

# A NEW APPROACH TO WOOD PROTECTION: PRELIMINARY STUDY OF BIOLOGICALLY SYNTHESIZED COPPER OXIDE NANOPARTICLE FORMULATION AS AN ENVIRONMENTAL FRIENDLY WOOD PROTECTANT AGAINST DECAY FUNGI AND TERMITES

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## ABSTRACT

Nanoparticles have addressed many challenges in science and technology and wood science research is one such field that has benefitted from application of metal nanoparticles. The metal nanoparticles that are commercially available for wood protection are synthesised by physical and chemical methods which produces toxic by-products and are expensive. The current study deals with a new approach for utilization of metal nanoparticle for wood protection in an ecofriendly and cost effective way. Metal nanoparticles were synthesised using plant extracts that are known to have wood preservative properties. The synergistic effects of the intrinsic property of plant extracts along with the biocidal property of metal nanoparticles were utilized. Copper oxide nanoparticles were synthesised using leaf extracts of Neem (*Azadirachta indica*), Pongamia (*Pongamia pinnata*), Lantana (*Lantana camara*) and extract of orange peel (*Citrus reticulata*). The effectiveness of the synthesised plant extract and copper oxide nanoparticle formulation is tested against wood decay fungi using agar mixed with the test substance. Graveyard test is employed to assess the effect of the copper oxide nanoparticle formulation against termites. Preliminary results are promising and studies are progressing to develop a stable and environmentally benign wood preservative formulation of metal nanoparticles and plant extracts.

**Keywords:** Antitermite activity, biological synthesis, brown-rot fungi, plant extracts, white-rot fungi, wood protection.

## INTRODUCTION

In last decade, nanoparticles have been reported to resolve many impeding technological challenges in various fields of science like engineering, medicine, water purification, catalysis etc. to name among a few (Klefenz 2004, Goodsell 2004, Chan and Nie 1998, Colvin *et al.* 1994, Tan *et al.* 2006, Lee *et al.* 2008, Pissuwan *et al.* 2006, Cai *et al.* 2008, Cao 2004). One such area in which nanoparticles, mainly metal nanoparticles have proved to be instrumental is wood protection. The metal nanoparticles used in wood protection are generally copper, zinc and boron. Lot of literature is available on the use of nanometals for wood protection (Clausen 2007, Clausen *et al.* 2010, Kartal *et al.* 2009, Akhtari and Arefkhani 2010, Nair *et al.* 2017). Terzi *et al.* (2016) reported the role of nanoparticles of ZnO, B<sub>2</sub>O<sub>3</sub>, CuO, TiO<sub>2</sub>, CeO<sub>2</sub> and SnO<sub>2</sub> in prevention of fungal decay, mold growth and termite attack on wood along with their effect on weathering properties and water repellency. Mishra *et al.* (2018) reported the impact of nanoparticles on the wood coating and preservation treatments based on survey of the registered patents. The report gave an overview of the scientifically most followed trends on nanoparticle utilization in wood science and wood protection depicted by recently filed patents. Even though nanoparticles have addressed various issues of wood science in a more efficient way than the traditional methods of employing metal salts, commercially available nanoparticles are synthesized by physical and chemical methods. The disadvantage of physical synthesis method is that they are energy intensive and are expensive. Chemical synthesis methods involve the use of many toxic chemicals which limits the applications of chemically synthesized nanoparticles, especially in medical field and are not environmental friendly (Vijayaraghavan and Ashokkumar 2017). Search for a biocompatible, non-toxic

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and ecofriendly method of nanoparticle synthesis lead to the development of biological synthesis route for production of nanoparticles. Biological synthesis of nanoparticles involves the use of biomaterials such as bacteria (Hulkoti and Taranath 2014), fungi (Dhillon *et al.* 2012), yeast (Moghaddam *et al.* 2015), virus (Dujardin *et al.* 2003), microalgae (Schrofel *et al.* 2011), macroalgae (Singaravelu *et al.* 2007) and plant extracts (Mittal *et al.* 2013). Of all these biomaterials, plant extracts are considered more advantageous for the synthesis due to their availability, renewable nature, simplicity of process, efficiency, rate and stability of synthesized nanoparticles and cost effectiveness (Vijayaraghavan and Ashokkumar 2017, Iravani 2011).

