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ARTÍCULO

Eco-friendly protection of pine wood using copper nanoparticles biosynthesized from *Cleistocalyx operculatus* leaf extracts

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Abstract:
This research investigates the eco-friendly biosynthesis of copper nanoparticles (CuNPs) from *Cleistocalyx operculatus* (water banyan) leaf extracts for the sustainable preservation of pine wood *Pinus kesiya* (khasi pine). The study specifically targets protection against common wood-decaying fungi, including *Aspergillus flavus* ATCC 9643, *Fusarium oxysporum* ATCC 48112, and *Penicillium citrinum* ATCC 9849, as well as termites. CuNPs were synthesized under optimized conditions: 80 °C for 30 minutes using a rotary evaporator, at pH 11, with 20 mM copper sulfate, and a 1.5:1 plant extract to precursor ratio. Comprehensive characterization using UV-Vis spectroscopy confirmed a prominent absorption peak at 595 nm. Scanning Electron Microscopy and Transmission Electron Microscopy further validated their spherical morphology, high crystallinity, and an average size of 2 ± 1 nm. Wood durability was evaluated through laboratory tests of fungal and termite resistance To assess the effects of different CuNPs treatments on fungal resistance and termite resistance, a one-way ANOVA was employed. Significant differences were found among the treatment groups ($P<0,05$). Duncan's multiple range test was performed using SPSS Statistics version 26, with statistical significance set at $\alpha = 0,05$. The findings demonstrated a clear concentration-dependent efficacy of CuNPs. Treated wood samples exhibited significantly enhanced fungal and termite resistance, displaying notably reduced weight loss (approximately 10-15 %) compared to untreated controls (20-30 %). Furthermore, even at the lowest tested dosage, a substantial termite mortality rate of 43,17 % was observed, highlighting the potent bioactivity of the biosynthesized CuNPs. These results support the potential of *Cleistocalyx operculatus*-mediated CuNPs as an environmentally sustainable substitute for conventional, often harmful, wood preservatives. This green approach offers a sustainable alternative for wood protection and shows promise for broader applications of eco-friendly nanomaterials.

Keywords*:* Biosynthesis, copper nanoparticles, *Pinus kesiya*, termites resistance, wood-decaying fungi, wood preservation.

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Introduction

Khasi pine (*Pinus kesiya* Royle ex Gordon) a member of the family Pinaceace, is native to the Himalaya region (Asia) including Burma, China, India, Laos, Philippines, Thailand, Tibet, and Vietnam (Cave and Walker 1994). Khasi pine (*Pinus kesiya* Royle ex Gordon) is a softwood tree that has been a valuable and effective material for construction and furniture making, for example, construction, boxes, flooring, ceilings, furniture, and resin (Thao *et al.* 2022, Xu *et al.* 2022). However, pinewood suffers from weak resistance against biotic (wood-decaying fungi and insects) (Baysal *et al*. 2021, Zanatta *et al.* 2022).

Pinewood preservatives have been used to enhance wood durability and even today, it keeps being an active field of research (Akhtari *et al.* 2013). Pinewood preservatives contain water-based preservatives, anti-corrosion oil mixtures, coal tar creosote preservatives, and pentachloroph preservatives. The main relevant water-based preservatives include CCA (chromium-plated copper arsenate), ACQ (quaternary ammonia copper), and CA (copper azole). Some wood preservatives have gradually been banned or restricted due to negative effects on environment, such as lindane, sodium pentachlorophenate, CCA, and so on (Zhou *et al.* 2016). Heat treatment,

an eco-friendly wood modification process, enhances the durability of pine wood but this method is ineffective against wood-decaying fungi and termites (Salman *et al.* 2017, Sivrikaya *et al.* 2015). Khasi pine (*Pinus kesiya* Royle ex Gordon), a widely utilized timber resource, faces significant preservation challenges due to its inherent susceptibility to biodegradation by wood-decaying fungi, alongside destructive insect pests like termites. This vulnerability necessitates effective preservation methods; however, conventional chemical treatments often present notable environmental and health risks. In recent years, nanomaterials have emerged as a pivotal component in the next generation of wood protection systems (Clausen *et al.* 2010, Shiny *et al*. 2019).

Nanoparticles exhibit superior penetration and more uniform absorption within wood compared to traditional materials (Aguayo *et al*. 2021). Specifically, the performance of Ag, Cu, ZnO, and TiO_2 nanoparticles has demonstrated improved durability and decreased deterioration of wood artifacts against termites, rot, mold, fungi, and ultraviolet rays (Corbu *et al.* 2023, Holy *et al.* 2022, Usmani *et al.* 2020, Zanatta *et al.* 2022). Prior studies have investigated the impregnation of radiata pine wood with CuNPs for their retention, absorption, permeation, and protective effects against wood-rotting fungi like *Gloeophyllum trabeum* and *Rhodonia placenta* (Aguayo *et al.* 2021). Furthermore, research has shown that CuNP-treated radiata pine (*Pinus radiata* D. Don) maintains superior mechanical properties compared to wood treated with micronized copper-impregnated samples, positioning CuNP-based preservatives as a promising candidate for alternative treatments (Aguayo *et al.* 2022).

