this study the use of UV-visible spectroscopy of sample was recorded on SPECTRO star Nano is equipped with BMG LABTECH's ultra –fast UV/Vis spectrophotometer, which allows users to measure full spectrum absorbance (220-1000 nm) in less than 1 second per well. The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded to detect the functional group of nanoparticle on Shimadzu FT-IR 8400 s instrument scanning from range 500–4000 $cm^{-1}$. The dynamic light scattering (DLS) was used to record the particle size of material on Anton paar instrument.

## Antifungal assay of metal nanoparticle *in vitro* condition

The antifungal activity of both synthesized metal oxide nanoparticles was studied against *Fusarium moniliforme Sheldon* fungal disease of sugarcane. Pure *Fusarium moniliforme Sheldon* fungal samples were isolated and then were grown on potato D-glucose agar (PDA) media for further experiment. The Wells were bored in each plate with the use of a sterilized borer. The green synthesized 2 ml nanoparticle was loaded into wells. The after loaded of nanoparticles the plate are raped to use of paraffin foil and plates were kept into incubator for 24 h at 28°C. The Zone of inhibition was observed on next three or Four day (Kirdat *et al.*, 2020). The zone of inhibition was assessed once the mycelial growth in the control plate extended to the edge of the Petri dish. The efficacy of growth inhibition (%) was then calculated using the method described by Margarita as follow:

$$\text{Inhibition Efficacy (\%)} = \frac{D_0 - D_a}{D_0} \times 100$$

Where,

$D_0$ is the diameter of mycelial growth in the control petri plates and $D_a$ is the diameter of mycelial growth in the plates treated with CuO NPs, respectively. (Hanh

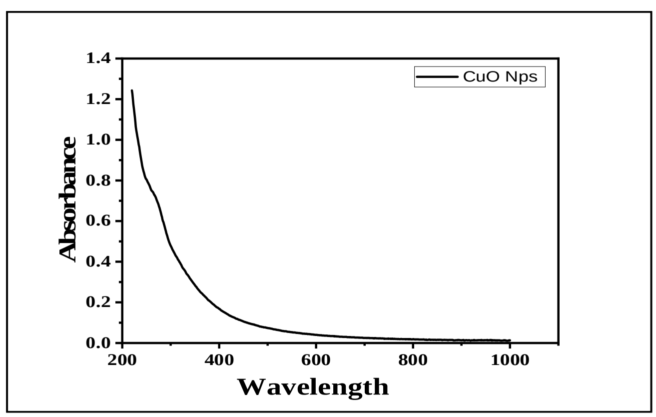


**Fig. 3:** Spectra observed in UV Spectrometer (Neem).

Thi Truong *et al*., 2023).

### Statistical analysis

The All fungal growth assays of copper oxide nanoparticle mediated neem leaf extract and copper oxide nanoparticle mediated tulsi leaf extract were carried out *in vitro* condition. The process are follow in triplicate and data are presented as the mean standard deviation on ANOVA tool to evaluate the interactions between both factors of concentration on inhibition efficacy of fungal growth for treated with nanoparticles (Treatment) and non- treated without nanoparticles (Control).

## Results and Discussion

### Synthesis of Copper oxide nanoparticle by using biological reduction

The biological reduction of copper sulphate pentahydrate salt as metal precursors is one of the most promising synthetic approaches to obtain metallic nanoparticles. The neem leaf extract and tulsi leaf extract act as reducing agents. The rate of growth of the nanoparticles depends on various parameters such as, the type of reductant, concentration of metal ions, pH and temperature. The green material also proved to be useful for its fast reaction kinetics of reduction. In this study, metal oxide nanoparticles were prepared by simple reduction of copper salts by using reducing agent. This process was eco-friendly, cost effective and time effective (Hanh Thi Truonget *et al.*, 2023). The smaller size of nanoparticles acts as potential antibacterial, antimicrobial, antifungal and antioxidant agent etc. The Neem leaf and its essential bioactive components such as Nimbin, 22,23-Dihydronimocinol have been demonstrated to exhibit pesticidal and antifungal, Insecticidal properties but other gedunin, nimbinin, mahmoodin bioactive component are present in root, bark, seed oil etc. is a part of neem plant shown many properties such as Antiviral, antifungal, and antibacterial, Insecticidal, and anti feedant etc. (Stephen Adusei *et al.,* 2022). In this study when reduction is occur the bioactive compound from neem leaf extract are bind to surface of metallic surface and exhibit potential antioxidant activity. The Due to different useful properties of neem and tulsi leaf is mainly used to synthesize metal oxide nanoparticle. (Kirdat *et al*., 2020).