Wood science researchers so far have not explored the possibility of utilizing biologically synthesized nanomaterials for wood protection. Plant extracts contain numerous organic molecules like carbohydrates, proteins, vitamins, alkaloids, enzymes etc. These molecules can act as either potential reducing or stabilizing agents for the synthesis of nanoparticles. In addition to the ecofriendliness and cost effectiveness, plant extract based synthesis combines the intrinsic properties of plants with the biocidal properties of nanoparticles via the synergistic effect. The plant extracts which have both wood preservative properties and are capable of synthesizing metal nanoparticles are to be selected. The synergistic effect of the plant extract with the biocidal nature of metal nanoparticles also contributes to the efficacy of a wood protectant. There are several factors that affect synthesis of nanoparticles using plant extracts, its characterization and application namely origin of plant material, method of extraction employed and the solvents used. The temperature employed, pH-value and the reaction time also plays an important role in the size and shape of nanoparticles produced (Vijayaraghavan and Ashokkumar 2017).

This research reports the synthesis of copper oxide nanoparticles using plant extracts and testing its efficacy as a wood preservative. Leaf extracts of Neem (*Azadirachta indica*), Pongamia (*Pongamia pinnata*), Lantana (*Lantana camara*) and extract of orange peel (*Citrus reticulata*) were used to synthesize copper nanoparticles. All these plants have reported wood preservative properties (Macias *et al.* 2005, Sotande *et al.* 2011, Machado *et al.* 2013, Venmalar 2017, Gupta *et al.* 2017) and are reported in the synthesis of metal nanoparticles (Ansilin *et al.* 2016, Sundrarajan *et al.* 2015, Majumder 2012).

## MATERIALS AND METHODS

### Chemicals

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was procured from Hi-Media Laboratories, Mumbai, India. Copper oxide (CuO) nanopowder (< 50 nm) was procured from Sigma Aldrich Chemicals Pvt. Ltd., Bengaluru, India and distilled water from Millipore was used.

### Plant materials and preparation of the extract

Leaves of *Azadirachta indica*, *Pongamia pinnata*, *Lantana camara* and orange peel (*Citrus reticulata*) were used for the synthesis of CuO nanoparticles. Leaves of these plants were collected from Nallal, Hoskote, Bangalore and orange peel was obtained from local market, Bangalore. Leaf extracts 20% by weight were prepared in d.w. filtered through Whatman filter paper No.1 and stored at 4° C for further use. Orange peels were dried under shade ( $26 \pm 1^\circ \text{C}$ ) and powdered. 10% by weight of the orange peel extract was prepared by refluxing the dried powder in d.w. for 2h. It is then filtered through Whatmann No1 and kept at 4° C.

### Synthesis of copper oxide nanoparticles

Copper oxide nanoparticles were synthesized using leaf extracts of the selected plants and orange peel extract. The extracts and precursor solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0,025M) were preheated separately in a water bath at 60° C for 30 minutes and mixed at 1:4 proportions (Majumder 2012). The resultant solution was stirred at 1000 rpm for 10 minutes and kept at room temperature for 3h. Presence of copper oxide nanoparticles was indicated by change in colour from bluish green to dark green. The solution thus obtained containing copper oxide nanoparticles was used for the experiment.

## Characterization of biologically synthesized CuO nanoparticles

The synthesized nanoparticles were characterized using Scanning electron microscopy (SEM) (Gemini Ultra 55 with ESB detector at 5,0kV) combined with Energy dispersive analysis X-ray (EDAX). A small quantity of nanoparticle formulation was dropped on to a small piece (5 mm x5 mm) of cleaned silicon wafer (drop casting). The wafer was dried thoroughly in air and was kept in a desiccator under vacuum for 48 h. Specimens were coated with gold and SEM images were recorded.

## Fungus culture

Actively growing fungal cultures of *Trametes hirsuta* (Wulfen: Fr.) Pilat (FRI No. 534) (white rot) and *Oligoporus placenta* (Fr.) Gilb. & Ryvarden (FRI No. 180) (brown rot) were obtained from the National Type Culture Collection, Dehradun, India and maintained on malt (2%) and agar (1,3%) media, at Institute of Wood Science and Technology (IWST), Bengaluru.

## Agar mixed with test substances (Poisoned food method)

The growth test on agar with the added test substance was performed to assess the antifungal activity of biologically synthesized CuO nanoparticles against wood decay fungi as per standard procedures (Nene and Thapilyal 2000, Das *et al.* 2010). Malt agar media was prepared with different concentrations of the extract containing the synthesized copper oxide nanoparticles ranging from 0,25; 0,5; 1; 5; 7 and 10 %. Commercially available copper oxide nanoparticles were also tested for comparing the efficacy of the synthesized copper oxide nanoparticle solution. Three replicates were prepared for each concentration and for each fungus. A mycelial disc of 6 mm diameter, cut out from the periphery of a 7 days old culture, was aseptically inoculated onto the centre of agar plates containing the test substances. Malt agar plates with 1mL of distilled water were used as positive control. Malt agar plates with extracts alone and precursor solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  served as negative controls. The inoculated plates were incubated at 25°C and the colony diameter was measured daily till the control plates reached the petri dish border. The percentage mycelia inhibition was calculated according to the following Equation 1.