Recognizing the compelling benefits of green chemistry, this study specifically focuses on the eco-friendly biosynthesis of CuNPs utilizing leaf extracts of water banyan (*Cleistocalyx operculatus* (Roxb.)) as both the reducing and stabilizing agent, a method that minimizes the use of hazardous

chemicals and promotes sustainable production. A current challenge involves thoroughly investigating the efficacy of these biosynthesized CuNPs in protecting pine wood from specified fungal decay and termite infestation, to establish a viable, environmentally benign alternative to traditional wood preservatives. Pham *et al.* (2024) effectively synthesized copper-chitosan nanoparticles by a reduction process, employing water banyan (*Cleistocalyx operculatus* (Roxb.)) leaf extract as a reducing agent and chitosan as a stabilizer. However, there is now no research on the production of CuNPs utilizing solely water banyan (*Cleistocalyx operculatus* (Roxb.)) leaf extract for the preservation of pine wood.

CuNPs, due to their nanoscale size, can be more readily absorbed into wood and distributed evenly, with their performance further enhanced by surfactants that aid dispersion. Their antifungal and termite-proof action stems from both copper ions and the ability of nanoparticles to penetrate fungal cells via endocytosis, disrupting DNA structure and inhibiting fungal growth (Civardi *et al*. 2015, Chatterjee *et al.* 2014). Studies have demonstrated superior protective effects of CuNPs against wood-degrading organisms. Akhtari *et al.* (2013) found that nano-copper treatments reduced termite-related wood loss from 46,8 % to 0,2 %. Similarly, Kartal *et al.* (2009) showed that wood treated with CuNPs or $CuSO_4$ experienced significantly reduced decay by *G. trabeum*. Biosynthesized copper oxide nanoparticles from *Lantana camara* extract have also proven effective in protecting wood against fungi and termites for at least six months (Shiny *et al.* 2019), supporting the potential of green-synthesized CuNPs as environmentally safe and efficient wood preservatives.

Despite the promising potential of nanotechnology in timber preservation, a significant gap remains in developing fully additive-free biosynthesis methods and applying them to high-value regional wood species. Current green-synthesis protocols often still rely on secondary chemical

stabilizers like chitosan, and the specific protective efficacy of these nanoparticles on the unique cellular structure of pine wood (*Pinus kesiya*) has yet to be explored clearly. Thus, the current study aims to carry out the following three main objectives. Firstly, biosynthesize CuNPs entirely from the extract of the leaf of water banyan (*Cleistocalyx operculatus* (Roxb.)), (without the addition of nanoparticle stabilizers). Secondly, investigate the impact of CuNPs on the growth of wood-decaying fungi, (specifically soft-rot fungi such as *Aspergillus flavus*, *Fusarium oxysporum*, and *Penicillium citrinum*). Finally, assess their potential for pinus wood protection against termites by CuNPs biosynthesized.

Material and methods

Material

The chemicals (Copper sulfate - 5 mM; 10 mM; 15 mM; 20 mM; 25 mM), sodium hydroxide - 0,1 M, Potato Glucose Agar medium) used were obtained from Sigma Aldrich Chemie (Germany). The fresh leaves of water banyan (*Cleistocalyx operculatus* (Roxb.)) were collected in Lamdong province, Vietnam. The leaves were processed immediately to avert biological deterioration.

Preparation of *Cleistocalyx operculatus* leaf extract

Water banyan (*Cleistocalyx operculatus* (Roxb.)) leaves were washed thoroughly 3 times in running tap water followed by sterile distilled water. Fresh leaves were dried at 50 ºC for 12 h and then homogenized by grinding. Leaf powder of 5 g was placed in 100 ml deionized water and kept in a water bath at 70 ºC ± 5 ºC for one hour. The cooled mixture was filtered using Whatman number 1 filter paper and securely stored in airtight glass bottles at 4 ºC for further experiments (Yugandhar *et al.* 2017).