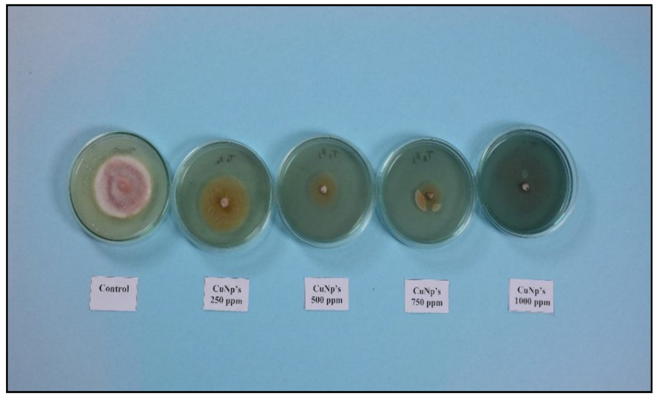

**Fig. 5:** Efficacy of Copper Nanoparticles against *Fusarium monilliforme*.

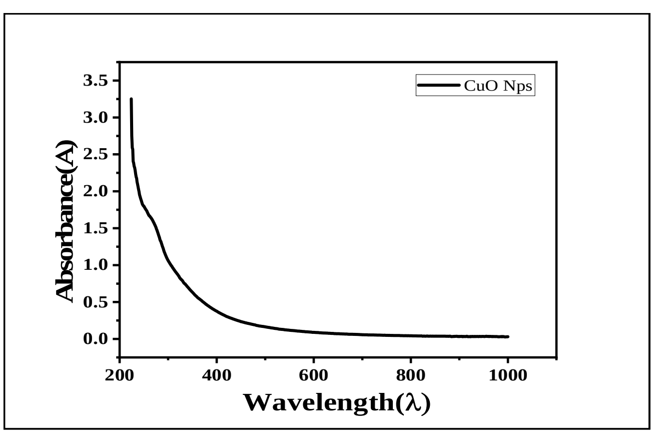


**Fig. 4:** Spectra observed in UV Spectrometer (Tulsi).

The mechanism for reduction of CuSO4.5H2O ions to copper oxide nanoparticles can be proposed as follows:

$$\underset{\text{Nimbin}}{C_{30}H_{36}O_9} + \underset{\text{pentahydrate Copper sulphate}}{CuSO_4.5H_2O} \longrightarrow CuO + SO_3^- + 5H_2$$

### UV-Vis Spectra Analysis of metal oxide nanoparticle

The UV-Visible spectral analysis of neem and tulsi leaf extract mediated CuO NPs was recorded in the wavelength range of 220-1000 nm at room temperature against distilled water as a reference. The absorption peak of neem leaf extract and tulsi leaf extract was obtained at 250nm and 330 nm. The absorption peak of neem and tulsi leaf extract mediated CuO NPs was exhibited at 250 nm and 330 nm. The absorbance wave length values are closely matched with the reported values is 346 nm and 350 nm (Ansilinet *et al*., 2016; Gurudevi *et al*., 2023).

### The Fourier transforms infrared spectroscopy (FTIR)

The Fourier transform infrared spectra of synthesized

**Table 1:** *In-vitro* testing of copper nanoparticles against *Fusarium monilliforme*.

| | R1 | R2 | R3 | R4 | Average |
|---|---|---|---|---|---|
| **Control (T0)** | 5.3 cm | 5.9 cm | 5.5 cm | 5.7 cm | **5.6 cm** |
| **250 ppm (T1)** | 5.7 cm | 5.4 cm | 5.3 cm | 4.7 cm | **5.2 cm** |
| **500 ppm (T2)** | 3.5 cm | 3.9 cm | 3.3 cm | 3.5 cm | **3.5 cm** |
| **750 ppm (T3)** | 3.3 cm | 3.3 cm | 3.8 cm | 5.0 cm | **3.8 cm** |
| **1000 ppm (T4)** | 1.9 cm | 2.0 cm | 1.7 cm | 1.5 cm | **1.7 cm** |

*Here, **R** denotes Replication and **T** denotes Treatment.

metal oxide nanoparticles were recorded between 500 and 4000 $cm^{-1}$. The different functional groups attached to synthesized nanoparticles were analysed by this spectroscopy. (Kirdat *et al.,* 2020).

**Anti-fungal activity**

Copper nanoparticles (CuNPs) have garnered significant attention for their antifungal properties. The unique characteristics of CuNPs, such as high surface area to volume ratio, catalytic activity, and ability to generate reactive oxygen species (ROS), make them effective against a variety of fungal pathogens. Copper nanoparticles exhibit potent antifungal activity through multiple mechanisms, including cell membrane disruption, ROS generation. Antifungal Activity of copper nanoparticles against *Fusarium monilliforme* was studied in *in vitro* conditions.

Samples of *Fusarium monilliforme* were Isolated from Pokkah boeng infected Sugarcane crop from Vasantdada Sugar Institute, Manjari. The pathogen was cultured on the PDA media with the given concentration of nanoparticles directly in the PDA media. The Recorded growth of pathogen was as mentioned in Table 1.

## Conclusion

The Peak of absorbance for Spectroscopy of Copper Nanoparticles synthesized from Neem and Tulsi was observed at 250 nm and 330 nm respectively. Best efficacy against *Fusarium monilliforme* was seen at 1000 ppm ($T_4$) *i.e* 1.77 cm in diameter and least results were observed at 250 ppm ($T_1$) *i.e.* 5.2 cm in diameter.

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