$$I = \frac{C - T}{C} \times 100 \quad (1)$$

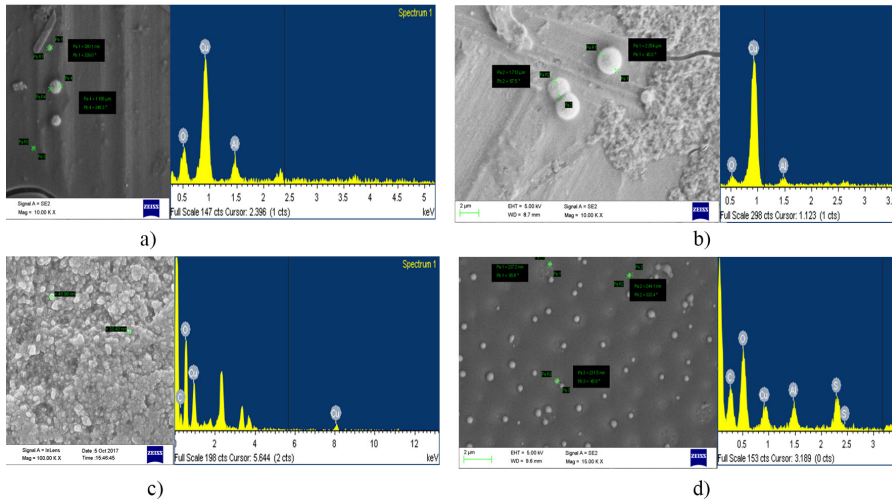
Where I is the inhibition, as percentage; C is the colony diameter of mycelia from control petri dishes, in millimeters; and T is the colony diameter (in mm) of mycelia from the petri dishes containing the sample. If the inhibitory ratio was greater than 20%, the test fungus would be considered inhibited and the minimal inhibitory concentration (MIC) for that fungus was then determined.

## Assessment of antitermite activity

Graveyard test (accelerated) as per the Methods for field testing of preservatives in wood (IS 4833, 1993) was done for assessing the antitermite activity of the CuO nanoparticle formulation. The experiment was done at the Institute of Wood Science and Technology, Bangalore and field evaluation was done at the termite test yard of IWST at Nallal, Karnataka. The termite fauna identified in this test yard were *Odontotermes horni* (Wasmann), *Odontotermes obesus* (Rambur), *Odontotermes redemanni* (Wasmann) and *Microtermes obesi* (Holmgren) (Sundararaj *et al.* 2003). Rubber wood stakes (*Hevea brasiliensis*) of 153 x 38 x 6,25 mm with long axis parallel to the grain of the wood, free from insect or fungal attack and from knots were air seasoned to constant weight. The rubber wood stakes were treated with the formulation by pressure impregnation method (30 min of vacuum followed by 60 min of 50 pounds).

RESULTS AND DISCUSSION

The synthesized CuO nanoparticles (CuO nps) using plant leaf extracts were characterized by SEM coupled with EDAX. SEM image confirmed the presence of CuO nanoparticles that are spherical in nature. The particles were well separated without agglomeration and the particle size remained within a range of 136 nm to 380nm in the case of CuO nps synthesized using *A. indica* leaf extract. In the case of CuO nps synthesized using *P. pinnata* leaf extract and *C. reticulata* peel extracts the particle sizes were in the range of 466nm to 2µm and 259nm to 519nm respectively. The particle size of CuO nps synthesized using *L. camara* leaf extract was in the range of 33nm to 46 nm. EDAX profile also confirmed the presence of copper and oxygen in major fraction (Figure 1).



**Figure 1:** Biologically synthesized CuO nanoparticles using plant extracts were characterized by SEM coupled with ED. a) Particle size of the synthesized copper oxide nanoparticles using *Azadirachta indica* leaf extract was between 136nm and 380nm. b) Particle size of the synthesized copper oxide nanoparticles using *Pongamia pinnata* leaf extract was between 466nm and 2µm. c) Particle size of the synthesized copper oxide nanoparticles using *Lantana camara* leaf extract was between 33nm and 46nm. d) Particle size of the synthesized copper oxide nanoparticles using *Citrus reticulata* leaf extract was between 259nm and 519nm.