Biosynthesis of the CuNPs

Various aspects significantly influence the biosynthesis of CuNPs, including reaction temperature, pH, duration, concentration of plant extract, utilized precursors, and mixing speed, all of which affect the synthesis process to achieve the required size and morphology of CuNPs (Caroling *et al.* 2015, Subha *et al.* 2017). The CuNPs biosynthesis process was studied under different conditions of $CuSO_4$ concentration (5 mM; 10 mM; 15 mM; 20 mM; 25 mM), extract and $CuSO_4$ ratio (1:0.5; 1:1; 1:1.5; 1:2), pH (5; 7; 9; 11; 13), temperature (50 ºC, 60 ºC, 70 ºC, 80 ºC, 90 ºC), and reaction time (5 min, 10 min, 20 min, 30 min, 40 min). CuNPs biosynthesis process was synthesized in a rotary evaporator. A visible transition in the solution's color from yellow to brown signified the formation of CuNPs. CuNPs were collected by centrifugation at 4000 rpm for 10 min. The precipitate was washed three times with distilled water for further experiments.

Characterization of biosynthesized CuNPs

The formation of CuNPs was inferred by visual observation followed by a UV-Vis spectrophotometer (Specord SmartSpecTM Plus, American) at a wavelength range from 400 nm to 700 nm. The shape and size of CuNPs were determined using Scanning Electron Microscopy (SEM, TM-1000, Hitachi, Japan) and Transmission Electron Microscopy (TEM, JEM-1230, JEOL, Akishima, Japan). The dimensions of CuNPs distributions from TEM images were assessed using ImageJ 1,53 t (64-bit) and Origin 9.1 (32-bit) (Zhang and Wang 2023).

Biological durability test

Pinewood specimens

Pine sapwood blocks were dried to the constant weight in an oven set at 70 ºC for 24 hours which measuring 50 mm x 25 mm x 15 mm (L x R x T) and 50 mm x 50 mm x 10 mm (L x R x T) were prepared for resistance to fungal decay test and termite test, respectively (Kadir *et al.* 2017). Wood specimens were initially vacuumed at 99 kPa for 37 min with the assigned CuNPs solution (1 mg/ml; 2 mg/ml; 3 mg/ml; 4,5 mg/ml; 6 mg/ml) in a vacuum chamber. Then, impregnation on the

wood specimens was applied again by submerging them for one hour (Ping 2015). A vacuum at 99 kPa for 10 min to empty the remaining solution. The negative control employed was wood impregnated with water banyan (*Cleistocalyx operculatus* (Roxb.)) leaf extract. The positive control employed was wood impregnated with WOODLIFE - CopperCoat Green Wood Preservative (Rust-Oleum® WOODLIFE® CopperCoat™).

Fungus culture

Three fungi were used for the determination of CuNPs protection efficiency *Aspergillus flavus* ATCC 9643, *Fusarium oxysporum* ATCC 48112, and *Penicillium citrinum* ATCC 9849 were bought from Medical Supply Company LTD. The fungal strains were reverted and cultured on a PGA (Potato Glucose Agar) medium.

Fungal resistance

Fungal resistance tests were performed according to the Evaluation of antifungal resistance based on the EN 113-2:2020 (2020) standard. Ten specimens exposed to CuNPs were incubated with isolated fungi at 22 °C ± 2 °C and 65 % ± 5 % RH for 16 weeks in PGA culture. The weight loss caused by a fungus was given by the following Equation 1:

Equation 1: $$WL(\%) = \frac{m_1 - m_2}{m_1} x100$$

Which: WL: weight loss (%)

m_1, m_2: dry weight of the sample before and after exposure to fungi (g)

The natural resistance classification was established according to the EN 113-2:2020 (2020) standard.

Termites test

Treated specimens were exposed to Asian subterranean termites (*Coptatermes curvignathus Holmgren*) according to EN 118:2013 (2013). Specimens were placed at the center of the test tubes that contained wet sand (1:4 v/v). A total of 250 termite workers were introduced into a test tube with 5 soldiers and 5 nymphs. The tubes were kept in a culturing chamber with air circulation at 22 °C ± 2 °C and 65 % ± 5 % RH for 8 weeks. The effect on the blocks was observed following the EN 118:2013 method. Ten samples of Pinus spp. were utilized to assess termite pathogenicity. At the conclusion of the testing period, the tubs were unsealed, and the quantities of live termite workers, soldiers, and nymphs were enumerated to ascertain the survival rate. Each wooden block was evaluated and visually assessed utilizing a standardized rating system (Table 1).

Table 1. Visual assessment rating of termite attack according to EN118 (2013).

Visual rating	Description
0	**No attack** No evidence of termite activity, erosion, or tunneling on the specimen surface.
1	**Attempted attack** One or more of the following, limited in extent: Superficial surface erosion too shallow to measure. Penetration to a depth of ≤ 0.5 mm restricted to small areas totaling no more than 30 mm². Any combination of the above.
2	**Slight attack** One or more of the following: Surface erosion up to 1 mm deep covering ≤ 1/10 of the specimen surface. A single tunnel up to 3 mm deep. Any combination of the above.
3	**Average attack** One or more of the following: Surface erosion < 1 mm deep over > 1/10 of the specimen surface. Surface erosion > 1 mm but < 3 mm deep covering ≤ 1/10 of the surface. Isolated tunnels > 3 mm deep that do *not* enlarge to cavities. Any combination of the above.
4	**Strong attack** One or more of the following: Surface erosion > 1 mm but < 3 mm deep covering > 1/10 of the specimen surface. Tunnels penetrating > 3 mm deep that enlarge to form noticeable cavities within the specimen. Any combination of the above.

Statistical analysis

The experiments were repeated 3 times. Statistical analysis was conducted using the SPSS Statistics version 26 in conjunction with analysis of variance (ANOVA). Duncan's multiple range test was used to analyze (statistical significance at α=0,05 level).

Results and discussion

Biosynthesis and characterization of CuNPs

Formation of CuNPs

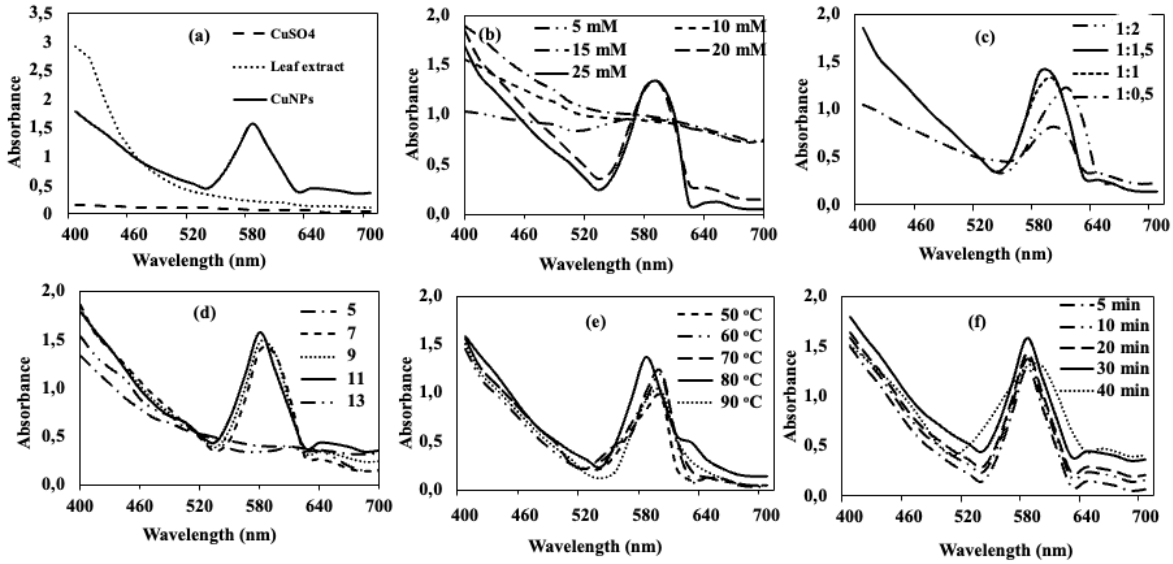

Figure 1: UV- vis of $CuSO_4$, leaf extract, and CuNPs. (a) Effect of the metal ion concentrations, (b) volume of extract: $CuSO_4$, (c) pH, (d) temperature, (e) reaction time, (f) formatted in CuNPs biosynthesis.

The color change from yellow to brown indicated the formation of CuNPs (Caroling *et al.* 2015, Thakur *et al.* 2014). The formation of CuNPs was monitored by UV-Vis spectrophotometer with the wavelength of absorbance from 400 nm to 700 nm (Chung *et al.* 2017, Manikandan and Sathiyabama 2015, Nasrollahzadeh *et al.* 2017, Nazar *et al.* 2018). In Figure 1a, there are no Surface Plasmon Resonance (SPR) peaks from 400 nm to 700 nm of leaf extracts and $CuSO_4$. SPR peak refers to the strong absorption of light at a specific wavelength due to the collective oscillation of surface electrons in metal nanoparticles like gold, silver, and copper. The SPR peak for CuNPs often occurs between 560 and 600 nm, influenced by particle size, morphology, and the surrounding medium of the nanoparticles (Maliki *et al.* 2022).

In this research, the SPR peak of the solution of leaf extract and $CuSO_4$ was depicted sharper at 595 nm. Therefore, CuNPs were biosynthesis successful from leaf extract (Chung *et al.* 2017, Manikandan and Sathiyabama 2015, Nasrollahzadeh *et al.* 2017, Nazar *et al.* 2018, Pradhan 2018). Several parameters play an important role in biosynthesized CuNPs including reaction temperature, pH, time, plant extract concentration, precursors used, and mixing speed which influence the synthesis process of CuNPs with desired size and morphology. Elevated temperatures frequently diminish particle size by expediting nucleation, however, the effects differ based on plant sources and post-synthesis processes, like as annealing. Extended reaction durations and aging generally result in larger particle sizes, whereas elevated quantities of plant extract facilitate the formation of smaller, more uniform nanoparticles owing to enhanced phytochemical richness. The selection and concentration of copper salt precursors influence both shape and size; nevertheless, further investigation is required for direct comparison. Ultimately, alkaline pH (exceeding 8) typically promotes the formation of smaller and more stable nanoparticles, whereas acidic circumstances result in bigger, agglomerated structures (Letchumanan *et al.* 2021).