Initiation of growth in control plates inoculated with *Trametes hirsuta* (white rot) and *Oligoporus placenta* (brown rot) was observed on 4<sup>th</sup> day of the experiment. On the 9<sup>th</sup> day the experiment was terminated as the control plates showed complete growth (Figure 2). The percentage of inhibition for *T. hirsuta* and *O. placenta* were calculated for all the plates (Table 1).

Sample→ Fungi↓	Control	CuO nanoparticle Std nanopowder (< 50 nm) 0,1; 0,25; 0,5; 1; 2; 5%	CuSO <sub>4</sub> ·5H <sub>2</sub> O 5,7,10%	<i>L.camara</i> leaf extract 5,7,10%	<i>L.camara</i> leaf extract with CuO nps 5,7, 10%
<i>Trametes hirsuta</i> (White rot)					
<i>Oligoporus placenta</i> (brown rot)					

**Figure 2:** Plates showing inhibition of growth on *Trametes hirsuta* Wulf. ex Fr. and *Oligoporus placenta* (Fr.) Gilb. & Ryvardeen at the end of the experiment (9<sup>th</sup> day) by CuO-*L.camara* np formulation.

There was no inhibition of fungal growth at concentrations 0,25; 0,5; 1; 5; 7 and 10% in all the plates till the end of the experiment in the case of plates having *A. indica*, *P. pinnata*, and orange peel (*C. reticulata*) extracts. Plates with CuO nanoparticles synthesized using *A. indica*, *P. pinnata* and orange peel (*C. reticulata*) extracts also showed no inhibition at concentrations ranging from 0,25; 0,5; 1; 5; 7 and 10%. Plates containing precursor solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  showed inhibition of up to 17% at a concentration of 10% which is not significant. *L. camara* leaf extract exhibited an inhibition of 9,66 % at a concentration of 10% against the white rot fungus *T. hirsuta*.

**Table 1:** Effect of biologically synthesized CuO nanoparticle against the wood decay fungi *Trametes hirsuta* and *Oligoporus placenta*\*.

Sample	Concentration (%)	Percentage of inhibition Ninth day	
		<i>T. hirsuta</i> (White rot)	<i>O. placenta</i> (brown rot)
Control	NIL	0,00	0,00
Copper sulphate precursor	5	3,00	2,30
	7	5,00	3,30
CuSO <sub>4</sub> 5H <sub>2</sub> O (0,025M)	10	17,00	17,30
CuO nanoparticle Std nanopowder (< 50 nm)	0,1	16,46	0,00
	0,25	46,80	0,00
	0,5	60,86	4,60
	1	58,83	4,03
	2	67,70	12,30
	5	71,60	51,60
<i>A. indica</i> leaf extract	0,25;0,5;1;5;7;10	0,00	0,00
<i>A. indica</i> leaf extract with CuO nps	0,25;0,5;1;5;7;10	0,00	0,00
<i>P. pinnata</i> leaf extract	0,25;0,5;1;5;7;10	0,00	0,00
<i>P. pinnata</i> leaf extract with CuO nps	0,25;0,5;1;5;7;10	0,00	0,00
	0,25;0,5;1;5;7;10	0,00	0,00
Orange peel ( <i>C. reticulata</i> ) extract	0,25;0,5;1;5;7;10	0,00	0,00
	0,25;0,5;1;5;7;10	0,00	0,00
Orange peel ( <i>C. reticulata</i> ) extract with CuO nps	0,25;0,5;1;5;7;10	0,00	0,00
	0,25;0,5;1;5;7;10	0,00	0,00
<i>L. camara</i> leaf extract	0,25	0,00	0,00
	0,5	0,00	0,00
	1	0,00	0,00
	5	1,30	0,00
	7	2,00	0,00
	10	9,66	0,00
<i>L. camara</i> leaf extract with CuO nps	0,25	0,00	0,00
	0,5	0,00	0,00
	1	0,00	0,00
	5	1,84	15,55
	7	48,88	22,96
	10	100,00	34,44

\*Mean of three replications.

In plates with CuO nanoparticles synthesized using *L. camara* leaf extract there was 48,88 % inhibition on the growth of the white rot fungus *T. hirsuta* and 22,96 % inhibition on the growth of brown rot fungus *O. placenta* at the end of experiment at a concentration of 7%. At 10% concentration there was complete inhibition of growth (100%) against *T. hirsuta* and an inhibition of 34,44 % was observed against *O. placenta*.

It revealed that even though CuO nanoparticle synthesized by *L. camara* leaf extract completely inhibited the growth of *T. hirsuta*, it is not capable of inhibiting the growth of *O. placenta* completely at a concentration of 10%. For further studies 10% of the sample is taken as minimal inhibitory concentration (MIC).