This research involved a sequential analysis of each factor, followed by the selection of optimal settings for subsequent studies. The factors investigated were executed in the following sequence:$CuSO_4$ concentration (5 mM; 10 mM; 15 mM; 20 mM; 25 mM), extract and $CuSO_4$ ratio (1:0.5; 1:1; 1:1.5; 1:2), pH (5; 7; 9; 11; 13), temperature (50 ºC, 60 ºC, 70 ºC, 80 ºC, 90 ºC), and reaction time (5 min, 10 min, 20 min, 30 min, 40 min) to biosynthesis CuNPs. The results were shown in Figure 1, which includes information on $CuSO_4$ concentration (Figure 1B), extract and $CuSO_4$ ratio (Figure 1c), pH (Figure 1d), temperature (Figure 1e), and reaction time (Figure 1f). CuNPs were biosynthesized successfully using leaf extracts of water banyan (*Cleistocalyx operculatus* (Roxb.)) under optimum conditions, including several steps.

Copper sulfate (20 mM) was prepared in deionized water, resulting in a blue solution. The leaf extract was added to the blue solution in a rotary evaporator with a ratio of 1:1.5, and the pH was adjusted to 11 with 0,1 M sodium hydroxide. The rotary evaporator was heated to a bath temperature of 80 °C. Subha *et al.* (2017) biosynthesized CuNPs using *Passiflora foetida* extract at a $CuSO_4$ concentration of 20 mM and pH = 11 at 30 minutes, yielding nanoparticles with antibacterial activity. Similarly, Nasrollahzadeh *et al.* (2017) synthesized CuNPs from *Plantago asiatica* leaves at 80 °C. This synthesis temperature was also used in studies involving *Aloe vera*, *Centella asiatica L.* and *Moringa oleifera* extracts (Galan *et al*. 2018, Nasrollahzadeh *et al.* 2017 Pradhan 2018). Notably, Nasrollahzadeh's team also reported successful CuNPs formation within 30 minutes of reaction time.

Morphology of CuNPs

Transmission electron microscopy and scanning electron microscope of CuNPs were used to visualize the size and shape of biosynthesized CuNPs (Figure 2). The range of size was from 1 nm to 47 nm with an average size of 2 nm (Figure 2) (Zhang and Wang 2023). Controlling nanoparticle size and demonstrating high stability of nanoparticle dispersion are very important features in nanoparticle biosynthesis. Despite the growing interest in CuNPs, several methodological and environmental limitations should be acknowledged. From a methodological perspective, maintaining consistent particle size, surface charge, and dispersion stability remains challenging, particularly when scaling up from laboratory synthesis to industrial production, where

minor variations in reaction conditions can significantly alter nanoparticle properties (Crisan *et al.* 2022). Environmentally, CuNPs may undergo leaching, dissolution, or transformation into ionic copper, raising concerns about their long-term fate and bioavailability in natural systems (Adeleye *et al.* 2017).

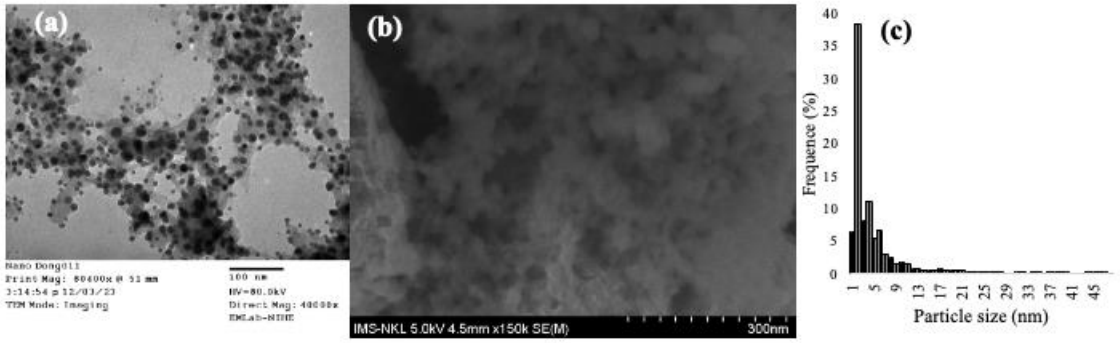

Figure 2: Biologically synthesized CuNPs using *Cleistocalyx operculatus* leaf extract. (a) TEM, (b) SEM, (c) histogram of CuNPs size distribution.

Biological durability test

Resistance soft-rot fungi of treated pinewood by CuNPs

Photographs of the pine wood samples, shown in Figure 3, display the CuNPs' effect on the colonization after 16 weeks of exposure to *Aspergillus flavus* ATCC 9643, *Fusarium oxysporum* ATCC 48112, and *Penicillium citrinum* ATCC 9849. Weight loss in pinewood for control (leaf

extract) and CuNPs-containing samples was determined after exposure to *Aspergillus flavus*, *Fusarium oxysporum*, and *Penicillium citrinum* (Figure 4). CuNPs inhibited the weight loss of wood compared to the control after exposure to wood-decaying fungi. Increasing CuNPs concentration improved the antifungal resistance and subsequently promoted the wood's resistance to decay. The weight loss of pinewood treated with leaf extract (control negative) when exposed to wood-decaying fungi ranged from 20 % to 30 %. Pine wood impregnated with modest quantities of CuNPs (1 mg/ml; 2 mg/ml; 3 mg/ml) was classified as fairly durable, exhibiting a mass loss of 10 % to 15 %. Elevating the concentration of CuNPs impregnated into wood to 4,5 mg/ml and 6 mg/ml demonstrated substantial protection, categorizing the wood within the durable group, with mass loss diminished by 10,25% ± 0,61% at 6 mg/ml and 9,02% ± 0,89% at 4,5 mg/ml.

Radiata pine wood impregnated with a solution of CuNPs containing 1 g/L and 3 g/L was capable of reducing mass loss to less than 5 % when wood comes into contact with wood-degrading fungi. The untreated pine wood sample has a mass weight of 25 - 30 %, comparable to the reference sample in this investigation (Aguayo *et al.* 2021).

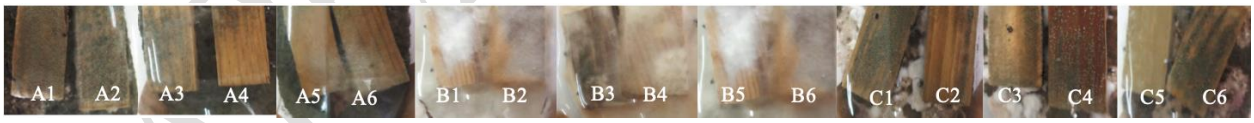

Figure 3: Photographs of pinewood samples exposed to biodeterioration at week 16 with fungi at the corresponding CuNPs-impregnated concentrations: control; 1; 2; 3; 4.5; 6 mg/ml. A1 – A6) correspond to exposure to *Aspergillus flavus* , B1 – B6) correspond to exposure to *Fusarium oxysporum*, C1 – C6) correspond to exposure to *Penicillium citrinum*.

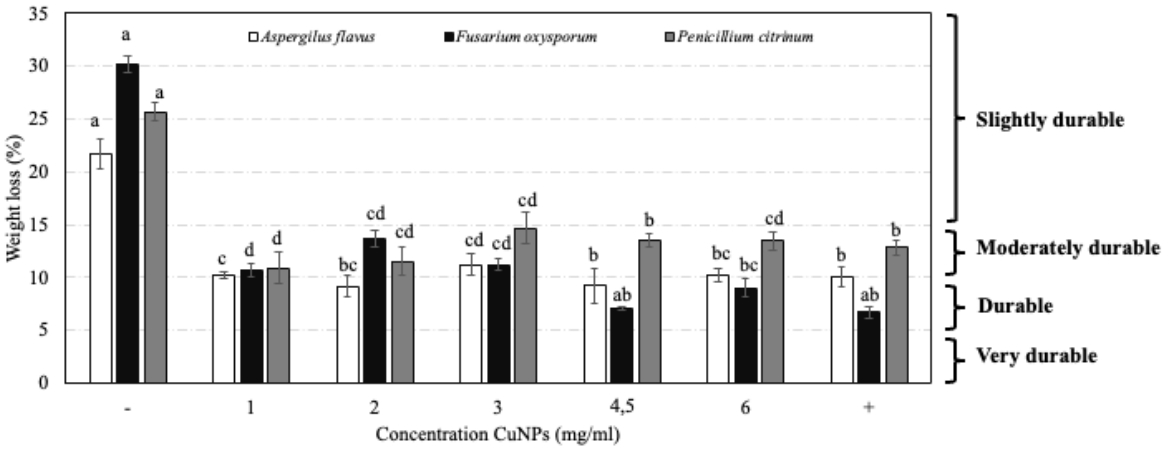

Figure 4: The figure antifungal activity at all concentrations of CuNPs (control negative; 1 mg/ml; 2 mg/ml; 3 mg/ml; 4,5 mg/ml; 6mg/ml, and control positive) with 3 fungal trains: *A. flavus, F. oxysporum, and P. citrinum* according to EN 113-2:2020 (2020). The letters abcd represent statistically significant differences in each other fungal strain of the Duncan test, P < 0,05.