Commercially available CuO nanoparticles were also tested against wood decay fungi for concentrations ranging from 0,1 to 5%. An inhibition of 71,6 % was observed against *T. hirsuta* and 51,6% against *O. placenta* at a concentration of 5% copper oxide nanopowder in distilled water. There was no complete inhibition on growth of both indicating the instability of the CuO nanoparticles in distilled water, as the particles that dispersed in distilled water without adding any stabilizer tend to aggregate and settle down. It corroborates the fact that the stability of the nanoparticles in the dispersing medium is one of the main factor that describes the properties of nanoparticles (Yu and Xie 2012, Nair *et al.* 2017, Nair *et al.* 2018).

Since there was no significant level of inhibition in the plates containing *L. camara* leaf extract and precursor solution of  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  (0,025M) alone, it can be concluded that the complete inhibition on growth of *T. hirsuta* observed in plates containing CuO nps synthesized by *L. camara* leaf extract is due to the presence of CuO nanoparticles synthesized or due to the synergistic effect of the plant extract and the synthesized CuO nanoparticles. The smaller size of CuO nanoparticles synthesized by *L. camara* leaf extract when compared to the size of CuO nanoparticles synthesized by other plant extracts might have also contributed to the inhibition on the growth of the decay fungi. The copper oxide nanoparticles synthesized in plant extracts were found to be stabilized by the components of the plant extract maintaining the properties of nanoparticles. This is in accordance with earlier reports that CuO nanoparticles synthesized with the help of plant extracts acts as both reducing and stabilizing agents (Lee *et al.* 2011, Khan *et al.* 2017). Many plant extracts have reported to have antifungal property and have wood preservative effects, also (Tascioglu *et al.* 2013). Gupta *et al.* (2017) reported that *L. camara* have wood protection properties as evidenced by its ability to improve the dimensional stability of wood. Since both CuO nanoparticles and *L. camara* extracts had wood preservative properties, the synergistic effect of both would be resulting in an effective ecofriendly, and less expensive wood preservative.

**Table 2:** Antitermite activity of biologically synthesized CuO nps plant extract formulation as per IS 4833 1993 (Graveyard test-accelerated).

No. of months after implantation → Samples ↓	1	2	3	4	5	6
Control	0,83	2,5	2,5	5,83	7,5	10,8
<i>A.indica</i> leaf extract	0	0	0	0	2,5	5
<i>A.indica</i> leaf extract - CuO-nps	0	0	0	0	0	0
<i>P. pinnata</i> leaf extract	0	0	0	0	0	5
<i>P. pinnata</i> leaf extract - CuO-nps	0	0	0	0	0	0
Orange peel ( <i>C. reticulata</i> ) extract	0	0	0	0	0	2,5
Orange peel ( <i>C. reticulata</i> ) extract - CuO- nps	0	0	0	0	0	0
<i>L. camara</i> leaf extract	0	0	0,8	2,5	5	7,5
<i>L. camara</i> leaf extract - CuO- nps	0	0	0	0	0	0

Damage (%) of rubber wood stakes. Mean of six replications.

There was no damage to the rubber wood samples treated with the formulation of copper nanoparticles

synthesized using all the plant extract after an exposure period of six months in the field. (Table 2). Evaluation is progressing to get a better idea regarding the antitermite activity of the plant extract CuO nps formulation under field conditions.

## CONCLUSIONS

Formulation of copper oxide nanoparticle and *Lantana camara* leaf extract was found to be effective as a wood protectant against two decay fungi as evidenced by growth test on agar with added test substances. This may be due to the smaller particle size of the CuO nps synthesized using *L. camara* extract. All the plant extract CuO np formulations are found to give protection against termites in the field for a period of six months and further evaluations are in progress for a better conclusion. Development of a sustainable plant extract based synthesis route for metal nanoparticles enables the possibility of combining the intrinsic property of plant extract and metal nanoparticles for potential application in wood protection. The resultant formulation may protect wood from biodeterioration in a more effective way without any harmful effects to the environment. However, decay tests with impregnated wood samples must follow to prove protecting activity also with wood. In view of a feasible use for wood protection it must be also investigated if the CuO nps fix to the woody cell wall because otherwise only an indoor use will be possible.

Furthermore, more fungi than only each one white rot and one brown rot species should be investigated because there are hundreds of different species involved in wood decay. In view of practice it is also remarkable that the CuO nps were more effective against a white-rot fungus because in many regions (e.g. Europe) softwoods are the main construction woods and these are mostly attacked by brown-rot fungi. Last, the costs of such wood treatment compared to other wood protection measures must be considered.

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