Soft rot is thought to be the initial degradation stage in a typical wood colonization process. The formation of cylindrical cavities inside secondary cell walls or cell wall erosion occurs after fungal enzymes attack the lignocellulose matrix in wood. Even while it only causes little mass loss (7 % - 8 % in 30 months in Scots pine) in comparison to brown and white rot, it is a superficial wood injury that can significantly reduce impact bending strength (Bridžiuvienė and Raudonienė 2013). Numerous investigations have been conducted on the antifungal mechanism of CuNPs against soft rot in wood (Hermida-Montero *et al.* 2019, Pariona *et al.* 2019, Aleksandrowicz-Trzcińska *et al.* 2018, Devipriya and Roopan 2017, Jafari *et al.* 2015, Pham *et al.* 2019). It can be explained the CuNPs create a biological interaction with the mycelium that modifies the structure and biological function of the cell membrane. At the same time, CuNPs release Cu^{2+} ions that react with oxygen, resulting call were damage, and destroying proteins, lipids, and nucleic acids.

Furthermore, another study suggested that forming a bond with sulfur-containing radicals to form sulfur-NPs, with consequent reduction in total lipid content (with an abnormal accumulation of fatty acid saturated) forms atypical for membrane lipid biosynthetic cascade (Ouda 2014, Ramyadevi *et al.* 2012, Theivasanthi and Alagar 2011, Usman *et al.* 2013, Valodkar *et al.* 2011). Some studies suggest that fungi cannot recognize CuNPs. Thus, nanoparticles penetrate the cell wall of the fungus creating an imbalance in the formation of reactive oxygen species in the fungal cell. In addition, fungal cells were penetrated by nanoparticles of small size, affecting the homeostasis process. Therefore, fungi were inhibited and did not grow in the presence of CuNPs (Akhtari *et al.* 2013, Civardi *et al.* 2016). Besides that, nanoparticles with sizes ranging from 1 nm to 100 nm can enhance the protection of wood, helping to improve the permeability of chemical compounds (biocides) contained in wood (Freeman and McIntyre 2008, Kartal *et al.* 2009, Borges *et al.* 2018).

The smaller size of CuNPs than the diameter of a window pit (typically 10000 nm) and membrane openings on a border pit (typically 400 nm to 600 nm) enhanced the penetration and distribution of nanoparticles in wood (Freeman and McIntyre 2008). Thus, CuNPs were biosynthesis in this research with a size ranging from 1 nm - 47 nm could be penetrated completely into the wood base and then inhibit the effect of decaying fungi by reducing the moisture availability in the wood, preventing the absorption of the moisture, or blocking the flow path.

Termite test

Table 1: The visual rating and wood consumption of Pinewood

Concentrations of CuNPs (mg/ml)	Visual rating (R^2 = 0,95)	Wood consumption (%)	Termite Mortality (%)
Control negative	4	13,75e ± 0,60	18,19
1	3	9,95d ± 0,60	43,17
2	3	8,62c ± 0,58	59,85
3	3	7,80c ± 0,86	59,49
4,5	1	2,05a ± 0,65	79,88
6	1	3,43b ± 0,53	84,64
Control positive	1	2,04a ± 0,08	100

a, b, c, d, e: indicates that in formulas with the same letter in the same column, there is no difference at the 0,05 significance; ± standard error.

Table 1 shows the term loss values and survival of termites indicating that CuNPs protected pinewood. Photographs of the pine wood samples tested with termites are shown in Figure 5. The percentage weight loss of Pine negative control samples was 13,75 % ± 0,60 % - visual rating of termites attacking wood at level 4 according to EN 118:2013 (2013) standard. This result is similar to the termite resistance study of *Pinus* spp. in Malaysia (untreated wood). Pinewood exposed to termites under laboratory conditions showed a wood consumption of 11,38 % (Kadir *et al.* 2017). Thus, pinewood is susceptible to subterranean termite attacks. The samples were impregnated with CuNPs at low concentrations (1 mg/ml; 2 mg/ml; 3 mg/ml) which the termite resistance increased but not much (9,95 % ± 0,6 %; 8,62 % ± 0,58 %; 7,8 % ± 0,86 %, respectively) with a visual rating of 3. The termite resistance increased with increasing copper nanoparticles at 4,5 mg/ml and 6 mg/ml, wood consumption was 2,05 % ± 0,65 % and 3,43 % ± 0,53 % respectively - visual rating at 1.

Additionally, increasing CuNPs concentration enhanced the termite mortality rate. CuNPs exert larvicidal effects by penetrating cell membranes, forming pits and pores that lead to cell death, as supported by Sap-lam *et al.* (2010). Inside larvae, CuNPs accumulate and bind to phosphorus- and sulfur-containing biomolecules like proteins and DNA, disrupting enzymatic activity and ATP

synthesis, ultimately causing organelle damage and mortality (Suman *et al.* 2013). Observations also showed that increasing CuNP concentrations led to higher termite mortality over time, confirming their dose-dependent toxicity (Sap-lam *et al.* 2010).

Tahir *et al.* (2022) investigated the anti-termite properties of copper nanoparticles synthesized from Phalsa (*Grewia asiatica* L.) Termites (*Heterotermes indicola*), were subjected to CuNPs, resulting in the greatest mortality rate among the termites. In this research, the wood samples impregnated with CuNPs at different concentrations illustrated different improvements in termite resistance. Besides that, the efficacy of wood protection remains comparable to that of commercially available treatments.

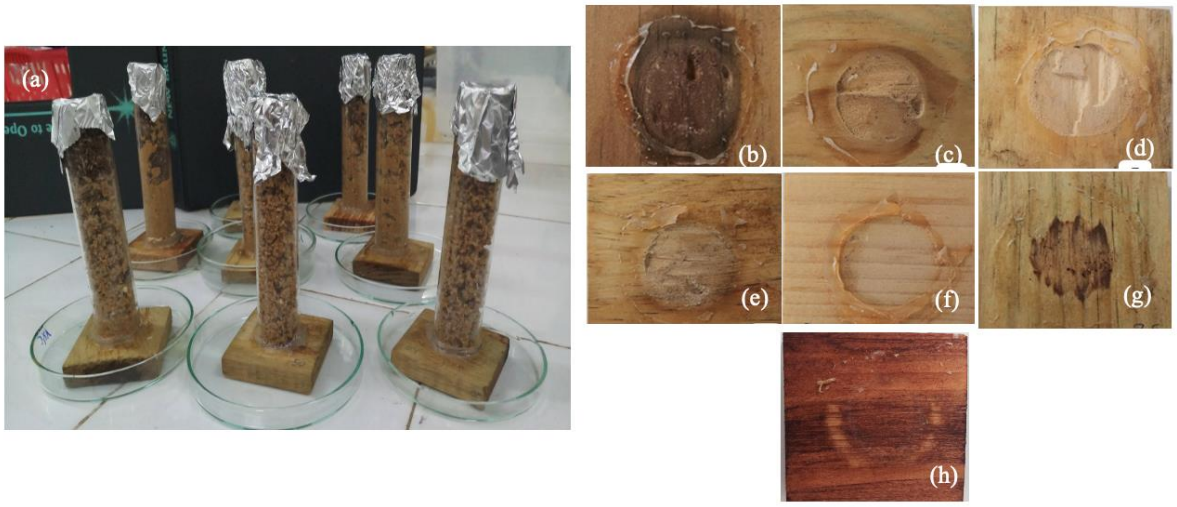

Figure 5: Photographs of pinewood samples exposed to biodeterioration with termites. a) Arrangement of wood blocks for EN 118:2013 (2013) test, b - h) The attacked pinewood species by *Coptatermes Curvignathus* were impregnated with CuNPs at concentrations of control negative; 1 mg/ml; 2 mg/ml; 3 mg/ml; 4,5 mg/ml; 6 mg/ml and control positive.

Conclusions

This study demonstrates the successful biosynthesis of copper nanoparticles (CuNPs) using Cleistocalyx operculatus leaf extracts under optimized conditions: 80 °C for 30 minutes using a rotary evaporator, at pH 11, with 20 mM copper sulfate, and a 1.5:1 plant extract to precursor ratio. The synthesized CuNPs exhibited spherical morphology, high crystallinity, and an average size of 2 ± 1 nm. The efficacy of these CuNPs in protecting pine wood from fungal decay and termite infestation was rigorously evaluated according to European standards EN 113-2:2020 (2020) and EN 118:2013 (2013), respectively. Pine sapwood impregnated with CuNPs at concentrations of 4,5 mg/ml and 6 mg/ml significantly increased wood durability, inhibited fungal growth, and enhanced termite resistance. Future research should prioritize evaluating the preservative's efficacy against a wider spectrum of aggressive white-rot and brown-rot fungi to ensure global versatility across diverse climatic conditions. Additionally, optimizing nanoparticle surface charge and dimensions will serve as a critical technical refinement to maximize deep-core penetration in large timber sections. These steps are essential for transitioning the current proof-of-concept into a high-efficiency industrial standard for sustainable wood protection.

Authorship contributions

T. B. T. T.: Methodology, validation, visualization, writing – original draft, writing – review & editing. T. T. A. L.: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, writing – original draft, writing – review & editing.

Declaration of interest

The authors declare no conflict of interest.

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